EFFECT OF BRIEF TRANSPLACENTAL EXPOSURE OF ORGAN CULTURES OF MOUSE EMBRYONIC LUNG TO THE ACTION OF URETHANE

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Following brief transplacental exposure to the action of high single doses of urethane, cultures of mouse embryonic lung tissues developed preadenomatous changes, consisting of diffuse hyperplasia of the epithelium. With increasing duration of the experiments, the incidence of these changes also increased, although the change from diffuse hyperplasia to focal hyperplasia or adenoma was never seen. The results of these experiments demonstrate that not merely the dose of urethane, but also the duration of its action, is important for the manifestation of its carcinogenic effect.

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In previous investigations adenomas of the lungs were obtained in organ cultures of mouse embryonic lung tissue through the transplacental action of urethane [3, 4]. The incidence of adenomas rose with an increase in the dose of urethane and in the duration of cultivation. Yet not only the dose of a carcinogen, but also the duration of its action, play an important role in the production of a visible carcinogenic effect [1]. The importance of the time factor increases especially in cases when the actual carcinogenic effect is due to an active metabolite and not to the substance itself, as can be postulated with respect to urethane [6, 8-11].

In the investigation described below a study was made of the morphological changes developing in an organ culture of mouse embryonic lung tissue following the transplacental action of single large doses of urethane.

EXPERIMENTAL METHOD

Experiments were carried out on line A mice. On the 18th-20th day of pregnancy female mice were given a single subcutaneous injection of 10% urethane solution in a dose of 60 or 90 mg. From 2 to 3 h later, when the animals were deeply anesthetized, they were sacrificed and the lungs taken from their embryos for subsequent organ cultivation. The technique is fully described in the previous communications [3]. Altogether 28 embryos from 4 females receiving urethane in a dose of 60 mg and 15 embryos from 2 females receiving 90 mg urethane were used, and 316 experimental and 341 control (embryonic lungs from intact females) explants were investigated after cultivation for 2, 5, 9, 12, 14, 17, 21, 24, and 26 days.

EXPERIMENTAL RESULTS

The morphological picture of the control cultures of embryonic lung tissue from intact mice in these experiments was the same as that described previously [3, 4, 6]. It will be seen in Table 1 that degenerative and necrotic changes in the control cultures were very slight at all periods of cultivation, but as the duration of the experiment increased, so also did their incidence.

Some of the explants in the experimental group of cultures were indistinguishable from the controls, but others showed changes similar to those described previously [3, 4, 6], with diffuse uniform or varied hyperplasia of the epithelium. The results given in Table 1 apply to both doses of urethane used, for no

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TABLE 1. Preadenomatous Changes in Organ Cultures of Mouse Embryonic Lung Tissue after Brief Transplacental Exposure to Urethane in Doses of 60 and 90 mg

Duration of cultivation (in days)		Total number of explants investigated	Morphological changes							
			absent		diffuse hyperplasia of epithelium		degenerative changes	widespread necrosis		complete absorption
			number of explants							
			abs.	%	abs.	%		partial	complete	
2	C	25	15	60,0				5	3	
	E	59	56	94,9				3		
5	C	35	21	60,0				8	2	3
	E	33	21	63,6	12	36,6				
9	C	42	24	57,1			2	16		
	E	59	26	42,5	19	32,2	6	13		
12	C	—	—							
	E	52	12	20,3	34	65,4	6			
14	C	30	23	76,6				2	2	3
	E	24	13	54,1	11	41,7	1	1	1	
17	C	151	68	45,0			49	31	4	17
	E	26	5	19,2	19	62,2		2		2
21	C	14	6	42,9				3	5	
	E	14	3	21,4	11	78,5		1		
24	C	20	7	35,0			5	3		5
	E	21	4	19,0	17	87,0				
26	C	33	7	21,2			12			14
	E	27	12	44,4	10	37,0	1	1	6	
Total	C	341	171	50,1			68	72	16	42
	E	316	149	47,1	133	42,0	8	27	8	2

Legend: C) control cultures; E) experimental.

difference in their carcinogenic action was observed. Diffuse hyperplasia of the epithelium was found in the experimental cultures after the 5th day of the experiment in 36.6% of cases. With an increase in the duration of the experiment the incidence of these changes rose, with slight fluctuations, to 80% (24th day of experiment), and then fell to 37% by the 26th day, when the experimental cultures began to develop degenerative changes. Diffuse hyperplasia is known to be the first stage of adenoma development [2, 5, 7, 9, 12]. However, progression to focal hyperplasia (the second stage), or more particularly into adenoma (the third stage), was not observed in these experiments.

Degenerative changes, disturbances of the alveolar structure of the lung tissue, necrobiosis, and necrosis were found much less frequently in the experimental cultures than in the controls: in only 10.9% of cases for the whole period of cultivation. Under the same conditions the control cultures appeared less viable, and degenerative changes were present in them in 49.9% of cases. Against the background of normal proliferation of the epithelium, preadenomatous changes were never observed in the control cultures.

Following the brief transplacental action of single large doses of urethane, cultures of mouse embryonic lung tissue thus developed diffuse hyperplasia of the epithelium. This condition, observed by many investigators, including the present writers, in experiments on animals [2, 5, 9, 12], is the first stage of development of adenoma of the lungs [6, 7]. In previous experiments to study adenoma development in vitro, the writers also observed diffuse hyperplasia of the epithelium developing in mouse embryonic lungs under the influence of urethane [3, 4]. Depending on the dose of the compound given and on the duration of the experiment, this diffuse hyperplasia turned into focal hyperplasia or adenoma. Administration of urethane was begun a few days before explanation, and the compound was given repeatedly at intervals of 1-2 days. Allowing for the rate of elimination of urethane from the body (24 h) [10], it can be concluded that each dose given had exerted its full effect. A clear relationship was thus revealed between the carcinogenic effect of the drug and the dose: administration of 60 mg led to diffuse and focal hyperplasia of the epithelium;

with a dose of 90-100 mg besides hyperplasia, adenomas appeared in 35.6% of cases, and with a dose of 120 mg in 66.6% of cases.

The same doses of urethane (60 and 90 mg) were used in the present investigation, but they acted for only 2-3 h. In this period of time naturally only part of the administered drug could be metabolized and could reach the lung tissue of the mouse embryos. This could explain, first, why the same affect was obtained from doses of 60 and 90 mg urethane, and second, why diffuse hyperplasia of the epithelium of the embryonic lung tissue did not progress into focal hyperplasia or adenoma, despite prolonged cultivation. Development of the carcinogenic effect is determined not only by the dose of a drug, especially urethane, but also by the duration of its action. The importance of dose and time of action of carcinogenic hydrocarbons in the case of skin carcinogenesis is confirmed by the results of Andrianov's experiments [1].

The brief transplacental action of urethane on mouse embryonic lung tissue thus induces preadenomatous changes in it in the course of subsequent organ cultivation. Similar changes must also be expected in the progeny of mice exposed to the action of this compound. This explains the danger from even brief contact between embryo and carcinogenic agents capable of inducing the development of preneoplastic changes in the postnatal period.

LITERATURE CITED

1. L. A. Andrianov, Byull. Éksperim. Biol. i Med., No. 1, 100 (1967).
2. L. A. Gritsyute, Vopr. Onkol., No. 5, 18 (1955).
3. T. S. Kolesnichenko, Vopr. Onkol., No. 12, 39 (1966).
4. T. S. Kolesnichenko, in: Tissue Culture in Oncology [in Russian], Moscow (1967), p. 269.
5. T. S. Kolesnichenko, Byull. Éksperim. Biol. i Med., No. 11, 87 (1968).
6. L. M. Shabad, Z. Krebsforsch., 70, 3 (1967).
7. L. M. Shabad, Precancer from the Experimental Morphological Aspect [in Russian], Moscow (1967).
8. E. Boyland and R. Nery, J. Biochem., 94, 198 (1965).
9. C. D. Larsen, Cancer Res., 7, 726 (1947).
10. A. M. Kaye, Cancer Res., 20, 237 (1960).
11. S. Rogers, J. Nat. Cancer Inst., 15, 1675 (1955).
12. M. B. Shinkin and M. J. Polissar, J. Nat. Cancer Inst., 16, 75 (1955).