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TUMORIGENESIS BY N-*n*-PROPYL-N-FORMYLHYDRAZINE IN MICE

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Summary.—Continuous administration of 0·04% N-*n*-propyl-N-formylhydrazine (PFH) for life in drinking water to 6-week-old randomly bred Swiss mice induced tumours of the lungs, preputial glands, liver and gall bladder. The tumour incidences in these 4 tissues were 91, 22, 8 and 6%, whereas in the untreated controls they were 25, 0, 0·5 and 0·5%, respectively. The higher dose of 0·08% PFH, given under identical conditions, induced only tumours of the lungs, liver and gall bladder in lower incidences, since the compound was too toxic for the mice. Histopathologically, the tumours were classified as adenomas and adenocarcinomas of the lungs, squamous-cell papillomas, and carcinomas and fibrosarcoma of preputial glands, benign hepatomas and liver-cell carcinoma, as well as adenomas and adenocarcinoma of the gall bladder.

The investigation is part of our structure/activity relationship inquiry aimed at revealing the mechanism of action of the N-alkyl-N-formylhydrazine series of chemicals.

THE PRESENT carcinogenesis study with N-*n*-propyl-N-formylhydrazine (PFH) in mice is a continuation of a structure/activity inquiry conducted with substituted hydrazines. Earlier investigations were performed in this species with structural homologues of PFH, including N-methyl-N-formylhydrazine (MFH), N-ethyl-N-formylhydrazine and N-*n*-butyl-N-formylhydrazine (Toth & Nagel, 1978; Toth *et al.*, 1979; Toth & Nagel, 1908*a*, *b*). At the beginning, we selected MFH as the first step in our inquiry because this chemical is an ingredient of the edible false morel mushroom *Gyromitra esculenta* (List & Luft, 1968; Schmidlin-Mészáros, 1975; Pyysalo & Niskanen, 1977). It is known that the human population is exposed to some extent, in certain areas, to this hazardous chemical (Miller, 1972). Subsequently, other N-alkyl-N-formylhydrazines were investigated to determine the possible relationship between chemical structure and tumour development at specific organ sites.

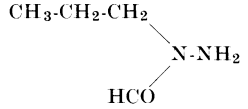
The current study proves the carcinogenicity of PFH given to Swiss mice at maximum tolerated dose levels in drinking water for life.

MATERIALS AND METHODS

Swiss albino mice from the colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, separated according to sex in groups of 5, and given Wayne-Lab blox diet in regular pellets (Allied Mills, Inc., Chicago, Illinois) and tap water or the chemical solution *ad libitum*.

The chemical used was N-*n*-propyl-N-formylhydrazine (PFH; Figure); mol. wt, 102·14; b.p., 98–100°C at 12 mm; purity >97%. PFH was synthesized in this laboratory in the following way (Kost & Sagitullin, 1963):

n-*Propylhydrazine*.—To a vigorously stirred solution of 1·0 kg (26·5 mol) of hydrazine monohydrate was added dropwise 285 g (2·3 mol) of *n*-propyl bromide. During the addition and 3 h after, the temperature was maintained at 45°C. The reaction mixture was con-



FIGURE—Chemical structure of N-*n*-propyl-N-formylhydrazine (PFH).

tinuously extracted for 48 h with 1 l of ethyl ether. The ether extract was dried (Na_2SO_4) and fractionally distilled until the volume was reduced to 250 ml. Solid KOH (20 g) was added and the *n*-propylhydrazine fraction (b.p. 119–21°C, lit. 119–20°C) was collected (yield 121 g, 71%). GLC and ^{13}C-NMR indicated the product was homogeneous and consistent with the assigned structure.

N-n-Propyl-N-formylhydrazine.—A solution of 44 g (1·0 mol) of methyl formate and 50 ml of absolute ethanol was added dropwise to a stirred solution of 74 g (1·0 mol) of *n*-propylhydrazine maintained at −15°C. The mixture was stirred for an additional 30 min at −15°C and warmed to room temperature over 4 h. The mixture was concentrated *in vacuo* and fractionally distilled. The N-*n*-propyl-N-formylhydrazine was collected at 105–107°C (14 mm) (yield 71 g, 70%). ^{13}C-NMR, IR and GLC analysis indicated the product was homogeneous and consistent with the assigned structure.

p-Nitrobenzaldehyde-N-propyl-N-formylhydrazone.—To ensure that the product was a 1,1-di-substituted hydrazine, a hydrazone was prepared. A solution of 755 mg (5·0 mmol) of *p*-nitrobenzaldehyde, 510 mg (5·0 (mmol) of PFH and 8·0 ml of methanol was refluxed for 2 h. Concentration and cooling produced yellow crystals. Recrystallization from 95% ethanol produced 1·03 g (88%) of the hyrazone (m.p. 144–5°C). ^{13}C- and ^{1}H-NMR were consistent with the assigned structure.

Analysis, calculated for C_{11}, H_{13}, N_3, O_3: C, 56·17; H, 5·53; N, 17·18. Found C, 55·92; H, 5·64; N, 17·82.

A toxicity study was performed before the chronic experiment. Eight dose levels of PFH, *viz.* 0·16, 0·08, 0·04, 0·02, 0·01, 0·005, 0·0025 and 0·00125% were administered in the drinking water for 35 days to Swiss mice. By taking into account 4 parameters: survival rates, body weights, chemical consumption levels and histological changes, the 0·08% dose was chosen for the lifelong treatment. This dose was, however, subsequently found to be too toxic in the chronic study, and an additional lower dose, 0·04%, was started separately. This toxicity technique was developed in our laboratory (Toth, 1972).

The solutions were prepared 3 times weekly, and the total consumption of water containing PFH was measured at the same intervals during the entire treatment. The solutions were contained in brown bottles because of the possible light sensitivity of the chemical. The 0·04 and 0·08% solutions of PFH were analysed by gas chromatography after standing 48 h at room temperature, and found to contain 98 and 97·5% respectively of the original compound. The chronic experimental groups and the controls were as follows:

Group 1: PFH was dissolved in the drinking water as a 0·08% solution and was given for the life span of 50 female and 50 male mice that were 6 weeks old at the beginning of the experiment. The average daily consumption per animal of water containing PFH was 4·2 ml for the females and 5·4 ml for the males. Thus the average daily intake of PFH was 3·4 mg for a female and 4·3 mg for a male.

Group 2: PFH was dissolved in the drinking water as a 0·04% solution and given for the life span of 50 female and 50 male mice that were 6 weeks old at the beginning of the experiment. The average daily consumption per animal of water containing PFH was 5·2 ml for the females and 6·2 ml for the males. Thus the average daily intake of PFH was 2·08 mg for a female and 2.50 mg for a male.

Group 3: As untreated controls, 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 moribund. Complete necropsies were performed on all animals. All organs were examined macroscopically and fixed in 10% buffered formalin. Histological studies were done on the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinals, at least 4 lobes of the lungs of each mouse, and on those organs with gross pathological changes. Sections from these tissues were stained with haematoxylin and eosin.

RESULTS

The survival rates after weaning are shown in Table I. The data demonstrate

TABLE I.—*Treatment and survival rate in N-n-propyl-N-formylhydrazine (PFH)-treated and control Swiss mice*

Group	PHF in drinking water for life	Initial No. and sex of mice	No. of survivors (age in weeks)														
			10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
1	0·08%	50♀	50	49	40	23	19	13	3	—							
		50♂	50	37	11	2	—										
2	0·04%	50♀	50	50	50	50	45	35	18	2	—						
		50♂	50	48	45	32	23	5	—								
3	Nil	100♀	100	99	98	92	83	77	70	55	45	30	16	6	1	1	—
		100♂	100	100	100	96	91	81	63	44	28	11	4	1	—		

that the treatments at both dose levels significantly shortened the survival time. The number and percentages of animals with tumours and their ages at death (latent periods) are summarized in Table II. The 4 statistically significant neoplasms were found in lungs, preputial glands, liver, and gall bladder, and are described in detail below:

Lung tumours

Of the females treated with the high dose, 22 (44%) developed 252 tumours in these organs. Of these, 19 mice had 188 adenomas and 3 mice had 50 adenomas and 14 adenocarcinomas. In the males treated with the high dose, 7 (14%) developed 54 adenomas.

Of the females treated with the low dose, 49 (98%) developed 751 tumours of the lungs. Of these, 18 mice had 177 adenomas and 31 mice developed 461 adenomas and 113 adenocarcinomas. In the males treated with the low dose, 42 (84%) developed 546 tumours in the lungs. Of these, 26 mice had 191 adenomas and 16 mice had 300 adenomas and 55 adenocarcinomas.

Macroscopically and histologically these lesions were similar to those described in this laboratory in this mouse strain (Toth & Shimizu, 1974; Toth *et al.*, 1979).

Preputial-gland tumours

Of the males treated with the high dose, 1 (2%) developed a squamous-cell carcinoma in this organ.

Of the males treated with the low dose, 11 (22%) developed tumours in this gland. Of these, 2 were classified as squamous-cell papillomas, 8 as squamous-cell carcinomas and the remaining one as a squamous-cell carcinoma jointly with a fibrosarcoma.

Grossly and histologically the above lesions were similar to those described earlier by other investigators (Hiraga & Fujii, 1977).

Liver tumours

Of the females treated with the high dose, 5 (10%) developed tumours in this organ. Of these, 4 were classified as benign hepatomas and the remaining one as a liver-cell carcinoma.

Of the mice treated with the low dose, 6 females (12%) and 2 males (4%) developed benign hepatomas.

Grossly and histopathologically the induced tumours were similar to those described earlier by us (Toth *et al.*, 1964).

Gall-bladder tumours

Of the females treated with the high dose, 5 (10%) developed tumours in this organ. Of these, 4 were classified as adenomas and the remaining one as an adenocarcinoma. In the males treated with the high dose, 2 (4%) developed adenomas of the gall bladder.

Of the mice treated with the low dose, 5 females (10%) and 1 male (2%) developed benign adenomas of this organ.

Macroscopically and histologically these tumours were similar to those published previously by us in other experiments (Toth & Nagel, 1978; Toth *et al.*, 1979).

TABLE II.—*Treatment and tumour distribution in PFH-treated and control Swiss mice*

Group	PFH in drinking water for life	Effective No. and sex of mice	Animals with tumours of: Lungs No.	%	Age at death*	Preputial glands No.	%	Age at death*	Liver No.	%	Age at death*	Gall bladder No.	%	Age at death*	Other tissues†
1	0·08%	50♀	22	44	62 (34–77)	—	—	—	5	10	64 (56–70)	5	10	51 (25–77)	2 Malignant lymphomas (29, 39) 2 Angiomas in spleens (67, 72) 2 Sq. cell carcinomas of clitoral glands (55, 56) 1 Sq. cell carcinoma of forestomach (56) 1 Angiosarcoma in spleen (54)
		50♂	7	14	39 (20–45)	1	2	26	—	—	—	2	4	28 (27–29)	1 Cholangiocarcinoma (21)
2	0·04%	50♀	49	98	66 (40–80)	—	—	—	6	12	68 (55–78)	5	10	66 (62/79)	1 Malignant lymphoma (41) 1 Cholangioma (75) 1 Angiosarcoma in liver (80) 1 Angiosarcoma in spleen (64) 1 Granulosa cell tumour (55) 1 Adenocarcinoma of ovary (77) 1 Adenocarcinoma of duodenum (65) 1 Sq. cell carcinoma of clitoral gland (66)
		50♂	42	84	52 (26–65)	11	22	50 (42–57)	2	4	60 (56–65)	1	2	39	3 Malignant lymphomas (19, 31, 36) 1 Fibrosarcoma, subcutaneous (59)

TABLE II.—*continued*

3	Nil	100♀	25	25	90 (29–129)	—	—	—	1	2	117	1	2	117	18 Malignant lymphomas (16, 48, 66, 76, 79, 87, 87, 90, 91, 92, 96, 98, 98, 104, 107, 116, 125, 128) 6 Angiomas in livers, uteri and ovaries (60, 90, 98, 108, 110, 125) 4 Angiosarcomas in livers and uteri (60, 82, 84, 111) 4 Papillomas of forestomachs (66, 90, 98, 101) 2 Adenocarcinomas of breasts (103, 120) 2 Fibrosarcomas, subcutaneous (79, 100) 1 Sarcoma, retroperitoneal (103) 1 Adrenocortical adenoma (117) 1 Granulocytic leukaemia (91) 1 Myxosarcoma of uterus (105) 1 Granulosa-cell tumour (49) 1 Polypoid adenoma of colon (84) 1 Adenocarcinoma of kidney (96) 1 Fibrosarcoma of submandibular gland (112) 1 Adenocarcinoma of submandibular gland (141) 1 Fibroma of breast (114)
		100♂	26	26	86 (37–113)	—	—	—	—	—	—	—	—	—	8 Malignant lymphomas (31, 70, 70, 87, 89, 93, 104, 106) 9 Angiomas in livers (69, 69, 75, 87, 93, 98, 98, 104, 115) 4 Angiosarcomas in livers (56, 82, 93, 97) 1 Fibrosarcoma, subcutaneous (91) 1 Adrenal cortical adenoma (93) 1 Adenoma of thyroid (93)

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

Other tumours

In a number of instances other types of neoplasms were also observed, and are listed in Table II. Since they occurred in low incidences, their appearances cannot be attributed to the treatment.

DISCUSSION

The present investigation shows that lifetime administration of 0·04% PFH in drinking water to randomly bred 6-week-old Swiss mice induced tumours of the lungs, preputial glands, liver and gall bladder. In females, the tumour incidences in these 4 tissues were 98 ($P < 0·00001$), 0, 12 ($P < 0·03$) and 10% ($P < 0·011$), respectively; in males they were 84 ($P < 0·00001$), 22 ($P < 0·0001$), 4, and 2% respectively. At the 0·08% dose level these tumour incidences in females were 44 ($P < 0·04$), 0, 10 ($P < 0·008$) and 10% ($P < 0·015$), while in males they were 14, 2, 0 and 4% respectively. In untreated controls, the corresponding tumour incidence was 25, 0, 1, and 1% in females and 26, 0, 0, and 0% in males. Statistical analysis was carried out by Fisher's exact probability test for 2×2 tables and by Peto's method (Armitage, 1971; Peto, 1974). Histopathologically, the tumours were classified as adenomas and adenocarcinomas of lungs, squamous-cell papillomas and carcinomas and fibrosarcoma of preputial glands, benign hepatomas and liver-cell carcinoma and adenomas, and adenocarcinoma of gall bladder.

Past studies have concerned the effect of chemical structure on tumour development As a first move, MFH, given at the maximum tolerated dose of 0·0039% to Swiss mice, induced tumours of lungs (77%), liver (46%), blood vessels (21%), bile ducts (7%) and gall bladder (10%) (Toth & Nagel, 1978; Toth *et al.*, 1979). Subsequently, N-ethyl-N-formylhydrazine administered at a 0·02% dose under conditions similar to those of MFH, produced tumours of the lungs (88%) blood vessels (79%), liver (13%), gall bladder (5%) and preputial glands (10%) (Toth & Nagel, 1980*a*). Furthermore, as a third step, N-*n*-butyl-N-formylhydrazine at a chronic dose of 0·04%, again given under conditions identical to those of the previous two compounds, elicited tumours of the lungs (87%), preputial (66%) and clitoral glands (10%) (Toth & Nagel, 1980*b*). Finally, the presently studied PFH at a dose of 0·04% gave rise to tumours of the lungs (91%), preputial glands (22%), liver (6%), and gall bladder (8%). In our untreated Swiss mice the tumours of lungs and blood vessels occur in moderate incidences, whilst tumours of liver and bile ducts are rarely seen. So far, tumours of gall bladder, preputial and clitoral glands have not been seen (Toth *et al.*, 1964; Toth & Shimizu, 1974, Toth & Nagel, 1978). From these findings it appears obvious that the lengthening of the N-alkyl chain influences, to a certain extent, the target tissues from which tumours emerge. The precise nature of the mode of action of these chemicals remains to be elucidated.

To date, 48 hydrazines, hydrazides and hydrazones were shown to be carcinogenic in experimental animals. These chemicals are indeed powerful carcinogens, since they induced tumours in over two dozen tissues and organs of mice, hamsters and rats (Júhász *et al.*, 1957; Biancifiori & Ribacchi, 1962; Morris *et al.*, 1969; Druckrey, 1970; Toth, 1975, 1980). It is of interest to note here that the human population is exposed to about one half of these compounds in the form of drugs, agricultural herbicides or industrial chemicals, and as naturally occurring substances such as ingredients of edible mushrooms and tobacco (Levenberg, 1960; List & Luft, 1968; Merck Index, 1976; Schmeltz *et al.*, 1977; LaRue, 1977).

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