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FORMYLHYDRAZINE CARCINOGENESIS IN MICE

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Summary.—Administration of 0·125% formylhydrazine in drinking water to 6-week-old randomly bred Swiss albino mice for life, induced lung tumours. Compared to untreated controls, the lung-tumour incidence rose from 15 to 94% in the females and from 22 to 100% in the males. The treatment had no detectable tumorigenic effect in other organs.

THE PRESENT WORK is part of systematic studies on the carcinogenicity and mode of action of hydrazine analogues. This class of chemicals is apparently widely distributed in the environment due to industrial, agricultural and medicinal use (The Merck Index, 1976). In addition they occur naturally in mushrooms, tobacco and moulds (Levenberg, 1961; List and Luft, 1968; Toth, 1975; Schmeltz, Abidi and Hoffmann, 1977; LaRue, 1977). Therefore the human population may be exposed to them to a considerable degree.

It was shown in earlier investigations that N-methyl-N-formylhydrazine, an ingredient of the edible false morel mushroom induced tumours in mice and hamsters (Toth, 1977; Toth and Nagel, 1978). Under conditions mimicking the milieu of the human stomach, N-methyl-N-formylhydrazine breaks down to methylhydrazine, a known tumour-inducer in mice and hamsters (Toth, 1977; Toth and Shimizu, 1973; Nagel *et al.*, 1977). Formylhydrazine was selected for this study to find whether this portion of the N-methyl-N-formylhydrazine molecule also posessess carcinogenic activity.

This report records the tumorigenicity of formylhydrazine administered in drinking water for life to Swiss mice.

MATERIALS AND METHODS

Swiss albino mice from the Eppley colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, separated according to sex into groups of 10, and given Wayne Lab-Blox diet in regular pellets (Allied Mills, Inc., Chicago Ill.) and tap-water or the chemical solution *ad libitum*, as described below.

The chemical used was formylhydrazine (FH, formic acid hydrazide) (Fig.), mol.wt 60·06, m.p. 54–56°C, purity > 99%, obtained from Aldrich Chemical Company, Inc. Milwaukee, Wisconsin.

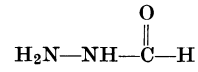

FIG. Chemical structure of formylhydrazine.

A toxicity study was carried out before the chronic experiment. Five dose levels of FH (1, 0·5, 0·25, 0·125 and 0·0625%) were administered in the drinking water for 35 days to Swiss mice. By taking into account 4 parameters—survival rates, body weights, chemical consumption and histological changes—the 0·125% dose was found to be suitable for the lifelong treatments. This toxicity technique was developed in this laboratory (Toth, 1972*a*).

The solution was prepared thrice weekly, and the total consumption of water containing FH was measured at the same intervals during the treatment period. The solution was contained in brown bottles because of the possible light sensitivity of the chemical. The 0·125% solution of FH used for the chronic experiment was analysed by gas chromatography after standing 48h at room temperature and was found to contain > 98% of the

original compound unchanged. The chronic-exposure group and the controls were as follows:

Group 1 —FH was dissolved in the drinking water as a 0·125% solution and was given for the life-span of 50 female and 50 male mice that were 6 weeks (43 days) old at the beginning of the experiment. The average daily consumption per animal of water containing FH was 4·9 ml for the females and 5·9 ml for the males. Thus the average daily intake of FH was 6·1 mg for a female and 7·3 mg for a male.

Group 2.—As an untreated control, 100 female and 100 male mice were kept and observed from weaning (5 weeks of age).

The experimental and control animals were carefully checked and weighed at weekly intervals, and the gross pathological changes were recorded. The animals were either allowed to die or killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and fixed in buffered 10% formalin. Histological studies were made on liver, spleen, kidney, bladder, thyroid, heart, pancreas, ovaries, testis, brain, nasal turbinae, and at least 4 lobes of the lungs of each mouse, as well as on those organs showing gross pathological changes. Sections from these tissues were stained routinely with haematoxylin and eosin.

RESULTS

The survival rates after weaning are recorded in Table I. As can be seen, the treatment in both sexes significantly shortened survival compared with that of untreated controls.

The numbers, and percentages of animals with tumours, and their ages at death (latent periods) are summarized in Table II. The detailed incidences of lung tumours are described below.

Lung tumours

Of the treated females, 47 (94%) developed 531 lung tumours. Of these, 23 mice had 149 adenomas and 24 mice had 331 adenomas and 51 adenocarcinomas. Their average age at death was 68 weeks, the first tumour being observed at the 43rd week and the last at the 89th week. In the treated males, 50 (100%) developed 400 such neoplasms. Of these, 26 mice had 114 adenomas and 24 mice had 237 adenomas and 49 adenocarcinomas. Their average age at death was 64 weeks, the first tumour being seen at the 37th week and the last at the 88th week.

Grossly and histopathologically, all observed lung lesions were similar to those described earlier (Toth, Magee and Shubik, 1964; Toth and Shimizu, 1974).

Other tumours

In a few instances, other types of tumours were found in the various groups shown in Table II. Because of their low numbers, their appearance cannot be attributed to the treatment.

DISCUSSION

The present finding demonstrates the tumorigenicity of formylhydrazine administered in drinking water daily to Swiss mice for life. The incidence of lung tumours increased from 15 to 94% in females and from 22 to 100% in males, when compared with the untreated con-

TABLE I.—*Treatment and Survival Rates in Formylhydrazine (FH)-treated and Control Swiss Mice*

Group	Treatment	Initial No. and sex	No. of survivors (age in weeks)												
			10	20	30	40	50	60	70	80	90	100	110	120	130
1	0·125% FH in drinking water daily for life	50♀	50	49	49	49	47	36	19	3	0				
		50♂	50	50	50	49	43	27	13	3	0				
2	Untreated controls	100♀	100	100	99	96	95	91	78	66	45	28	13	2	0
		100♂	100	98	92	88	80	62	36	17	11	3	2	1	0

TABLE II.—*Tumour Distribution in FH-treated and Control Swiss Mice*

Group	Treatment	Effective No. of mice	Animals with tumours of: Lungs No.	%	Age at death*	Other tissues*
1	0·125% FH in drinking water daily for life	50♀	47	94	68 (43–89)	1 Angiosarcoma of liver (82) 1 Angioma of liver (73) 1 Angioma of ovary (70)
		50♂	50	100	64 (37–88)	3 Angiomas of liver (50,72,77) 2 Angiosarcomas of liver (66,78) 1 Fibroma of spleen (82)
2	Untreated	100♀	15	15	90 (67–116)	20 Malignant lymphomas (31,33,47,76,79,85,86,87,89,89,91,91,94,99,102,103,104, 111,114,116) 2 Angiomas of ovary (88,119) 2 Angiomas of liver (81,114) 2 Angiosarcomas of liver (74,75) 2 Fibrosarcomas, subcutaneous (101,102) 2 Sex cord mesenchyma tumours (107,114) 1 Angiosarcoma of uterus (101) 1 Angiosarcoma of lymph node (86) 1 Adenoacanthoma of ovary (71) 1 Adenoma of ovary (110) 1 Adenoma of thyroid (88) 1 Adenocarcinoma of breast (127) 1 Adenocarcinoma of duodenum (86) 1 Carcinoma of glandular stomach (64)
	control	100♂	22	22	70 (40–124)	8 Malignant lymphomas (28,62,67,78,84,91,92,112) 2 Fibrosarcomas subcutaneous (69,92) 2 Adenomas of thyroids (69,92) 2 Hepatomas (69,94) 2 Angiomas of liver (70,92) 1 Angiosarcoma of liver (77) 1 Angiosarcoma of pararenal fat (81) 1 Angioma of anal gland (57) 1 Adenoma of parathyroid (78)

* Mean and range.
† Age (in weeks) at death in parentheses.

trols. The statistical analysis, carried out using Fisher's exact test (Armitage, 1971) for 2 × 2 tables, shows that treated females $P < 0{\cdot}0001$ and in males $P < 0{\cdot}0001$, the incidence of lung neoplasms was significantly higher than in the corresponding controls Histological examination showed the characteristic appearance of adenomas and adenocarcinomas of the lungs.

The edible wild mushroom, the false morel *Gyromitra esculenta* (Miller, 1972) contains up to 500 parts/10^6 N-methyl-N-formylhydrazine (Schmidlin-Mészáros, 1974; Wallcave and Conrad (unpubl.). This compound, administered orally, induced tumours in the lungs, liver, gall bladder and bile ducts of Swiss mice (Toth and Nagel, 1978) and in nearly the same organs in Syrian golden hamsters (Toth, 1977). Under certain *in vitro* conditions, and also in the mouse stomach, N-methyl-N-formylhydrazine breaks down to methylhydrazine (Nagel *et al.*, 1977), which in earlier investigations produced lung tumours in mice (Toth, 1972*b*), as well as malignant histiocytomas and tumours in the caecum of hamsters (Toth and Shimizu, 1973). The current study is the next logical step along this line to assess the possible tumorigenicity of the remaining portion of the N-methyl-N-formylhydrazine molecule. Formylhydrazine exhibited strong tumour-inducing action here, and this helps to explain the highly carcinogenic nature of N-methyl-N-formylhydrazine. In addition, the present work is also a continuation of studies designed to reveal the relative carcinogenic potencies of mono- and dialkyl-hydrazines. A series of compounds, such as methyl-, 1,2-dimethyl-, and 1,1-dimethyl-hydrazines, provided valuable information on the relationship between chemical structure and carcinogenic activity (Toth and Wilson, 1971; Toth, 1972*b*, 1973).

To date, around 40 hydrazine derivatives are known to induce tumours in laboratory animals (Toth, 1975, 1978). Interestingly enough, the human population is exposed to about half of these compounds. It appears therefore that this class of chemicals is relevant to environmental carcinogenesis, even though it has thus far received limited attention. Twenty of these 40 hydrazines were first shown to be carcinogenic in this laboratory.

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