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Lack of Carcinogenicity of Quercetin in F344/DuCrj Rats

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Quercetin was administered at dietary levels of 0(control), 1.25 and 5.0% to groups of 50 male and 50 female rats for 104 weeks, and then all animals were maintained without quercetin supplement for a further 8 weeks. At 5.0% quercetin, both sexes showed growth retardation throughout the study. There were no treatment-ascribed effects regarding clinical signs, mortality, urinalyses or hematology. Although serum glucose in 5.0% quercetin-treated males was significantly decreased and some relative organ weights in 5.0% groups showed statistically significant increases, these latter changes seemed to be related to the growth retardation. An increased incidence of non-neoplastic hyperplastic polyps in the cecum was noted in the 5.0% males. The incidences of cystic changes and fibroadenomas of the mammary gland, and foci (areas) of hepatocellular alteration in the 5.0% females, and liver bile duct proliferations in the 5.0% males were significantly decreased. No proliferative lesions of the urinary bladder related to treatment with quercetin were found in any rats. The incidences of several other nonneoplastic and neoplastic lesions which demonstrated statistically significant changes appeared to be related to the growth retardation or to be within the normal range, and therefore none was considered to be significant biologically. Thus, the investigation did not demonstrate any clear carcinogenic effect of quercetin on F344 rats at dietary levels of up to 5.0%.

Key words: Quercetin — F344 rat — Lack of carcinogenicity

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is widely distributed in many edible plants, and is therefore an integral part of the human diet.[1] In addition, naturally occurring flavonoids, including quercetin, and their synthetic analogs have attracted extensive interest owing to their therapeutic properties, including anti-inflammatory and anti-tumor effects.[2, 3]

Quercetin has been reported to induce mutations, chromosomal aberrations, sister chromatid exchanges, DNA strand breaks and cellular transformation *in vitro*.[4–14] Although quercetin has been generally considered to be non-toxic and non-carcinogenic,[15] two recent long-term studies indicated that it might be an ileal and urinary bladder carcinogen[16] or a liver carcinogen in albino rats.[17] However, these carcinogenic effects were not found in other long-term studies of quercetin using rats,[18–20] mice,[21, 22] or hamsters.[23] Furthermore, Morino *et al.*[23] reported that quercetin did not possess any initiating activity for two-stage skin carcinogenesis and it was clearly demonstrated that quercetin also did not act as a skin tumor promoter,[24] rather inhibiting the tumor-promoting activity of established promoters for mouse skin two-stage carcinogenesis.[25–27] In addition, it was demonstrated in our laboratory that quercetin does not act as a tumor promoter in initiation-promotion studies of the rat urinary bladder[28] or liver carcinogenesis.[29]

The objective of the present investigation was to clarify the equivocal carcinogenicity, particularly for the ileum, urinary bladder and liver, of the environmental mutagen quercetin, and also to evaluate its toxic potential.

MATERIALS AND METHODS

Chemicals The quercetin used in this study was supplied by the National Institute of Hygienic Sciences, Tokyo, and was manufactured by Tokyo Kasei Co. Ltd., Tokyo. The purity was determined to be at least 99.4% (analyzed by Japan Food Research Laboratories, Tokyo). Quercetin was incorporated into powdered CRF-1 diet (Charles River Japan, Inc., Kanagawa), at concentrations of 0, 1.25 and 5.0%. Analyses of the samples showed that the actual levels of quercetin in food, nominally containing 1.25 and 5.0%, were 1.24±0.04 and 5.02±0.15%, respectively (analyzed by Japan Food Research Laboratories).

Animals and maintenance Three hundred F344/DuCrj rats (150 males and 150 females) were obtained from Charles River Japan, Inc. The rats were 6 weeks old at the commencement of the experiment and were housed five to a plastic cage with hardwood chips for bedding. The room temperature was kept at 22±2°C and the relative humidity at 60±10% with a 12-h light/dark cycle. A positive air pressure was maintained with more than 15 air changes per hour.

Experimental procedure Groups of 50 male and 50 female rats were given diet containing 0, 1.25 or 5.0% quercetin for 104 weeks and then normal diet for a

further 8 weeks. The selection of the highest dose was based on previous reports and also obeyed the guidelines for oncogenicity studies, which recommend 5% as the maximum dietary level. The animals were observed daily for abnormalities; rats showing signs of ill-health were isolated, to be returned to the group if their condition improved but otherwise to be killed and autopsied. Individual body weights were recorded weekly for the first 14 weeks and then every other week. Food and water consumption were measured over the 2-day period before each weighing. During week 112, urine samples were obtained from 10 rats in each group and analyzed with Multistix for pH, protein, glucose, bilirubin, ketones, occult blood and urobilinogen. After 112 weeks, surviving animals were deprived of food, but not water, overnight and then killed under ether anesthesia by exsanguination (blood samples collected) from the abdominal aorta. Hematological determinations included erythrocyte counts, leukocyte counts, measurement of hemoglobin concentrations and hematocrit values, platelet counts (Coulter Electronic counter) and differential leukocyte counts. After separation, the serum was analyzed for glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, total cholesterol, total protein, albumin:globulin ratio, urea nitrogen, glucose and albumin, the analyses being performed at the Chunichi Clinic Center, Ohgaki, Japan.

Gross observations were performed at autopsy, and detailed examinations of the luminar surfaces of intestines and urinary bladder were also carried out using a dissecting microscope after fixation. The following organs of each rat were weighed and organ-to-body-weight ratios were determined: brain, heart, liver, spleen, kidneys, adrenals and testes or ovaries. Samples of these organs and of the salivary glands, trachea, lungs, thymus, lymph nodes, stomach, small intestines, large intestines, pancreas, urinary bladder, pituitary, thyroid, adrenals, prostate, seminal vesicle, epididymis, skin, mammary gland, skeletal muscle, eyes, Harderian glands, spinal cord, sciatic nerve and any other tissues of abnormal appearance were fixed in 10% buffered formalin. Preserved tissues to be examined microscopically were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Histopathological examinations were also performed on rats that died spontaneously and those that were killed upon becoming moribund.

Statistical analyses Data on cumulative mortality and tumor incidence were analyzed by the two-sided Fisher's exact probability test. Other data were analyzed using Student's *t* test.

RESULTS

No clinical signs related to quercetin treatment were apparent in any of the rats during the 112 weeks of the experiment. At the end of study, the survival rates of males fed 0, 1.25 and 5.0% quercetin were 56, 66 and 68% and those of females were 66, 62 and 72%, respectively. There were no significant differences in mortality between controls and treated animals during the 112 weeks of the study (Fig. 1).

Body weights were significantly reduced in both sexes receiving the 5.0% dose from week 1 to the termination, and in 1.25% males and females sporadically (Fig. 2). The food consumption of controls and treated animals showed no clear differences. For the two treated groups, the intake of quercetin calculated from the nominal dietary levels, the mean food consumption and the mean

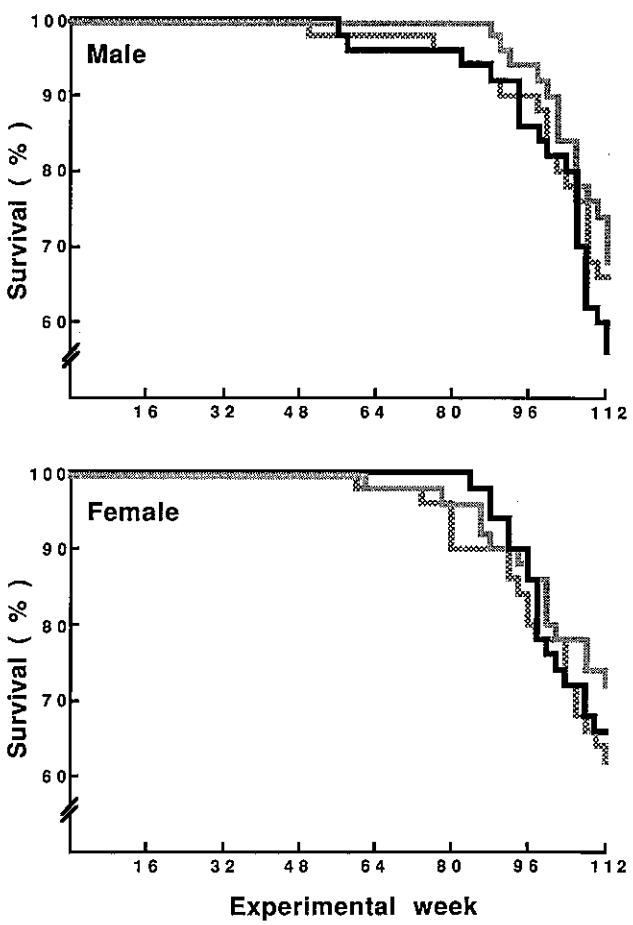

Fig. 1. Survival rates of male and female F344 rats on diet containing quercetin at a concentration of 0 (■), 1.25 (▒) or 5.0% (▓).

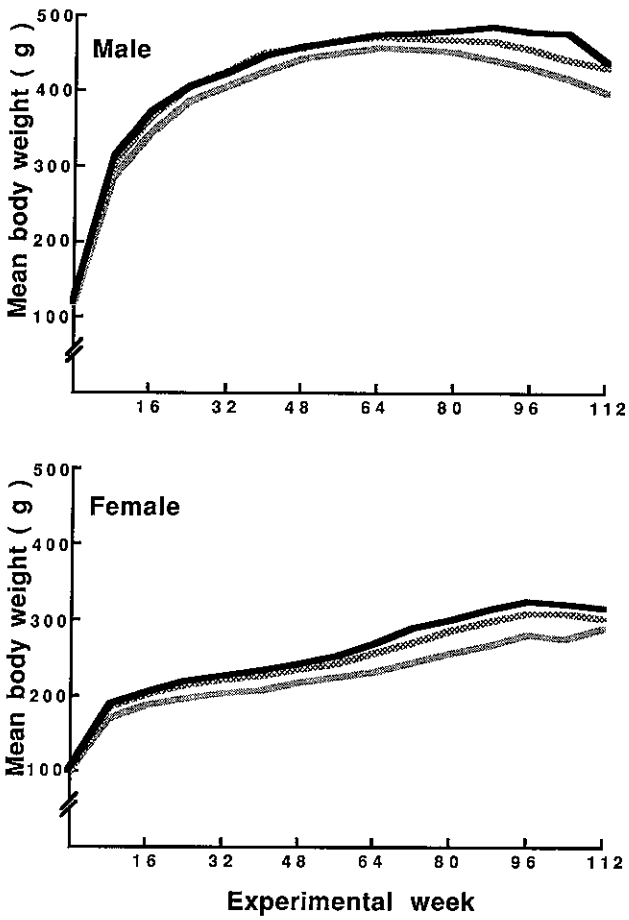

Fig. 2. Growth curves of male and female F344 rats on diet containing quercetin at a concentration of 0 (■), 1.25 (▒) or 5.0% (▓).

body weight for each group, was higher in the first 3 months than during the remainder of the experiment. Excluding the first 3 months, the average quercetin intakes for males and females, respectively, were 427 and 497 mg/kg body weight/day with 1.25% quercetin, and 1926 and 2372 mg/kg body weight/day with 5.0% quercetin.

The urine analyses and hematological examinations did not reveal noteworthy changes in any of the groups. Serum glucose values were decreased in the 5.0% males, and this seemed to be related to the observed marked growth retardation. There were no other differences in serum chemistry between control and quercetin-treated groups of either sex.

On detailed gross observation, a higher incidence of raised areas of the cecum, which occurred as single lesions, was recorded in the 5.0% males when compared to the controls. No stone formation in the urinary bladder was found in any of the rats and there were no treatment-related changes in other organs. Although statistically significant increases of relative organ weights of brain in both sexes receiving 5.0% quercetin, and of the kidneys in the 5.0% male group were observed, all values were within the normal range for the F344/DuCrj strain of rats and seemed to be related to the reduced body weights (Table I).

Proliferative (nonneoplastic) and neoplastic lesions of the intestines, liver and urinary bladder, which were suspected to be target organs in previous reports, are summarized in Table II. Adenocarcinomas of the small intestine were found in a control male and in a 1.25% male, but no tumors were seen in the 5.0% group. Hyperplastic polyps of the cecum were found in both sexes administered 5.0% quercetin, but the increased incidence was only statistically significant in males. The mucosal tubular glands were elongated, but no reduction in number of goblet cells or epithelial atypia was evident histologically (Fig. 3). An adenoma and two adenocarcinomas of the cecum in the 5.0% males, as well as two colon adenomas in the 5.0% females were observed. However the differences from the zero control values were not statistically significant.

Table I. Final Body (g) and Relative Organ Weights (% Body Weight) of F344 Rats Given Quercetin in the Diet (Mean±SD)

Sex	Group	No. of rats	Final body weight (g)	Brain	Liver	Spleen	Kidneys
Male	0%	28	424±49	0.50±0.08	2.88±0.41	0.33±0.11	0.67±0.10
	1.25%	33	420±57	0.51±0.09	3.05±0.44	0.43±0.31	0.68±0.10
	5.0%	34	387±43**	0.55±0.07*	2.78±0.53	0.47±0.53	0.73±0.10*
Female	0%	32	304±34	0.63±0.07	2.42±0.39	0.28±0.22	0.68±0.19
	1.25%	31	292±31	0.66±0.08	2.40±0.26	0.37±0.44	0.64±0.08
	5.0%	36	284±32*	0.68±0.08*	2.60±0.51	0.50±0.79	0.66±0.08

*, ** Significantly different from the control group values at $P<0.05$ or 0.01, respectively.

Table II. Nonneoplastic-proliferative and Neoplastic Lesions Developing in the Intestines, Liver and Urinary Bladder of F344/DuCrj Rats Fed Quercetin-containing Diet

Site and type of tumor	Males			Females		
	0%	1.25%	5.0%	0%	1.25%	5.0%
Effective no. of rats	50	50	50	50	50	50
Small intestine						
Adenocarcinoma	1 (2)	1 (2)	0	0	0	0
Leiomyoma	1 (2)	0	0	0	0	0
Cecum						
Hyperplastic polyp	0	0	11(22)[a]	0	0	2 (4)
Inflammatory polyp	0	0	0	0	0	1 (2)
Adenoma	0	0	1 (2)	0	0	0
Adenocarcinoma	0	0	2 (4)	0	0	0
Sarcoma, NOS	0	0	1 (2)	0	0	0
Colon						
Adenoma	0	0	0	0	0	2 (4)
Liver						
Focus (area) of cellular alteration	19(38)	21(42)	14(28)	25(50)	22(44)	10(20)[b]
Hyperplastic (neoplastic) nodule	2 (4)	2 (4)	3 (6)	2 (4)	0	0
Bile duct hyperplasia	47(94)	44(88)	0[a]	1 (2)	0	0
Urinary bladder						
Transitional cell hyperplasia	0	1 (2)	1 (2)	0	0	3 (6)
Papillomatosis	0	0	1 (2)	0	0	1 (2)
Transitional cell papilloma	1 (2)	0	0	0	1 (2)	0
Leiomyoma	1 (2)	0	0	0	0	0

a), *b*) Significantly different from control values at $P<0.001$ or 0.01, respectively.

The incidence of foci (areas) of cellular alteration in the liver was significantly decreased in the 5.0% females, although that of hyperplastic (neoplastic) nodules was not affected. No hepatocellular carcinomas developed in any of the rats in the study. Bile duct hyperplasia, found in control males at a high incidence, was not a feature in the 5.0% male group (Fig. 4). In the urinary bladder, no treatment-related changes were found. Transitional cell hyperplasias, papillomatosis and transitional cell papillomas were observed sporadically.

Neoplastic lesions other than those in the aforementioned organs are summarized in Table III. The incidence of fibroadenomas of the mammary gland in the 5.0% females was significantly decreased when compared to the control. Malignant lymphoma/leukemia was increased in the 1.25% males, but it was not considered to be treatment-related, since there was no dose dependency.

On histopathological examination, a wide range of non-neoplastic lesions were evident (data not shown). The lesions were similar to those considered usual in aged F344 rats. In several instances decreased incidences of non-neoplastic lesions achieved statistical significance: for example hyperplasia of the pituitary or prostate. On the contrary, an increased incidence of chronic nephropathy were observed in the 1.25 and 5.0% males, although values were within the normal range of compiled data in our laboratory, and therefore seemed to be not biologically significant.

DISCUSSION

The present investigation of the carcinogenicity of quercetin revealed a treatment-related growth retardation suggesting toxicity, although there were no biologically significant findings with regard to other clinical signs, survival rate, urinalyses, hematology and relative organ weights.

The hyperplastic polyps of the cecum which were found in both sexes receiving the 5.0% dose, and at statistically significant incidences in males, appeared to correlate with the raised areas observed on detailed gross examination with a dissecting microscope. These lesions were considered to be of non-neoplastic nature, since no epithelial atypia was found histopathologically. To our knowledge, no spontaneous occurrence of this lesion has been reported,[30–32] though Newberne *et al.*[33] did describe cecal enlargement and hyperplasia (reversible) together with soft stools or diarrhea in rats treated with modified food starches. These symptoms were lacking in the pres-

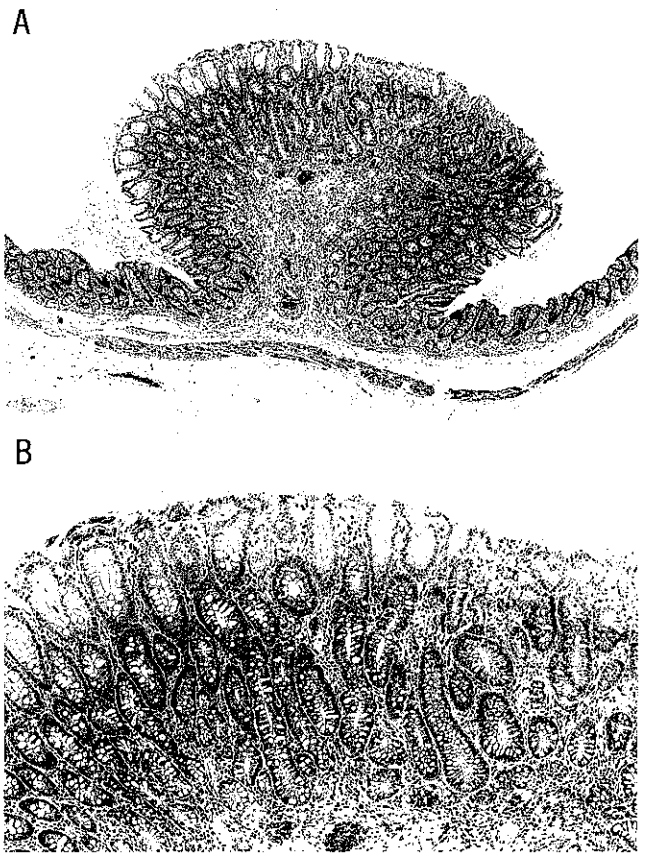

Fig. 3. Hyperplastic polyp of the cecum in a male on diet containing quercetin at 5.0% concentration. The mucosal tubular glands are elongated, although no reduction in number of goblet cells and no epithelial atypia are evident. H-E, ×40 (A), ×100 (B).

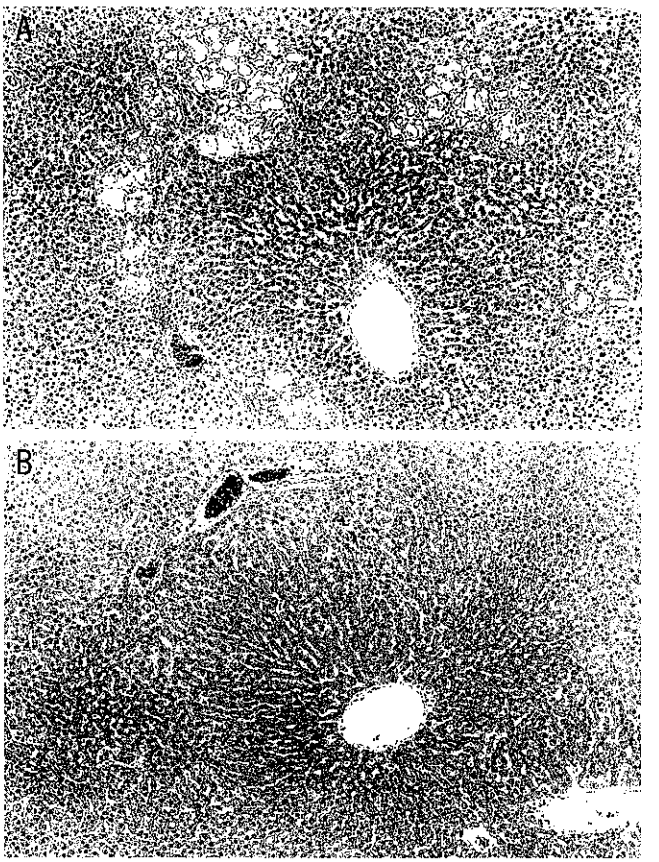

Fig. 4. (A) Bile duct hyperplasia of the liver in a control male. (B) Normal appearance of the liver in a male on diet containing quercetin at a concentration of 5.0%. H-E, ×100.

ent study, and the etiology of hyperplastic polyps developing in rats given quercetin is presumably different. Although an adenoma and two adenocarcinomas of the cecum or two adenomas of the colon, which are rare spontaneous tumors in aged F344 rats[30–32] were observed in the 5.0% males or females, respectively, the numbers were not statistically significant. Furthermore, the high incidences of ileal tumors in rats fed quercetin-containing diet observed by Pamukcu *et al.*[16] were not a feature of this investigation.

Pamukcu *et al.* also reported that administration of quercetin at a dietary level of 0.1% to both sexes of albino rats for 58 weeks resulted in urinary bladder tumor development. Detailed microscopic examination of urinary bladder sections in the present experiment did reveal low incidences of transitional cell hyperplasia and papillomatosis in animals treated with 5.0% quercetin, but no transitional cell papillomas and carcinomas developed in either sex, and even though special attention was paid to possible signs of papillary or nodular hyperplasia, which has been recognized as a preneoplastic lesion in this organ,[28, 34] these lesions were conspicuously lacking. Other investigators also failed to find any urinary bladder tumors in laboratory animals maintained on quercetin-containing diet at doses of up to 10%.[15–23] Moreover, Hirose *et al.* demonstrated that quercetin did not possess either tumor promotion or initiation activity for two-stage carcinogenesis of the rat urinary bladder.[28]

Ertürk *et al.* demonstrated an increased frequency of hepatomas and biliary adenomas in female F344 rats fed 2% or 1% quercetin for up to 139 weeks.[17] These findings were, however, not confirmed by other long-term studies including the present experiment.[15, 16, 18–23] On the contrary, the incidence of foci (area) of hepatocellular alteration was decreased significantly in

Table III. Incidences of Tumors Developing in Organs Other than the Intestines, Liver and Urinary Bladder of F344/DuCrj Rats Maintained on Diet Containing Quercetin

Site and type of tumor	Males			Females		
	0%	1.25%	5.0%	0%	1.25%	5.0%
Effective no. of rats	50	50	50	50	50	50
Heart						
Sarcoma, NOS	0	0	0	0	1 (2)	0
Spleen						
Lymphoma	0	0	0	1 (2)	0	0
Fibroma	0	1 (2)	0	0	0	0
Hemangiosarcoma	0	0	1 (2)	0	0	0
Leiomyosarcoma	0	0	1 (2)	0	0	0
Pituitary						
Adenoma, pars distalis	14(28)	12(24)	9(18)	20(40)	19(38)	17(34)
Adenoma, pars intermedia	1 (2)	1 (2)	1 (2)	0	1 (2)	0
Craniopharyngioma	0	1 (2)	0	0	0	0
Carcinoma, pars distalis	0	2 (4)	1 (2)	2 (4)	4 (8)	0
Thyroid						
Follicular cell adenoma	1 (2)	0	0	0	0	0
C-cell adenoma	5(10)	8(16)	3 (6)	3 (6)	0	2 (4)
Follicular cell carcinoma	0	0	1 (2)	0	0	0
C-cell carcinoma	0	3 (6)	1 (2)	1 (2)	1 (2)	1 (2)
Parathyroid						
Adenoma	1 (2)	0	0	0	1 (2)	0
Adrenals						
Pheochromocytoma	4 (8)	8(16)	6(12)	2 (4)	1 (2)	0
Ganglioneuroma	2 (4)	0	0	0	0	0
Cortical carcinoma	0	0	0	2 (4)	0	0
Malignant pheochromocytoma	2 (4)	1 (2)	2 (4)	1 (2)	0	0
Malig. ganglioneuroma-pheochromocytoma	0	0	0	2 (4)	0	0
Lungs						
Adenoma	1 (2)	2 (4)	0	1 (2)	0	0
Adenocarcinoma	1 (2)	0	1 (2)	0	0	0
Esophagus						
Fibroma	0	0	0	0	1 (2)	0
Stomach						
Squamous cell papilloma	0	0	0	0	1 (2)	0
Squamous cell carcinoma	0	0	0	1 (2)	0	0
Pancreas						
Acinar cell adenoma	0	0	1 (2)	0	0	0
Islet-cell adenoma	2 (4)	1 (2)	2 (4)	0	0	0
Kidneys						
Adenoma	1 (2)	0	0	0	0	0
Adenocarcinoma	0	1 (2)	0	0	0	0
Transitional cell carcinoma	0	1 (2)	0	0	0	0
Nephroblastoma	1 (2)	0	0	0	0	0
Testes						
Interstitial cell tumor	48(96)	47(94)	47(94)	—	—	—
Malignant interstitial cell tumor	0	1 (2)	1 (2)	—	—	—
Prostate						
Adenoma	9(18)	12(24)	6(12)	—	—	—
Adenocarcinoma	3 (6)	1 (2)	1 (2)	—	—	—
Preputial/clitoral gland						
Adenoma	2 (4)	4 (8)	3 (6)	1 (2)	2 (4)	4 (8)
Adenocarcinoma	1 (2)	1 (2)	2 (4)	0	0	0

Table III — continued

Site and type of tumor	Males			Females		
	0%	1.25%	5.0%	0%	1.25%	5.0%
Mammary gland						
Adenoma	1 (2)	0	0	1 (2)	0	0
Fibroadenoma	6(12)	11(22)	9(18)	15(30)	8(16)	4 (8)[a]
Adenocarcinoma	0	0	1 (2)	0	2 (4)	0
Ovaries						
Granulosa/Theca cell tumor	—	—	—	1 (2)	0	0
Uterus						
Adenoma	—	—	—	1 (2)	0	0
Endometrial stromal polyp	—	—	—	8(16)	10(20)	12(24)
Adenomatous polyp	—	—	—	0	1 (2)	0
Adenocarcinoma	—	—	—	1 (2)	1 (2)	3 (6)
Endometrial stromal sarcoma	—	—	—	2 (4)	1 (2)	1 (2)
Leiomyosarcoma	—	—	—	2 (4)	0	0
Fibrosarcoma	—	—	—	1 (2)	0	0
Sarcoma, NOS	—	—	—	0	1 (2)	0
Skin/subcutis						
Squamous cell papilloma	3 (6)	2 (4)	3 (6)	0	0	0
Fibroma	11(22)	9(18)	10(20)	3 (6)	2 (4)	4 (8)
Lipoma	3 (6)	0	0	0	0	0
Lymphangioma	0	1 (2)	0	0	0	0
Squamous cell carcinoma	1 (2)	0	0	0	0	0
Basal cell carcinoma	0	1 (2)	1 (2)	0	0	1 (2)
Sebaceous carcinoma	1 (2)	0	0	0	0	0
Fibrosarcoma	1 (2)	0	1 (2)	0	0	0
Malignant fibrous histiocytoma	2 (4)	1 (2)	0	1 (2)	1 (2)	1 (2)
Liposarcoma	0	1 (2)	0	0	0	0
Sarcoma, NOS	0	0	1 (2)	0	0	0
Malignant schwannoma	0	0	0	1 (2)	0	0
Brain						
Granular cell tumor	0	0	1 (2)	0	0	0
Astrocytoma	1 (2)	0	0	0	0	0
Mixed glioma	0	0	0	0	0	1 (2)
Spinal cord						
Astrocytoma	0	1 (2)	0	0	0	0
Peripheral nerve						
Malignant schwannoma	0	1 (2)	0	0	0	0
Thoracic cavity						
Mesothelioma	1 (2)	0	0	0	0	0
Abdominal cavity						
Lipoma	0	0	2 (4)	0	0	0
Liposarcoma	0	0	0	0	1 (2)	0
Mesothelioma	5(10)	3 (6)	0	0	0	0
Malignant fibrous histiocytoma	0	0	0	0	0	1 (2)
All sites						
Malignant lymphoma/leukemia	6(12)	18(36)[a]	14(28)	8(16)	14(28)	17(34)
Site unknown						
Malignant fibrous histiocytoma	0	1 (2)	0	0	0	0
Others						
Odontoma	0	1 (2)	0	0	0	0
Lipoma	0	0	1 (2)	0	0	0
Osteosarcoma	0	0	0	1 (2)	0	0

a) Significantly different from control group values at $P<0.01$.

the 5.0% females, whereas those for hyperplastic (neoplastic) nodules were similar in treated and control groups. Moreover, quercetin did not induce preneoplastic glutathione *S*-transferase placental form-positive foci in a medium-term bioassay system using F344 rats.[29] It has been reported that quercetin does not enhance the phase I or phase II enzyme activities in rat liver,[35] in contrast to the marked induction observed with the established liver tumor promoter, phenobarbital. The available evidence thus suggests that quercetin is neither a liver tumor promoter nor an initiator. Since the reduction in bile duct hyperplasia found in the 5.0% males was also noticed in another carcinogenicity study together with a clear body weight reduction,[36] it was considered likely to be related to the growth retardation.

Earlier studies have shown that quercetin can inhibit the promoting activity of known skin tumor promoters,[25-27] while not exerting any first-step modification potential for skin carcinogenesis.[24] No evidence of modifying effects on incidences of spontaneous skin tumors was found, however, in any long-term studies of quercetin itself.[15-23] The several instances of reduced non-neoplastic lesion incidences which achieved statistical significance in the present experiment might be related to quercetin administration, but the biological significance remains unclear. Most of the non-neoplastic lesions observed in this study occurred with similar frequencies in both control and treated animals. In conclusion, although quercetin has previously been indicated to possess carcinogenic potential for the ileum, urinary bladder and liver in rats, it was not carcinogenic for F344/DuCrj rats in the present study.

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