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LOWER CARCINOGENICITY OF URETHANE FOR THE LUNGS OF ATHYMIC NUDE MICE THAN FOR IMMUNOLOGICALLY NORMAL MICE OF THE SAME LITTERS

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After two injections of urethane in a dose of 1 mg/g adenomas of the lungs developed in 2 of 10 nude mice and in 7 of 10 normal animals from the same litter. The mean number of adenomas per mouse was 0.2 ± 0.13 and 1.2 ± 0.36 respecitvely ($P < 0.05$).
KEY WORDS: *nude mice; urethane; immunologic surveyance.*

Mutant nude mice (nu/nu), characterized by congenital absence of the thymus, with consequent inability to form immunologic reactions of cellular type [4, 7, 10], provide a promising model for the study of the role of immunity in carcinogenesis. However, the short life span of these animals when kept under ordinary conditions (mean 3-4 months) makes it difficult to use them for comparison with normal mice with respect to the frequency of development of spontaneous tumors. They are more suitable for experiments with induced carcinogenesis. Several reports of the induction of tumors in nude mice by means of viruses and methylcholanthrene have already been published [10]. However, urethane was used for this purpose in only one investigation, despite its many advantages: solubility in water, comparatively low toxicity, highest activity after early postnatal administration, and so on. Tumors of the lungs induced by urethane can be detected macroscopically 1.5-3 months after injection. The multiple character of these tumors increases the reliability of the results, even when the number of animals used in the experiments is small. All this suggests that urethane carcinogenesis in athymic nude mice is a suitable model for testing Burnet's hypothesis [3] of the role of immunologic surveyance in carcinogenesis.

In the investigation described below an attempt was made to use this model for the above purpose.

EXPERIMENTAL METHOD

Experiments were carried out on nude (nu/nu) and immunologically normal (+/+ and +/nu) mice belonging to the same litters. The mice were obtained in 1972 from the Institute of Animal Genetics (Edinburgh, Scotland) and kept since that time in the animal house of the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, by mating heterozygous +/nu individuals. One quarter of the animals born under these circumstances have the nu gene in a homozygous state and are clearly distinguished from phenotypically normal +/+ and +/nu mice by the absence of hair. On April 15, 1975, urethane was injected intraperitoneally in a dose of 1 mg/kg into all the nude and an equal number of normal mice of the same sex in each of the 14 available litters. After 7 days a further injection of the same dose of carcinogen was given. The animals remained under observation until death of one of the partners (this was always a nude mouse), after which the other partner was immediately killed. The lungs were removed and fixed in 10% formalin. The adenomas were counted by examining the lungs under the MBF-1 loupe with a magnification of 16×. Altogether 10 pairs of mice were studied; 6 pairs in which the nude mice died earlier than 2 weeks after injection of the carcinogen were excluded.

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TABLE 1. Induction of Lung Tumors with Urethane in Immunologically Normal and Athymic nu/nu Mice from the Same Litters

No. of pair of litter	Sex of animal	Age at time of injection of urethane, days	Life span, days		Number of adenomas of lungs per mouse	
			total	after 1st injection of urethane	in immunologically normal mice +/+ and +/nu	in immunologically deficient nu/nu mice
1	Females	54 and 61	152	98	0	0
2	»	49 and 56	75	25	0	1
3	»	41 and 48	157	116	1	0
4	»	28 and 35	87	59	1	0
5	»	15 and 22	72	57	0	0
6	»	12 and 19	74	62	3	0
7	Males	12 and 19	81	69	1	0
8	Females	11 and 18	80	68	1	0
9	»	8 and 15	57	49	2	0
10	Males	8 and 15	67	59	3	1
	Total number of mice with adenomas of the lungs				7	2
					$P<0,05$	
	Total number of adenomas				12	2
	Mean number of adenomas per mouse				1,2±0,36	0,2±0,13
					$P<0,05$	

EXPERIMENTAL RESULTS

Details of the experiments and their results are given in Table 1. As a result of the action of urethane 70% of the normal mice developed adenomas of the lungs, 3 of them having more than one tumor. Both the frequency of development of the adenomas and their number per mouse increased with a decrease in the age at which the animals received the carcinogen, in agreement with data in the literature on this question [1]. As regards immunologically deficient nude mice, contrary to expectation according to the theory of immunologic surveyance, the number of lung tumors was not greater, but significantly smaller, than in the normal animals (in 2 of 10 mice). In attempts to explain this fact from the immunologic standpoint, it is not the concept of immunologic surveyance that must be invoked, but the opposite "immunostimulation theory." In fact, in normal mice immunity can be considered to stimulate the development of lung tumors, whereas immunodepression, by removing this stimulation, reduces their frequency. However, this hypothesis is contradicted by evidence showing that early thymectomy either does not affect or stimulates urethane carcinogenesis in mouse lungs [10].

Differences in the sensitivity of normal and athymic mice to the carcinogenic action of urethane may be connected with the pleiotropic effect of the nu gene on lung tissue, making it less predisposed to neoplastic degeneration, and also with its influence on the activity of the systems responsible for metabolic conversion of the carcinogen. Since it is urethane itself and not its metabolic products [8] that has a direct carcinogenic action, it is evident that an increase in the rate of metabolism of urethane in the body can lead to a decrease in the frequency of tumors induced by it. So far as the writers are aware, no investigation of the activity of enzymes hydroxylating urethane in nude mice has yet been published.

Finally, the possibility cannot be ruled out that the low frequency of tumors in nude mice in the present experiments was due to their relatively slow growth. For instance, whereas at the age of 4-5 days the mean body weight of the normal mice used in the experiments was 3.9 g, that of their siblings from the same litter did not exceed 2.5 g. Even at the age of 2 months, the nude mice were 33-50% lighter than the normal mice. This was evidently connected with disturbance of the function of their pituitary–adrenal and thyroid systems [9], which are concerned in the control of growth. At the same time, it has been shown that growth-inhibiting procedures also inhibit urethane carcinogenesis in mice [2, 5], whereas agents stimulating growth also stimulate urethane carcinogenesis [6]. In the writers' view,

it was the slow growth of the mice in the present experiments that was the cause of the relatively small number of tumors induced in them. However, whether this hypothesis is correct or not, the results are evidence that immunity does not play the decisive role in determining the frequency of development of lung tumors, at least of those induced by urethane, in mice. It is determined to a much greater degree by hormonal and, evidently, by certain other factors, and also by the local peculiarities of the target tissue.

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