EFFECT OF AGE ON INDUCTION OF INTESTINAL TUMORS IN MICE BY 1,2-DIMETHYLHYDRAZINE

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Data on the effect of age changes on tumor induction in laboratory animals are extremely contradictory [1]. In a previous investigation [4], in which experimental animals were treated with 1,2-dimethylhydrazine (DMH) and remained under observation until natural death, the frequency of intestinal tumors was the same in young and old mice although the lifespan of the old animals was much shorter than that of the young mice. This suggested that intestinal tumors in young mice began to appear later than in old mice.

Definite technical difficulties arise in the organization of experiments to study the effect of aging on carcinogenesis. When small doses of a carcinogen are used, the old animals may not survive long enough for neoplasms to appear. If large doses of carcinogen are used, on the other hand, the final frequency of tumors may be equal. The optimal variant is evidently to use a comparatively high dose of carcinogen and to kill the animals at different times after the beginning of exposure in order to discover differences in the early stages of carcinogenesis.

EXPERIMENTAL METHOD

Female CBA mice obtained from the Stolbovaya nursery were used. The experiment began simultaneously on mice of three age groups: 2 months (young), 8 months (adult), and 12 months (old). All the mice obtained from the nursery were kept after their removal and until the experiments began in the animal house of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. At the beginning of the experiments the mice weighed 18.9, 39.3, and 40.5 g in the young, adult, and old groups respectively. DMH·2HCl was dissolved in distilled water and injected into the mice weekly for 25 times subcutaneously in a dose of 8 mg/kg body weight, calculated as base (0.1 ml of solution/10 g body weight). The mice were weighed individually before each injection of the carcinogen. The mice were killed with ether once every 2 weeks starting with the 26th week after the beginning of DMH administration; animals for sacrifice were chosen on a random number table. The large intestines of all the mice killed or dying in the course of the experiment was opened along its whole length and the number of tumors found was recorded. After fixation of the intestine in 10% formalin solution for histological investigation, all the obvious tumors and also areas of intestinal wall suspected of having tumors were removed. Sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The frequency of tumors in the adult and old mice were about the same (Table 1), but in the young mice at all stages it was considerably lower. Differences in values obtained in the young animals, on the one hand, and the adult or old animals on the other hand, were highly significant ($P < 0.001$; $\chi^2 = 13.1$–53.3). Differences in the values obtained for the old and adult mice were not significant.

In the course of the experiments, 2, 8, and 13 mice died respectively in the groups of young, adult, and old animals. Tumors of the large intestine also were found in some of these mice. The total frequency of tumors (in killed and dying animals) was 13.1, 61.0, and

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TABLE 1. Frequency (in %, M ± m) of Intestinal Tumors in Animals Killed at Different Times after Beginning of DMH Injection

Group of animals	Age, months	Body weight of mice, g		Time after injection of DMH, weeks				Total
		at beginning of experiment	19 weeks later	26—29	31	33	35	
Young	2	18,9±0,85	34,7±3,6	0/25 0	1/19 5,3±5,1	5/20 25,0±9,7	5/18 27,8±10,6	**11**/82 13,4±3,8
Adult	8	39,3±4,1	35,6±4,9	9/15 60,0±12,6	4/12 33,3±13,6	11/12 91,7±8,0	9/12 75,0±12,5	33/51 64,7±6,7
Old	12	40,5±3,8	33,6±4,1	13/17 76,5±10,3	13/19 68,4±10,7	13/15 86,7±8,8	15/17 88,2±7,8	49/68 72,1±5,4

Legend. Numerator — number of animals with intestinal tumors, denominator — number of animals killed at the given time.

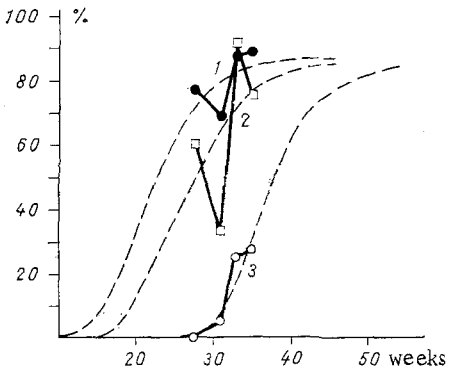

Fig. 1. Number of mice with tumors of large intestine (in % of number of animals killed) at different times after injection of DMH. 1) Old, 2) adult, 3) young animals. Abscissa, time after injection of DMH (in weeks); ordinate, number of mice with tumors of large intestine (in %). Broken lines — curves approximating data of time — effect relationship by means of the function of a lognormal distribution.

69.1% respectively in the groups of young, adult, and old mice. The frequency of tumors with infiltration of the subjacent tissues was 6, 30.5, and 54.3% respectively. The frequency of invasive tumors in the old mice, incidentally, was significantly higher than in the young and adult groups ($P < 0.05$). This is a particularly important finding, for the adult and old animals received equal absolute doses of DMH. The most malignant types of tumors (mucous carcinoma, tumors with invasion of the whole thickness of the intestinal wall) were found more often in the old mice, but the difference from the adult mice in this case was not significant.

Since the body weight of the adult and old mice at the beginning of the experiment was greater than that of the young mice, and since the dose of DMH depended on body weight, the young animals received a smaller absolute dose of DMH. In the course of injection of DMH the body weight of the adult and old mice fell, whereas that of the young mice rose; by the 19th week of the experiment the body weight was the same in animals of all groups (Table 1). Ultimately the adult and old mice received a total individual dose of DMH which was 1.3 times greater than that received by the young mice. Under clinical conditions at the pres-

ent time drug dosages are based on surface area (in mice this is determined from body weight by reducing by a power of 2/3). If in the present experiment the carcinogen had been given in a dose calculated per square meter of body surface of the animal, the total individual dose of DMH received by the adult and old mice would have been 1.2 times greater than that received by the young mice. The frequency of tumors in the adult and old animals would have been somewhat lower (by 10-15%).

In a previous investigation [4] in which DMH, in the same sessional dose, was injected into young mice for 30 weeks, the frequency of tumors in the large intestine toward the end of the period of observation (47 weeks) was 72%. When 25 injections of DMH were given the frequency of tumors in the large intestine was 70% 50 weeks after the beginning of exposure. As was pointed out above, the ultimate effect, assessed from the frequency of tumors in the young and old mice, was the same.

The time—effect relationship for the action of carcinogens is known to be described well by the function of a lognormal distribution. By applying this mathematical model to the experimental data, approximate curves of the time—effect relationship were obtained for young, adult, and old mice (Fig. 1). It could be estimated by these approximate curves that the medium latent period of tumor development in the large intestine of the young mice was approximately 1.8 times longer than in old mice and 1.4 times longer than in adult mice. Such differences as there were between the old and adult mice [1] were not statistically significant. Considerable age differences were thus found in induced carcinogenesis of the large intestine, manifested as a significant shortening of the latent period of tumor development in the old and adult animals compared with young mice.

It was shown previously [2] that if DMH is injected into rats aged 4, 8-10, and 18 months (dose calculated per kilogram body weight) the number of animals with tumors was the same in all groups, but the multiplicity of tumors was less in the rats aged 18 months, in which, however, the tumors were larger and exhibited destructive growth more often. In the old rats the indices of proliferation of the epithelium of the large intestine, especially at the bottom of the crypts where the stem cells are located, were significantly lower than in young mice. The authors cited concluded that changes developing in the body during aging inhibit the initiating action of the carcinogen but accelerate growth of tumors which have already arisen. It was shown later [7] that if DMH is injected (dose calculated per kilogram body weight) into rats of two lines aged 35, 120, and 210 days, the frequency of intestinal tumors was lower in females of one line in the older age group, whereas in animals of the other line, age did not affect the frequency of intestinal tumors in either females or males. We know that DMH is a carcinogen with systemic action, metabolized in the liver with the formation of conjugation products of the metabolite with glucuronic acid. These conjugation products are broken down in the intestine under the influence of bacterial β-glucuronidase, with isolation of the carcinogenic metabolite [2, 4]. Metabolic oxidation of carcinogens has been shown to take place more slowly in the liver of old mice, but the metabolites thus formed have greater mutagenic activity than those formed in young mice. It has also been found [6] that the β-glucuronidase activity of the large intestine of old rats is 1.5-2 times higher than that of young rats, the increase in activity starting from the age of 6-8 months. These facts evidently allow the accelerating action of the special nature of metabolism of the carcinogen in old animals on carcinogenesis in the large intestine to be to some extent explained.

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