

Renal Carcinogenicity of Concurrently Administered Fish Meal and Sodium Nitrite in F344 Rats

Fumio Furukawa,^{1,4} Akiyoshi Nishikawa,¹ Hitoshi Ishiwata,² Michihito Takahashi,¹ Yuzo Hayashi¹ and Masao Hirose¹

¹Division of Pathology and ²Division of Food Additives, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501

The effects of long-term concurrent administration of powdered fish meal and sodium nitrite were examined in F344 rats. A total of 600, 6-week-old rats were divided into 6 male and 6 female groups, each consisting of 50 animals. Rats in groups 1–3 and 7–9 were respectively fed diets supplemented with 64%, 32% and 8% (basal diet) fish meal, and simultaneously given 0.12% sodium nitrite in their drinking water. Groups 4–6 and 10–12 were respectively given 64%, 32% and 8% fish meal and tap water. At the 104th week, all surviving animals were killed and examined histopathologically. Treatment with fish meal dose-dependently increased the incidences and multiplicities of atypical tubules, adenomas and renal cell carcinomas in sodium nitrite-treated males. Females were less susceptible than males for renal tumor induction. In males given the 64% fish meal diet alone, the incidence and multiplicity of atypical tubules were also significantly increased as compared with the 8% fish meal alone case. Nephropathy was apparent in fish meal-treated groups in a clear dose-dependent manner, irrespective of the sodium nitrite treatment, and was more prominent in males than in females. Dimethylnitrosamine was found in the stomach contents after 4-week treatment with 64% fish meal plus 0.12% sodium nitrite, at a level twice that in the 8% fish meal plus 0.12% sodium nitrite group. The results clearly indicate that concurrent administration of fish meal and sodium nitrite induces renal epithelial tumors. Further studies are required to elucidate how nephropathy and nitrosamines produced in stomach contents may contribute to the observed renal tumor induction.

Key words: Powdered fish meal — Sodium nitrite — Renal cancer — Nitrosamine — Nephropathy

Sodium nitrite is widely used in Japan and throughout the world as a food additive to preserve and color cured meat and/or fish, being contained in flour, macaroni and soybean flour, and especially abundant in pickles.¹⁾ Nitrates can also be reduced to nitrites in the human oral cavity by bacteria.¹⁾ This fact might be environmentally important, since nitrates are present in foods, e.g. vegetables, in considerably higher quantities than nitrites.¹⁾ It has been reported that sodium nitrite has mutagenic activity in various mutagenicity tests,²⁾ and continuous oral administration of 0.3% sodium nitrite for at least one year caused significant increase in the incidence of forestomach papillomas in Wistar rats.³⁾ However in another study, it failed to exert carcinogenicity in F344 rats.⁴⁾ It is well known that sodium nitrite and secondary amines contained in many foods are precursors of *N*-nitroso compounds, reacting under the acidic conditions of the stomach to form nitrosamines or related carcinogens.^{5,6)} They therefore constitute one of the most important risk factors for human cancer.⁷⁾ Fish meal contains abundant secondary amines.

Moreover, volatile *N*-nitrosoamines such as dimethylnitrosamine (DMN), *N*-nitrosopyrrolidine and *N*-nitrosothiazolidine were detected in 32 commercial Japanese samples.⁸⁾ From the amounts of nitrosamines detected in fish, fish products, meat products, beer and cheese, and the average daily intake in Japan, human exposure to nitrosamines has been estimated to be 0.5 $\mu\text{g}/\text{head}/\text{day}$.⁹⁾

A high protein diet is regarded as an additional risk factor for human cancer. Epidemiological studies have suggested, for example, a link with cancer of the colon and rectum¹⁰⁾ in particular, as well as the breast¹¹⁾ and kidney.¹²⁾ Experimental studies have also provided evidence that high protein intake enhances carcinogen-induced proliferative lesion development in diverse tissues including the liver¹³⁾ and colon.¹⁴⁾ For example, with aflatoxin B₁-induced rat hepatocarcinogenesis, treatment with a high protein (20% casein) diet significantly increased the development of preneoplastic γ -glutamyltranspeptidase-positive foci as compared with the low protein (5% casein) case.¹³⁾ Topping and Visek reported elevated incidences of tumors in the colon, small intestine and sebaceous glands in rats fed 15% and 22.5% protein diets as compared to those fed a 7.5% diet.¹⁴⁾

⁴To whom correspondence should be addressed.
E-mail: furukawa@nihs.go.jp

In the present study, the carcinogenic potential of the two factors, fish meal and sodium nitrite, either alone or in combination was examined in F344 rats.

MATERIALS AND METHODS

Animals A total of 600 F344 rats, 300 of each sex (5-week-old), purchased from Charles River Japan Inc. (Kanagawa), were maintained on the basal diet (CRF-1) and tap water for one week as an acclimation. They were housed five to a polycarbonate cage with sterilized soft-wood chips as bedding in a barrier-sustained animal room conditioned at 24±1°C and 55±5% humidity. The basal diet was analyzed for contaminants twice a year and it was ascertained that contaminants such as pesticides, metals, benzo[a]pyrene and aflatoxins were not contained at appreciable levels.

Experimental design A total of 600, 6-week-old F344 rats, were divided into 6 male and 6 female groups, each consisting of 50 animals. Rats in groups 1–3 (male) and 7–9 (female) were respectively fed diets supplemented with 64%, 32% and 8% (basal diet) fish meal, and simultaneously given 0.12% sodium nitrite in their drinking water for 104 weeks. Groups 4–6 (male) and 10–12 (female) were respectively given 64%, 32% and 8% fish meal and tap water for 104 weeks. The fish meal (white fish meal) was made from walleye pollack and cod (Oriental Yeast Co., Ltd., Tokyo), and contained 9.1% water, 65.4% protein, 6.8% fat and 18.1% ash. Sodium nitrite (Wako Pure Chemical, Osaka) was found to be very stable when dissolved in water (data not shown). The sodium nitrite solution was renewed twice weekly. The animals were observed daily. Body weights were measured every 4 weeks, and food consumption and water intake every week. At the end of week 104, all animals were killed under ether anesthesia and completely autopsied. Moribund or dead animals were also autopsied completely to determine the development of tumors. Those killed at week 44 or later were included in the effective numbers. The brain, lungs, heart, liver, spleen, adrenal glands, kidneys and testes of each animal, where appropriate, were weighed. Tumor masses, as well as all organs and/or tissues were fixed in 10% buffered formalin and paraffin-embedded sections were routinely prepared and stained with hematoxylin and eosin, as well as periodic acid-Schiff (PAS) for the kidney.

Analyses for nitrosamines For analyses of *N*-nitrosamines in the stomach contents and serum, groups of five male F344 rats (5-week-old, Charles River Japan Inc.) were given 64% fish meal plus 0.12% sodium nitrite, 64% fish meal, 8% fish meal plus 0.12% sodium nitrite or 0.12% sodium nitrite alone for 4 weeks and then blood samples were collected from the aorta under ether anesthesia. Stomach contents were collected and frozen at

–80°C until analyzed. Analysis was performed twice using a thermo-energy analyzer (TEA-502, Shimadzu Inc., Tokyo).⁹⁾

Statistics Differences between mean values were analyzed statistically using analysis of variance (ANOVA). The incidences of tumors were compared with Fisher's exact probability test.

RESULTS

Growth and survival curves Growth curves for groups 1 and 4, and particularly groups 7 and 10 were depressed as compared with those of other groups (Fig. 1). As shown in Figs. 2 and 3, final survival rates in groups 1, 4, 7 and 10 were 50%, 14%, 50% and 30% respectively, much lower than in the other groups (72–84%).

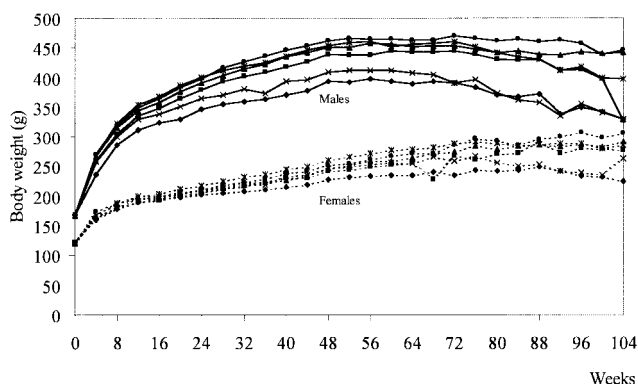


Fig. 1. Body weight curves for F344 rats treated with fish meal alone or with sodium nitrite. —◆— group 1, —■— group 2, —▲— group 3, —×— group 4, —*— group 5, —●— group 6, ---◆--- group 7, ---■--- group 8, ---▲--- group 9, ---×--- group 10, ---*--- group 11, ---●--- group 12.

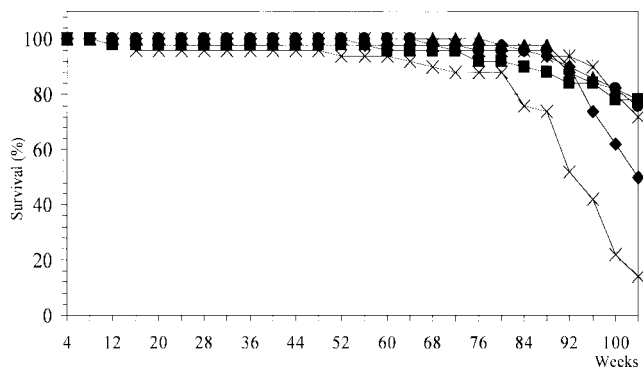


Fig. 2. Survival curves for male rats given fish meal alone or with sodium nitrite. ◆ group 1, ■ group 2, ▲ group 3, × group 4, * group 5, ● group 6.

Intake of fish meal and sodium nitrite Data for total intake of fish meal and sodium nitrite, estimated from the food and water consumption data, are shown in Table I. Mean daily intake of fish meal, and total intake of fish meal and sodium nitrite were clearly related with fish meal dose levels. Sodium nitrite intake also dose-dependently increased with the dose of fish meal.

Tumors of the kidneys Macroscopically, tumors of the kidney were sometimes seen as round yellowish-white or grayish-white projections from the renal cortex. Most grossly appeared as yellowish-white small nodules in cut sections. Neoplastic and preneoplastic lesions observed in the tubular epithelium of kidneys were histologically classified as atypical tubules, renal cell adenomas and carcinomas. Atypical tubules (Fig. 4) consisted of usually single or sometimes 2–3 tubules with mild atypia. The cell cytoplasm was clear, eosinophilic, or basophilic with a granular or a foamy appearance. Adenomas (Fig. 5) appeared as

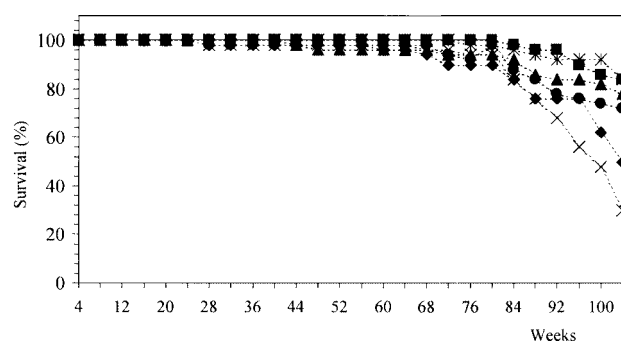


Fig. 3. Survival curves for female rats given fish meal alone or with sodium nitrite. ◆ group 7, ■ group 8, ▲ group 9, × group 10, * group 11, ● group 12.

solid, cystic or papillary nodules larger than a single glomerulus and were located in the cortex. They are well circumscribed with thin, fibrous capsules or pseudocapsules and compressed neighboring tissues. Renal cell carcinomas (Fig. 6) were usually larger than adenomas, and more

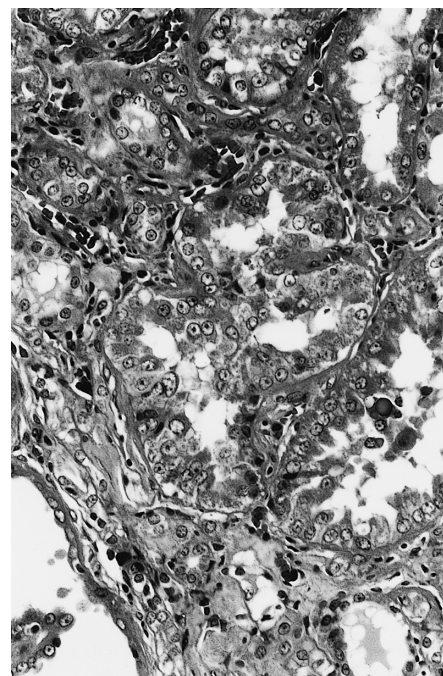


Fig. 4. Atypical tubules with mild atypia. The cytoplasm is basophilic with a granular appearance. The affected tubules are slightly larger than normal tubules. (Group 1: 64% fish meal plus 0.12% sodium nitrite treatment).

Table I. Food and Water Consumption by Rats Given Fish Meal Alone or with Sodium Nitrite

Group, Treatment	Food consumption		Fish meal	Water consumption		Sodium nitrite	
	Daily intake (g/animal/day)	Total intake (g/animal/2 years)	Total intake (g)	Daily intake (ml/animal/day)	Total intake (ml/animal/2 years)	Total intake (g)	
Males	1, 64% FM+SN ^{a)}	16.2	11802.9	7553.9	41.4	30116.4	37.6
	2, 32% FM+SN	15.5	11294.6	3614.3	26.6	19397.4	24.2
	3, 8% FM+SN	14.9	10880.3	870.4	18.3	13346.0	16.7
	4, 64% FM	16.9	12309.0	7877.8	46.3	33699.9	0.0
	5, 32% FM	15.9	11541.5	3693.3	28.5	20767.3	0.0
	6, 8% FM	14.8	10790.9	863.3	20.3	14781.8	0.0
Females	7, 64% FM+SN	11.6	8428.7	5394.4	27.0	19637.0	24.5
	8, 32% FM+SN	11.0	7988.1	2556.2	18.6	13527.5	16.9
	9, 8% FM+SN	10.4	7558.7	604.7	13.1	9562.1	12.0
	10, 64% FM+SN	11.9	8678.6	5554.3	28.1	20454.6	0.0
	11, 32% FM+SN	11.3	8257.0	2642.3	19.9	14512.1	0.0
	12, 8% FM+SN	10.7	7811.1	624.9	14.3	10381.1	0.0

a) FM, fish meal; SN, 0.12% sodium nitrite.

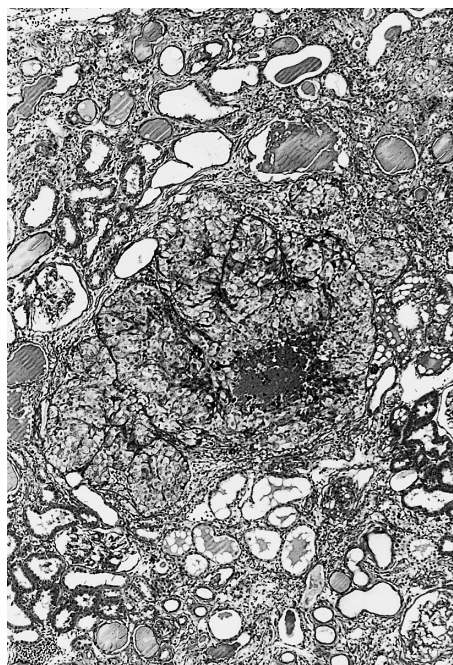


Fig. 5. Adenoma appearing as a solid nodule consisting of closely packed polygonal cells. It is well circumscribed with a thin, fibrous capsule or pseudocapsule and compresses the neighboring tissue. (Group 1: 64% fish meal plus 0.12% sodium nitrite treatment).

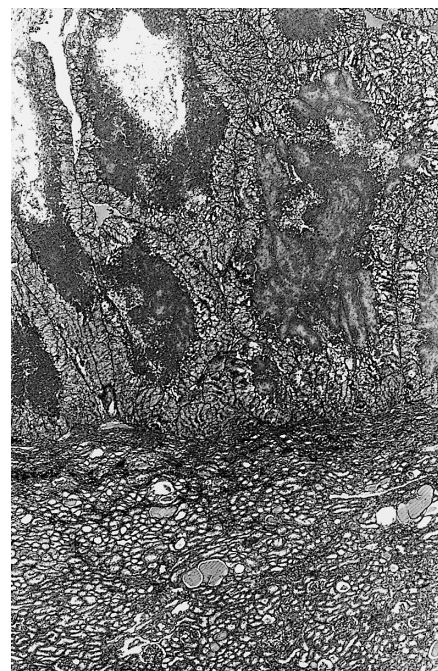


Fig. 6. Renal cell carcinoma is sharply demarcated from the neighboring tissue, showing extensive necrosis and hemorrhage. (Group 1: 64% fish meal plus 0.12% sodium nitrite treatment).

Table II. Incidence and Multiplicity of Renal Proliferative Lesions in Rats Given Fish Meal Alone or with Sodium Nitrite

Group	Effective no. of rats	Renal proliferative lesions ^{a)}					
		Atypical tubules		Adenoma		Adenocarcinoma	
1	49	36 (73.4) ^{c,d)}	1.95±1.82 ^{b,d)}	33 (67.3) ^{c,e)}	1.40±1.33	28 (57.1) ^{c,e)}	1.61±1.98 ^{b,d)}
2	47	20 (42.2) ^{b,g)}	0.61±0.84 ^{b,f)}	12 (25.5) ^{c,g)}	0.38±0.73	7 (14.8) ^{c,g)}	0.19±0.53
3	49	2 (4.0)	0.04±0.19	1 (2.0)	0.02±0.14	0	
4	47	9 (19.1) ^{h)}	0.18±0.38 ^{h)}	1 (2.1)	0.02±0.14	0	
5	49	0		0		0	
6	47	0		0		0	
7	48	22 (45.8) ^{j,l)}	0.92±1.30 ^{i,k)}	8 (16.6) ^{i,l)}	0.23±0.56 ^{i,k)}	1 (2.0)	0.02±0.14
8	43	5 (11.6)	0.12±0.33	1 (2.3)	0.03±0.16	0	
9	47	3 (6.3)	0.07±0.26	1 (2.1)	0.02±0.14	0	
10	49	2 (4.0)	0.04±0.20	0		0	
11	50	1 (2.0)	0.04±0.14	0		0	
12	45	0		0		0	

a) Incidence and mean±SD.

b,c) $P < 0.05$, $P < 0.01$ compared to group 3.

d,e) $P < 0.05$, $P < 0.01$ compared to group 4.

f,g) $P < 0.05$, $P < 0.01$ compared to group 5.

h) $P < 0.01$ compared to group 6.

i,j) $P < 0.05$, $P < 0.01$ compared to group 9.

k,l) $P < 0.05$, $P < 0.01$ compared to group 10.

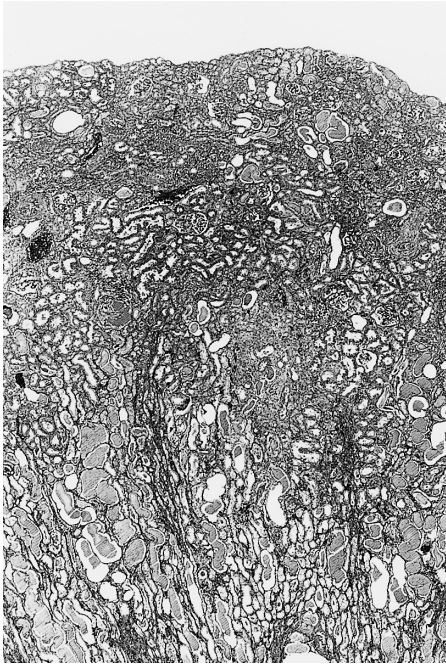


Fig. 7. Severe nephropathy in a rat characterized by fibrosis, dilatation of tubules and hyaline casts. (Group 1: 64% fish meal plus 0.12% sodium nitrite treatment).

anaplastic and atypical in nature. Hemorrhage and necrosis were usually present and sometimes invasion to surrounding tissues was seen.

As shown in Table II, in males, the incidences and multiplicities of renal cell carcinomas, adenomas and atypical tubules dose-dependently increased in the fish meal plus sodium nitrite-treated groups. Respective incidences were 57.1%, 67.3% and 73.4% for group 1, 14.8%, 25.5% and 42.2% for group 2 and 0%, 2.0% and 4.0% for group 3. Values for the highest (group 1) and middle dose (group 2) were statistically significantly different from the lowest dose level (group 3) and the respective groups without sodium nitrite (groups 4 and 5). Interestingly, in male rats treated with 64% fish meal without sodium nitrite (group 4), an adenoma was found in one rat and the incidence (19.1%) and multiplicity (0.18 ± 0.38) of atypical tubules were significantly increased as compared with the 8% fish meal case (group 6). In females, although a carcinoma was only found in one rat given 64% fish meal plus sodium nitrite (group 7), the multiplicity of adenomas and atypical tubules were significantly increased in this group as compared with 8% fish meal plus sodium nitrite (group 9), and 64% fish meal alone groups (group 10). Two rats and one rat treated with 64% and 32% fish meal alone (groups 7 and 8), respectively, had atypical tubules.

Nephropathology Chronic nephropathy was observed (Fig. 7), characterized by an increase of glomerular

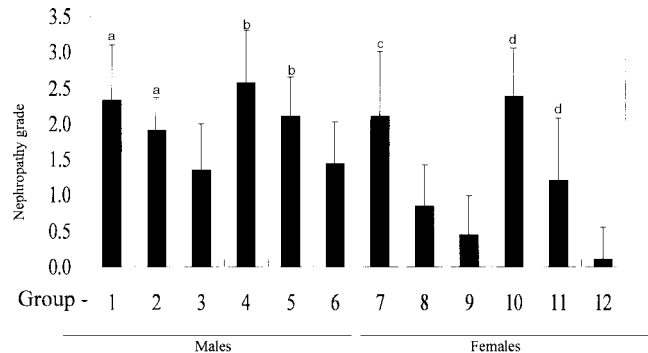


Fig. 8. Grade of nephropathy in rats given fish meal alone or with sodium nitrite. a, $P < 0.01$ compared to group 3; b, $P < 0.01$ compared to group 6; c, $P < 0.01$ compared to group 9; d, $P < 0.01$ compared to group 12.

mesangial PAS-positive materials, thickening of mesangial basement membranes, fibrous thickening, proliferation of epithelial cells, crescent formation and hyalinization in Bowman's capsules. Tubular lesions included luminal dilatation with flattening of epithelia and colloid-like hyaline casts. Grading of nephropathy in rats according to the rate of appearance of tubules with hyaline casts was judged as follows (severe: +++, >50%; moderate: ++, 20–50%; slight: +, 5–20%; normal: –, <5%). As shown in Fig. 8, chronic nephropathy developed in all groups. Grades of nephropathy dose-dependently increased, irrespective of the sodium nitrite treatment. However, the degree of development in females was less severe than in males.

Other tumor incidences and distribution Table III summarizes data for histopathologic diagnosis of tumors observed in various organs other than the kidney. In all male groups, tumors of the testis were the most frequent, followed by tumors in the hematopoietic organs, adrenals, thyroid and liver. The incidences of follicular cell adenomas in the thyroid gland were low in male rats fed 64% fish meal with or without sodium nitrite treatment, statistical significance being achieved with group 1 (0%) as compared to group 3 (30%). The incidences of leukemias showed similar tendencies in males and females. Liver adenomas tended to be dose-dependently decreased in sodium nitrite-treated males. In females, endometrial polyps were the most common tumors, with no intergroup variation.

Non-neoplastic lesions such as calcification in the gastrointestinal tract and lung, gastric erosion, osteosclerosis and granuloma of the liver were more severe in group 4 as compared to group 6.

Levels of N-nitrosamines in blood and stomach contents As shown in Table IV, DMN was detected in the stomach contents of all groups. The level was greatest in rats given 64% fish meal plus 0.12% sodium nitrite

Table III. Sites and Types of Tumors in Rats Given Fish Meal Alone or with Sodium Nitrite

		Group:											
		1	2	3	4	5	6	7	8	9	10	11	12
		No. of rats:											
		48	49	50	47	47	47	49	46	47	49	50	45
Thyroid	C-cell adenoma	0	0	2	0	0	1	1	5	1	0	0	1
	C-cell carcinoma	1	1	2	0	2	2	0	3	0	0	1	1
	Follicular cell adenoma	0 ^{a)}	1	6	1	2	4	0	0	3	0	1	1
	Follicular cell carcinoma	2	0	1	0	2	2	0	4	2	0	0	1
Adrenal	Pheochromocytoma	9	10	6	2	11	6	3	1	0	0	0	0
	Malig. pheochromocytoma	1	1	2	0	0	1	6	2	2	1	3	0
HPS	Leukemia	1	2	5	2	6	9	1	1	5	2	6	6
Lung	Adenoma	1	0	2	0	1	0	1	0	0	0	0	1
	Adenocarcinoma	3	0	0	0	0	1	1	0	0	0	0	0
Liver	Adenoma	2	4	9	4	3	4	1	1	0	0	2	1
	Adenocarcinoma	1	0	0	0	1	2	1	1	0	0	0	0
	Hemangioma	0	1	0	1	0	0	0	0	0	0	0	0
	Hepatoblastoma	0	1	0	0	0	0	0	0	0	0	0	0
	Fibrosarcoma	1	0	0	0	0	0	0	0	0	0	0	0
	Liposarcoma	0	0	0	1	0	0	0	0	0	0	0	0
	Histiocytic sarcoma	0	0	0	0	0	1	0	0	0	0	0	0
Testis	Interstitial cell tumor	46	42	49	38	43	43						
Uterus	Endometrial polyp							12	11	10	10	14	9
	Endometrial sarcoma							1	0	1	1	1	2
	Adenoma							0	3	0	0	0	0
	Adenocarcinoma							2	1	0	0	0	0

HPS: hematopoietic system.

a) $P < 0.05$ compared to group 3.

Table IV. Levels of Dimethylnitrosamine in Stomach Contents and Blood of Male Rats Given Fish Meal Alone or with Sodium Nitrite for 4 Weeks

Treatment	Sample No.	Stomach content	Blood
64% fish meal+0.12% sodium nitrite	5	5.8±1.1	ND
64% fish meal	5	2.5±1.0	ND
8% fish meal+0.12% sodium nitrite	5	3.3±3.6	ND
8% fish meal	5	3.0±0.9	ND

ND: less than 1 ppb.

(5.8±1.1 ppb), followed by 8% fish meal plus 0.12% sodium nitrite (3.3±3.6 ppb), 8% fish meal (3.0±0.9 ppb) and 64% fish meal (2.5±1.0 ppb). DMN was not detected in the serum in any groups. No other nitrosamines, including diethylnitrosamine, were detected in any of the samples.

DISCUSSION

The present study clearly demonstrated that long-term concurrent administration of high fish meal diet and sodium nitrite induces high yields of kidney tumors in

rats. Oral administration of 0.3% sodium nitrite for at least one year in Wistar rats was earlier reported to cause a significant increase (18%) in the incidence of forestomach papillomas, but not kidney tumors,¹⁵⁾ although in another experiment using F344 rats, sodium nitrite was not carcinogenic.⁴⁾ Several experiments have demonstrated that combined treatment with secondary amines and sodium nitrite results in tumors in several organs.^{16, 17)} Kitano *et al.* reported that in a rat multi-organ carcinogenesis bioassay system, the incidence of tumors and/or preneoplastic lesions in the forestomach and esophagus was increased by exposure to methylurea plus sodium nitrite.¹⁸⁾ Also

liver tumors were induced by the treatment with morpholine and 0.2% nitrate^{19, 20)} or aminopyrine and 0.1% sodium nitrite in rats,²¹⁾ and forestomach and lung tumors were induced by amines or methylurea and 0.2% sodium nitrite in mice.²²⁾ Under the acidic conditions of the stomach, methylurea is formed from creatine-containing foods, such as smoked or dried bonito fish products and fried bacon, and *N*-methyl-*N*-nitrosourea was found in the gastric contents at 2 h after combined administration of methylurea and sodium nitrite.¹⁸⁾ *N*-Nitroso compounds are well known strong carcinogens, closely related to food and nutrition, and constitute one of the most important risk factors in man. In the present study, 5.3 ppb of DMN was detected in the stomach contents with 64% fish meal plus 0.12% sodium nitrite treatment. It is well established that DMN can induce only tumors in the liver and kidney in rats.²³⁾ However, in a carcinogenicity study with up to 50 ppm in Porton rats, only liver tumors were found²⁴⁾ and similarly, long-term dietary administration of 10 ppm DMN failed to induce kidney tumors.²⁵⁾ Since the DMN level of stomach contents in the present study was much lower than these values, it is not likely that DMN alone was responsible for the kidney tumor formation. The Joint FAO/WHO Expert Committee on Food Additives established an accepted daily intake for nitrite of 0–0.06 mg/kg body weight, expressed as nitrite ion, which is equivalent to 0.06 mg/kg/day for a person.²⁶⁾ The maximum intake dose applied in group 1 in the present study was about 1 g/kg/day for a rat.

Recently, a new mutagen, 2-chloro-4-methylthiobutanoic acid (CMBA), has been isolated from salt-nitrite-treated Sanma hiraki fish²⁷⁾ and shown to exert possible initiating and promoting activity in the rat stomach.²⁸⁾ CMBA causes replicative as well as unscheduled DNA synthesis in the stomach pyloric mucosa of rats²⁹⁾ and its chemical structure suggests that it may be derived from methionine in the presence of salt and nitrite.³⁰⁾ Gastric tumors were not observed in the present experiment. Nitrosamines and CMBA might have been too small in amount, if present at all, to play a role in gastric tumor induction in the present study. Therefore CMBA may not play a role in tumor induction in the present experiment, possibly due to insufficient production. However, it cannot be precluded that some other unknown mutagen or carcinogen is generated by fish meal diet mixed with sodium nitrite.

Wynder *et al.* reported that mortality rates from male renal cancer in 15 countries were highly correlated with *per capita* consumption of animal fat and animal protein.³¹⁾ Mellegaard *et al.* found a positive association between risk of renal cell carcinoma and intake of fat, protein and carbohydrates in a population-based case-control study in Denmark, indicating that the total energy intake, rather than particular energy sources, is important.¹²⁾ However,

Armstrong and Doll observed that a high intake of animal protein was most strongly correlated with increased renal cancer incidence.¹⁰⁾ Animal data are limited on the effects of a high protein diet on kidney carcinogenesis. Clinton *et al.* showed that a casein-based high protein diet did not modify azoxymethane-induced renal carcinogenesis in the promotion period, but rather inhibited it during the initiation period.³²⁾ Protein-deficient diet rather enhanced DMN-induced renal carcinogenesis in rats.^{33, 34)} A decreased incidence of renal cell adenomas was also observed in hamsters fed a low fat and low protein diet either before or after administration of *N*-nitrosobis(2-oxopropyl)amine.³⁵⁾ It is likely that dietary protein also modifies renal carcinogenesis in human, but experimentally, high protein enhancement may not occur.

In the present study, 64% fish meal-administered male rats (groups 1, 4, 7 and 10) showed particularly severe chronic nephropathy, regardless of sodium nitrite treatment. It is well known that kidney tumors are associated with α_{2u} -globulin-induced nephropathy.³⁶⁾ Hydroquinone exposure for 2 years has been reported to induce renal cell adenomas and simultaneously to exacerbate spontaneous chronic progressive nephropathy (CPN) in male F344 rats.³⁷⁾ The findings that 1) grade of CPN and induction of atypical tubules and adenomas ran in parallel, 2) atypical tubules are precursor lesions of adenomas, 3) atypical tubules, adenomas and adenocarcinomas were located only within areas of severe chronic nephropathy and not within normal portions of renal parenchyma, suggested that stimulation of tubular proliferation was partly responsible for the hydroquinone-related adenoma formation.³⁷⁾ In the present study, the grade of chronic nephropathy also paralleled the dose of fish meal diet and was associated with increased frequencies of atypical tubules, adenomas and carcinomas. Therefore it is likely that both nitrosamines and nephropathy contribute to renal tumor induction by a high fish meal diet.

In conclusion, the results of the present study clearly indicate that concurrent administration of fish meal and sodium nitrite exerts strong renal carcinogenicity in rats. A high fish meal diet itself may in fact act as a weak kidney carcinogen. Further studies are now required to elucidate whether nitrosamines, other mutagenic compounds, or other factors are involved in the development of renal tumors.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Research on Environmental Health from the Ministry of Health and Welfare of Japan.

(Received September 16, 1999/Revised October 29, 1999/
Accepted November 4, 1999)

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