# TRANSPLACENTAL EFFECT OF URETHANE ON DNA SYNTHESIS IN MOUSE EMBRYONIC LUNG TISSUE IN ORGANOTYPICAL CULTURE

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The dynamics of DNA synthesis in organ cultures of line A embryonic mouse lung tissue (intact and exposed to the transplacental action of urethane) was studied by an autoradiographic method. Under the transplacental influence of urethane inhibition of DNA synthesis took place in the embryonic lungs on the first day. The labeling index fell by about two-thirds. It recovered during the next two days, and subsequently was higher than normally. The maximal labeling index in the experimental explants occurred on the 7th day of culture, i.e., on the 8th-10th day after transplacental action of urethane on the lung tissue *in utero*. The labeling index fell toward the 15th day of culture, but was still higher than in the intact explants; by the 21st day in intact cultures DNA synthesis had ceased completely, whereas it still continued in the experimental cultures.

KEY WORDS: Urethane; transplacental action; organ cultures of the lungs; DNA synthesis.

It was shown previously that during organ culture of embryonic target tissues exposed to the transplacental action of carcinogens and, in particular, of urethane, hyperplastic precancerous foci of proliferation and tumors develop in the lungs [2-6]. Under these circumstances the rate of survival of the experimental cultures was considerably increased compared with the control, especially at late stages of the experiment.

In chemical carcinogenesis the appearance of tumors and of morphologically detectable precancerous changes in the target tissues is preceded by a series of earlier changes detectable by other methods. One of these changes is inhibition of DNA synthesis in sensitive cells and inhibition of their passage through the mitotic cycle. Early changes have been studied mainly during the action of carcinogen on cultures of connective tissue cells [1]. Several papers have been published on changes in DNA synthesis during the development of adenomas in the lungs induced in adult mice by carcinogenic hydrocarbons and urethane [7-11].

Experiments were carried out to study the dynamics of DNA synthesis during transplacental carcinogenesis induced in embryonic target tissues in organ culture.

This paper gives the results of an autoradiographic study of DNA synthesis in organ cultures of embryonic lung tissue of intact mice and of mice exposed to the transplacental action of urethane.

## EXPERIMENTAL METHOD

Experiments were carried out on embryonic lungs of line A mice, which are distinguished by a high frequency of spontaneous adenomas of the lungs and are sensitive to the pneumotropic carcinogenic action of urethane. DNA synthesis was studied repeatedly 1, 7, 15, and 21 days after the beginning of culture of the lung tissue from 16-, 18-, and 21-day intact and experimental embryos. The transplacental effect of urethane was investigated at different times of embryogenesis, on the 15th, 17th, and 20th days. The lung tissue of the experimental embryos was explanted 1 or 3 days after exposure to urethane — on the 16th, 18th, and 21st days respectively of embryogenesis, so that the level of DNA synthesis could be compared in the

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experimental and control embryos. A single subcutaneous injection of urethane in 10% solution in a dose of 1 mg/g body weight was given to the pregnant females. Organ culture was carried out by the method described previously [2, 6]. Thymidine-$^3$H was added to the nutrient medium in a concentration of 1 μCi/ml 24 h before fixation of the explant. After histological treatment serial paraffin sections were cut from the explant to a thickness of 3 μ; the dewaxed sections were coated with photographic emulsion which was developed after exposure for 4 weeks at 4°C; the sections were stained with hematoxylin. In all the experiments 8 to 10 explants were taken at each time and the labeling index was determined in them, by counting at least 1000 cells in each explant. Only epithelial cells were counted. Student's t-test was used for the statistical analysis of the results.

## EXPERIMENTAL RESULTS

Autoradiographic study of DNA synthesis in explants of embryonic lung tissue from intact A mice showed that toward the end of embryonic development the proportion of DNA-synthesizing cells fell. For instance, in lung explants from 16- and 18-day embryos incubated for 24 h in medium containing thymidine-$^3$H the labeling index was 18.1 and 19.4%, respectively (Table 1), and in 21-day embryos 13.3%. After culture for 7 days DNA synthesis remained at a high level in the lungs of the 16- and 18-day embryos but in the lungs of the 21-day embryos it was reduced by about one-third. After culture for 15 days DNA synthesis was sharply reduced in lung explants of embryos of all ages studied, and after 21 days it ceased altogether.

The transplacental action of urethane had a marked effect on DNA synthesis in explants of mouse embryonic lung tissue. The dynamics of DNA synthesis in the experimental cultures depended on the time elapsing between administration of urethane to the mother and explantation of the embryonic lung tissue and on the age of the embryos. For instance, in the case of explantation of lungs from 16- and 18-day embryos one day after transplacental exposure to urethane (series I) the labeling index in 1-day-old cultures was about one-third of that in the corresponding cultures of embryonic lungs from intact mice (control). In the case of explantation of embryonic lungs 3 days after exposure to urethane (series II) the labeling index in the experimental cultures of the same age was actually a little higher than in the corresponding control (Tables 1 and 2).

After culture for 7 days the labeling index in the experimental explants of series I was about 4 or 5 times higher than after the first day in culture. In the experimental explants of series II a very small increase was observed in the proportion of labeled cells compared with the initial level. As a result, the level of DNA synthesis at this time in the experimental cultures of both series was almost identical (Table 2). Compared with the corresponding control (Table 1), it is evident that the labeling index in the experimental explants in the lungs of 18- and 21-day embryos was 1.5-2.5 times higher (Table 2).

On the 15th-21st day in culture a considerable decrease was found in the number of labeled cells in all series of experiments. However, the intensity of DNA synthesis under these conditions was higher in the experimental explants. For instance, on the 15th day of culture the proportion of DNA-synthesizing cells in the experimental cultures was 9.1-18% and in the control cultures 3.8-7.6%.

By the 21st day of culture DNA synthesis in the control cultures had completely ceased, whereas in the experimental explants of lungs from 18- and 21-day embryos the labeling index was 1.1-3.2, and in the lungs of 16-day embryos 11.7%.

The transplacental action of urethane thus caused initial inhibition, followed by stimulation of DNA synthesis in the embryonic lung tissue of mice. Judging from the results of these experiments, inhibition takes place on the first day after administration of urethane to the pregnant female. During the next two days DNA synthesis is restored, and after 3 days it is actually at a higher level than normally. During organ culture the number of DNA-synthesizing cells in the experimental cultures reached a maximum on the seventh day of culture, i.e., 8-10 days after the transplacental action of urethane on the lung tissue *in utero*. Inhibition and subsequent stimulation of DNA synthesis take place in the lungs of mice also following postnatal exposure to urethane and other carcinogens [7-11]. However, judging from results published by different workers, recovery and maximal incorporation of label during subsequent stimulation of DNA synthesis under these circumstances were observed later than after the transplacental action of urethane on organ cultures of embryonic lung tissues studied in the present experiment. This may be due both to differences in the level of proliferation of the lung tissue cells in embryos and adult mice and also to the conditions of explantation *in vitro*.

TABLE 1. Dynamics of DNA Synthesis in Organ Cultures of Embryonic Lung Tissue of Intact Mice (M ± m)

| Duration of culture, days | Labeling index in epithelium of explants of lung tissue taken at different times of embryonic development of mice | | |
|---|---|---|---|
| | day of embryonic development | | |
| | 16th | 18th | 21st |
| 1 | 18,0±1,1 | 19,4±0,75 | 13,3±1,4 |
| 7 | 23,0±0,5 | 18,0±2,0 | 9,9±0,1 |
| 15 | 4,3±0,7 | 7,6±0,16 | 3,9±0,7 |
| 21 | 0 | 0 | 0 |

TABLE 2. Dynamics of DNA Synthesis in Organ Cultures of Embryonic Lung Tissue of Mice Exposed to Transplacental Action of Urethane (M ± m)

| Duration of culture, days | Labeling index in epithelium of explants of lung tissue taken at different times of embryonic development of mice | | | |
|---|---|---|---|---|
| | exposure to urethane 1 day before explantation | | exposure to urethane 3 days before explanation | |
| | day of embryonic development | | | |
| | 16th | 18th | 18th | 21st |
| 1 | 5,4±0,5 | 7,0±1,4 | 23,4±1,3 | 19,5±1,1 |
| 7 | 25,7±1,6 | 25,9±2,2 | 31,9±0,6 | 24,0±0,3 |
| 15 | 18,0±1,0 | 9,1±0,1 | 12,5±1,0 | 10,8±2,0 |
| 21 | 11,7±3,3 | 1,1±0,5 | 3,2±0,5 | 2,5±0,3 |

Comparison of the dynamics of DNA synthesis in organ cultures of embryonic lungs of intact mice (control) and of mice exposed to the transplacental action of urethane shows that in the latter case the number of DNA-synthesizing cells was much higher and they were found in the late stages of culture, when DNA synthesis in the control had ceased completely (Tables 1 and 2). This fact evidently reflects the growth-stimulating action of carcinogenic agents, including urethane, on organ cultures of embryonic target tissues, which the writers demonstrated previously [2, 3, 5-6].

Previous investigations also showed that the growth-stimulating effect and the intensity of the morphological changes in embryonic tissue cultures depend on various factors and, in particular, on the dose of the carcinogen and the sensitivity of the tissue [2-4, 6]. In the present investigation a dose of urethane causing the development of hyperplastic changes in the epithelium in cultures of embryonic tissue from A mice was used. In this connection it would be interesting to study the dynamics of DNA synthesis in the lungs of sensitive and resistant mice exposed to different doses of urethane, including doses inducing adenomas of the lungs *in vitro*.

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