

Absence of Carcinogenic Effects of Chronic Feeding of Snuff in Inbred Syrian Hamsters

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Absence of Carcinogenic Effects of Chronic Feeding of Snuff in Inbred Syrian Hamsters. HOMBURGER, F., HSUEH, S.-S., RUSFIELD, A. B., LAIRD, C. W., AND VAN DONGEN, C. G. (1976). *Toxicol. Appl. Pharmacol.* **35**, 515-521. Fifty male inbred Syrian hamsters, aged 60-90 days, received a diet reduced in caloric content by 20% methylcellulose, or a diet containing 20% snuff (powdered tobacco), or 50 (5 mg) gavages of 20-methylcholanthrene (MC) in addition to chow, or 50 (0.5 mg) gavages of MC (assumed to be a noncarcinogenic dose) with a cellulose-containing diet, or 50 (0.5 mg) gavages of MC with a diet containing 20% snuff. These chronic feeding studies failed to reveal any carcinogenic or cocarcinogenic effects of snuff. Presence in serum of cotinine derived from nicotine, together with food consumption and body weight studies, showed adequate snuff intake. Tumors in the MC-fed animals demonstrated the susceptibility of the two inbred lines of Syrian hamsters used in this study. The only effect of snuff noted was a slower growth of the animals in one of the inbred lines but not in the other. The conclusion is warranted that 20% snuff in the diet is neither carcinogenic nor cocarcinogenic for these animals.

From time to time, smoking and/or the use of chewing tobacco have been implicated in the etiology of oral carcinomas (Friedell and Rosenthal, 1941). Controlled animal experimentation on the effects of a widely used form of chewing tobacco, known as snuff, is lacking. In previous studies we observed no adverse effects when carcinogen-susceptible inbred Syrian hamsters were exposed for 51 weeks to daily periods of chewing snuff inserted into their oral cavities by means of a special bit (Homburger, 1971). It was felt that lifelong exposure of inbred Syrian hamsters to oral consumption of snuff admixed with the diet, beginning at 60 to 90 days of life, would constitute a more severe oral and gastrointestinal exposure than any other method could provide. The studies herein reported were designed to determine: (a) whether snuff when consumed in the diet was a carcinogen or had any other detectable effects and (b) whether snuff administered in the diet could act as a cocarcinogen, enhancing the effects of a known carcinogen, 20-methylcholanthrene (MC), administered in doses too small to be, by themselves, carcinogenic.

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METHODS

Male inbred Syrian hamsters of two carcinogen-susceptible lines, BIO 15.16 and BIO 87.20 (Homburger *et al.*, 1972) were purchased from TELACO, Bar Harbor, Maine, at 50 days of age. The animals were kept on Purina mouse pellets until the start of the study. The experimental design is shown in Table 1. The experimental diets

TABLE 1
EXPERIMENTAL DESIGN

Group number ^a	BIO strain	Feeding regimen
I	15.16	Snuff admixed to diet (chow); testing effect of snuff alone
II	87.20	
III	15.16	Cellulose admixed to diet (chow); testing effect of caloric restriction (negative control)
IV	87.20	
V	15.16	5 mg/animal \times 50 MC by stomach tube plus diet (chow); testing carcinogenicity of MC and demonstrating susceptibility of animals (positive control)
VI	87.20	
VII	15.16	0.5 mg/animal \times 50 MC by stomach tube plus cellulose in diet (chow); testing the effect of a small dose of MC assumed to be noncarcinogenic
VIII	87.20	
IX	15.16	0.5 mg/animal \times 50 MC by stomach tube plus snuff in diet (chow); testing the cocarcinogenic effect of snuff
X	87.20	

^a Each group included 50 male hamsters.

were started when the animals reached 60–90 days of age. Snuff was obtained fresh from the manufacturer once a month and was admixed to Purina chow in a Hobart blender. The snuff was a popular-type, damp snuff widely sold and used throughout the country.

Food consumption and body weights were measured every 3–4 days for the first year of the study. The study was planned to last for at least 2 years, or until the animals would die. At the time of death or sacrifice, a complete autopsy was done, and histological slides of the abnormal tissues were prepared.

In seven animals of each group from those surviving for 2 years in the BIO 87.20 strain, blood pressure was measured by direct cannulation of the abdominal aorta, placing the tip of the cannula at the level of the left renal artery. For this procedure, animals were tranquilized with 1 mg of acetyl promazine and 5 mg of meperidine hydrochloride and anesthetized with 0.25 mg/kg of pentobarbital sodium. This procedure has been found to have only minimal effects upon blood pressure and electrocardiogram (ECG). The pressure was measured through a pressure transducer with a Grass recorder. Each pressure was validated by direct manometric reading and electronically calibrated for each animal. ECG was recorded at the same time, and blood was taken for determination of packed cell volume in microcapillary tubes and for cotinine determination by the method of Langone *et al.*, 1974.⁴

⁴ The authors are indebted to Dr. Helen Van Vunakis of Brandeis University, Waltham, Massachusetts, for the analyses of cotinine.

Autopsies were performed on all animals found dead and reasonably well preserved and on all sacrificed animals. Special care was taken to inspect the oral cavity, the cheek pouches, and the nasopharynx and larynx. All larynxes and urinary bladders, distended by injection of fixation fluid, were sectioned for histology, as were all organs appearing grossly abnormal.

Statistical analysis of significance was made by Student's *t* test when values or incidences in groups of equal size were compared (Snedecor, 1962) or by Fisher's exact test when values or incidences in groups of unequal size were considered (Oldham, 1968).

RESULTS

Food consumption was somewhat reduced in the animals receiving chow containing snuff. The difference was, however, small in both strains and not statistically significant. In the BIO 87.20 hamsters receiving snuff and chow, there was a significant slowing of growth so that at the end of the study the animals receiving snuff and chow weighed about 15–20% less than those receiving the same regimen plus MC gavage. The smaller animals of the BIO 15.16 line showed the same trend, but in this strain the difference was not statistically significant. This is analogous to the marked weight reduction observed in BIO 87.20 animals inhaling cigarette smoke which did not occur in BIO 15.16 animals similarly treated (Bernfeld *et al.*, 1974).

Throughout the 2 years of the study, only 10 unprogrammed deaths occurred prior to the end of year 1 of the study, except in the animals receiving the high dose of MC (5 mg/animal \times 50). Twenty-two of the 100 animals receiving the carcinogen died during year 1. All animals except those receiving the high dose of MC thus showed the survival pattern previously noted as typical of these strains and again observed in the chow and cellulose controls.

The incidence of neoplasms is shown in Table 2. In the controls, malignant tumors were limited to adrenals, forestomach, stomach, intestines, liver, and lymphatic tissue. Among the cellulose-fed controls, there were 12 tumor-bearing animals of the BIO 15.16 strain and 22 of the BIO 87.20 strain. Many of these malignant neoplasms and benign adenomas occurred, as expected, in the adrenals. This is the "normal" tumor distribution in hamsters of this age (Van Hoosier *et al.*, 1971). It also closely resembles observations made by us on these same inbred strains (Homburger *et al.*, 1972).

The animals fed snuff alone showed a tumor spectrum and incidence nearly identical to that of the controls. Clearly, snuff was not carcinogenic. It is especially noteworthy that there were no tumors of the oral cavity, salivary glands, esophagus, nasopharynx, larynx, urinary bladder, gonads, or ear ducts in any of these animals.

The BIO 87.20 strain appeared to be especially susceptible to induction of cancer of the stomach. The lower dose of MC, which was intended to be a noncarcinogenic initiating dose, caused six carcinomas of the stomach (vs zero in controls), a difference which is significant ($p < 0.05$). However, no cocarcinogenic effect of snuff was observed.

The response of the animals to the high dose of carcinogen (50 \times 5 mg of MC by stomach tube) demonstrated that these hamsters were indeed susceptible to MC. There were fewer adrenal tumors in MC-treated animals (due to their earlier deaths), but they had a high incidence of gastrointestinal tumors and more lymphoid system tumors than either controls or snuff-treated animals.

TABLE 2
PRESENCE OF MALIGNANT NEOPLASMS IN HAMSTERS FED VARYING DIETS

Organ	BIO strain	Cellulose (36♂; 33♀)		Snuff (44♂; 54♀)		MC (high dose) (49♂; 50♀)		Snuff + MC (low dose) (40♂; 40♀)		Cellulose+MC (low dose) (39♂; 37♀)	
		T ^a	M ^a	T	M	T	M	T	M	T	M
Adrenal	15.16	3	2	10	1	1 ^b	0 ^b	3	0	4	1
	87.20	14	1	14	1	2 ^b	0 ^b	7	2	9	2
Forestomach	15.16	5	3	4	0	35	8	1	1	9	4
	87.20	2	1	4	1	35	12	2	1	4	3
Stomach	15.16	0	0	0	0	0	0	2	2	0	0
	87.20	0	0	0	0	19	19	6	6	6	6
Small intestine	15.16	0	0	0	0	4	4	0	0	0	0
	87.20	0	0	0	0	4	4	0	0	0	0
Large intestine	15.16	1	1	1	1	25	25	1	1	2	2
	87.20	0	0	0	0	27	26	0	0	0	0
Liver	15.16	0	0	1	0	0	0	1	1	1	0
	87.20	2	2	2	0	2	2	5	2	4	3
Lymphoma (including spleen)	15.16	3	1	1	1	3	3	1	0	7	1
	87.20	3	2	0	0	0	0	2	2	7	1
Skin (sc)	15.16	0	0	0	0	6	2	0	0	0	0
	87.20	1	0	0	0	0	0	0	0	1	1
Total neoplasms	15.16	12	7 (19) ^c	17	3 (7)	74	42 (85)	9	5 (12)	23	8 (20)
	87.20	22	6 (18)	20	2 (4)	89	63 (100)	22	13 (32)	31	16 (43)

^a T, total neoplasms (benign and malignant); M, malignant neoplasms, carcinoma, sarcoma, lymphomas.

^b Low incidence due to early deaths.

^c Values in parentheses are percentages of animals on which an autopsy was performed.

* Values in parentheses are percentages of animals on which an autopsy was performed.

TABLE 3
SUMMARY OF PHYSIOLOGICAL PARAMETERS AND MEASUREMENTS OF COTININE CONCENTRATION IN SEVEN BIO 87.20 HAMSTERS

Group	Statistics	Cotinine concentration	Hematocrit (% RBC)	Heart rate (beat/min)	Blood pressure		
					Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)
I	\bar{X}	--	41.9	321	124	90.6	101.2
Aged 87.20♂♂, pellet-fed	SD	—	2.7	25	14.4	6.6	6.7
II	\bar{X}	0.0	40.8	260	91.6	68.0	75.3
87.20♂♂, cellulose:chow	SD	0.0	6.1	78	43.7	31.6	35.9
III	\bar{X}	0.0	38.0	286	109.8	79.7	88.8
87.20♂♂, MC + cellulose:chow	SD	0.0	5.9	88.5	45.0	40.0	41.3
IV	\bar{X}	1.72	41.9	318	88.3	66.7	73.9
87.20♂♂, MC + snuff:chow	SD	1.69	6.7	27.9	36.2	26.5	29.7

SNUFF FEEDING IN SYRIAN HAMSTERS

The only nontumorous lesions observed in the histological study of various organs were the degenerative changes common to aging hamsters. There was no recognizable change in the distribution pattern of these non-neoplastic lesions associated with any of the treatments.

Physiological parameters. These studies are based on groups of seven BIO 87.20 hamsters only, sacrificed at 24 months. The results are summarized in Table 3 and show that heart rate and blood pressure were not affected by snuff feeding. The ECG tracings also were unaffected by snuff feeding.

In the same animals, the packed cell volume was not affected by snuff feeding but was relatively low in all groups. This may be due to the advanced age of the animals or may be characteristic of the strain.

Cotinine in serum was detectable only in those animals having consumed snuff in amounts far exceeding those observed in sera of human smokers. This is proof that significant amounts of nicotine were absorbed and metabolized by these animals (Table 3).

DISCUSSION

This study revealed that inbred Syrian hamsters accept diets containing 20% (by weight) of methylcellulose. Since methylcellulose is known to pass the intestinal tract without being absorbed (Braun *et al.*, 1974), this represents a simple means for adjustment of caloric intake. In the present study, substitution of 20% of the laboratory chow did not cause differences in the weight gain from previously published growth curves for the two inbred strains studied (TELACO, 1974; Bernfeld *et al.*, 1974). Snuff, which consists of powdered tobacco leaves and stems, was equally well accepted in the hamster diet. The effect upon growth of a 20% substitution of the diet by snuff was minimal in BIO 15.16 inbred hamsters and somewhat more marked in the BIO 87.20 line. This is in agreement with similar observations of differences in the effects upon weight of smoke inhalation in these same two strains (Bernfeld *et al.*, 1974). When 50 feedings by stomach tube of 0.5 ml of corn oil containing 0.5 mg of MC were added, the growth-inhibiting effect of the snuff was overcome.

The continued ingestion of a 20% snuff-containing diet beginning at 60 to 90 days of age had no other effects. No tumors were produced, and no pathological changes were demonstrable. The animals used in this study had previously been shown to be susceptible to cancer induction by MC feeding. This was confirmed in the present study. No cocarcinogenic effect could be demonstrated when snuff feeding was combined with the administration of subcarcinogenic doses of MC. The presence of cotinine in the serum provides proof of the absorption of nicotine from the ingested snuff.

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