[CANCER RESEARCH 48, 5754–5758, October 15, 1988]

Inhibition of 7,12-Dimethylbenz(*a*)anthracene- and *N*-Nitrosomethylurea-induced Rat Mammary Cancer by Dietary Flavonol Quercetin[1]

Ajit K. Verma,[2] Jeffrey A. Johnson, Michael N. Gould, and Martin A. Tanner

Department of Human Oncology, University of Wisconsin Clinical Cancer Center, Madison, Wisconsin 53792

ABSTRACT

The effects of dietary supplementation of flavonol quercetin on both 7,12-dimethylbenz(*a*)anthracene (DMBA)- and *N*-nitrosomethylurea-induced mammary cancer in female Sprague-Dawley rats were determined. Quercetin diet was started 1 wk before intragastric instillation of DMBA (65 mg/kg of body weight) or i.v. injection of *N*-nitrosomethylurea (50 mg/kg of body weight) and was continued during the entire period (20 wk) of the experiment. Dietary quercetin inhibited both the incidence and the number of palpable rat mammary tumors; rats fed on 2% quercetin had 25% less incidence of mammary cancer, while the average number of mammary tumors per rat was reduced by 39% at 20 wk post-DMBA administration compared to animals on a control diet. In a separate experiment, a 5% quercetin diet elicited a greater inhibitory effect on the induction of rat mammary tumors by DMBA than was observed with a 2% quercetin diet. The inhibitory effect of quercetin on mammary tumor incidence in rats on 2% and 5% diets and on tumor multiplicity in animals on a 5% diet was statistically significant ($P < 0.05$). In addition, the risk of the development of a palpable tumor (as determined by the nonparametric estimate of the hazard function) in the quercetin-fed group was lower than the group on control diet throughout the course of the experiment. Furthermore, 5% dietary quercetin significantly inhibited ($P < 0.05$), although to a lesser extent than observed in DMBA-induced tumor formation, both the incidence and the number of palpable mammary tumors per rat induced by *N*-nitrosomethylurea. Dietary quercetin did not elicit any detectable sign of toxicity. The gain in body weight in rats on the quercetin diet and the quantity of diet consumed per rat per week were similar to those for rats on the control diet.

INTRODUCTION

The flavonoids constitute an integral part of the human diet. It has been estimated that an average dietary intake of flavonoids is approximately 1 g per day (1, 2). Flavonoids are widespread among food plants including vegetables and fruits, and the flavonol quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Fig. 1) is found in the edible portion of the majority of dietary plants (*e.g.*, citrus, berries, leafy vegetables, roots, tubers and bulbs, herbs and spices, legumes, cereal grains, tea, and cocoa) (1).

A number of flavonols of edible plants are mutagenic and genotoxic in *in vitro* assays, but the results of their carcinogenicity in a number of experimental animal models are inconsistent (3). Among flavonoids, mutagenicity is largely confined to flavonols, and quercetin is one of the most potent mutagens in the Ames test (4–9). Quercetin is directly mutagenic to both TA98 and TA100 strains of *Salmonella typhimurium*. Flavonol glycosides (*e.g.*, rutin) are nonmutagenic unless they are hydrolyzed to the free aglycone (quercetin) (5). The mutagenicity of the flavonols and flavonol glycosides appears to be very dependent upon the absence of excision repair capability, as well as the presence of the pkM101 plasmid which enhances error-prone repair of DNA lesions (3). Elimination of either or both of these factors results in virtually complete loss of mutagenic activity (3).

Two independent investigations using Chinese hamster ovary cells (10) and V79 cells (11) concluded that quercetin did not induce forward mutations at the HGPRT, APRT, and Na^+/K^+-ATPase loci. Furthermore, quercetin caused a marginal, but not significant, increase in sister chromatid exchange (10, 11). However, quercetin did induce significant increases in *TK* locus mutation in both Chinese hamster ovary and V79 cells (10, 11). The results were not affected by the addition of an exogenous enzyme preparation (11).

Pamukcu *et al.* (12) found that quercetin, one of the components (tannins, shikimic acid, catecholamines, pterosin, pterolactam, kaempferol, and rutin) isolated from bracken fern, was a urinary bladder and intestinal carcinogen when given in a special commercial chow to a specific strain of Norwegian rats. Additionally, investigators from the same laboratory, Erturk *et al.* (13), reported in an abstract that quercetin fed to female Sprague-Dawley and Fischer 344 rats was also a hepatocarcinogen but, to the contrary, quercetin in a short-term test for genotoxicity did not induce DNA repair in rat hepatocytes (14). In several independent studies by others (5, 15–20), quercetin or rutin in the diet, as high as 10%, was observed to be inactive in eliciting tumors in rodents.

Accumulating evidence lends support to the facts that quercetin and certain related flavonoids may be inhibitors of carcinogenesis (21–25). Thus, flavonoids were found to inhibit metabolism of carcinogens *in vitro* in isolated liver microsomes (26–28). Furthermore, quercetin fed, at the 4% level, to C57BL/6J mice inhibited benzo(*a*)pyrene-induced nuclear damage in colonic epithelial cells (29). It is also noteworthy that a number of hydroxylated flavonoids including quercetin were found to inhibit the mutagenic activity of bay-region diol-epoxides of benzo(*a*)pyrene (30). If topically applied in conjunction with the tumor promoter TPA[3] to mouse skin, certain flavonoids (quercetin) inhibited skin tumor formation (21, 22, 24). However, little is known about the effects of dietary flavonoids on chemically induced tumors in experimental animal models, which are relevant to determining the relationships between diet and carcinogenesis.

The present study was designed to evaluate the effect of dietary quercetin on the induction of rat mammary tumors induced by DMBA or NMU. Data indicating that dietary quercetin inhibits both DMBA- and NMU-induced rat mammary cancer are summarized in this paper.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from Aldrich Chemical Co., Milwaukee, WI, and also from Kodak, Rochester, NY. NMU was purchased from Sigma Chemical Co., St. Louis, MO. Quercetin dihydrate was obtained from Aldrich.

Animals and Diets. Virgin female Sprague-Dawley rats, 45 to 50 days old, were purchased from Harlan Sprague-Dawley, Madison, WI. All

Received 2/26/88; revised 6/13/88; accepted 7/11/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] The work was supported by American Institute of Cancer Research Grant 86-A45-REV to A. K. V.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz(*a*)anthracene; NMU, *N*-nitrosomethylurea.

rats were fed powdered Wayne Lab-Blox commercial diet and were housed in light-, humidity-, and temperature-controlled rooms. Food and water were available *ad libitum*. The rats were kept in a normal rhythm of 12-h-light and 12-h-dark periods. Quercetin was blended into the powdered Wayne Lab diet and stored in sealed containers at 4°C. The nutrient concentration of the experimental diet was diluted by the addition of quercetin. We do not believe that this dilution is of sufficient concern; thus the unmedicated diet was undiluted.

Tumor Induction Experiments. Mammary tumors in female Sprague-Dawley rats were induced by a single intragastric administration of DMBA (65 mg/kg of body weight) in 0.7 to 1.0 ml of sesame oil (31). Rat mammary tumors were also induced by i.v. injection of NMU (50 mg/kg) (32). NMU, in Isopac vials containing 1 g of NMU and approximately 350 mg of a 5% solution of acetic acid as a stabilizer, was dissolved in 0.9% sodium chloride solution. The rats were started on quercetin diet a week before carcinogen administration. Fresh diet was added to the protected feeders once a week with feed weigh-back being recorded. The difference between the weight of the feed added during the experimental period and that of the feed removed provided close estimates of feed consumption. The rats were weighed weekly for the first 8 to 10 wk of the experiment and every other week thereafter. The rats were palpated at least biweekly in the experiment using a 2% quercetin diet and weekly in the two 5% quercetin experiments. The tumor induction experiments were terminated at 20 wk post-carcinogen administration. At the end of the experiment, all rats received a complete postmortem examination. Tumors were fixed in 10% buffered formalin, and sections were stained for histopathological examination. The significance of the difference in the tumor multiplicity data obtained from the control and the quercetin-fed rats was determined with a one-sided *t* test (33).

RESULTS

The effect of 2% dietary quercetin on DMBA-induced rat mammary tumors is illustrated in Figs. 2 to 4. In this experiment, the quercetin diet was started 1 wk before carcinogen administration. The quercetin diet significantly ($P < 0.05$) inhibited the induction of palpable rat mammary tumors at 7, 9, 13, 14, 16, and 20 wk post-DMBA treatment. The number of tumors per rat at 20 wk was 2.3 ± 0.5 and 1.4 ± 0.3 in control and quercetin diet groups, respectively. This represents a 30% reduction in tumor yield for those animals fed a quercetin diet ($P < 0.03$, *t* test, one-sided). The data also indicate that feeding 2% quercetin to untreated (not given DMBA) rats for 20 wk did not induce palpable mammary tumors (Fig. 2*A*).

The time to first palpable tumor is presented in Fig. 2*B*. An analysis of the data via the log-rank test (33) demonstrated a significant difference between rats fed a control and 2% quercetin diet ($P < 0.0025$). Furthermore, the quercetin group had a lower percentage of rats with tumor than the control group during the entire period of the experiment (Fig. 2*B*). The average consumptions of food by rats on control (18.2 ± 2.4 g/rat/day) and quercetin (15.2 ± 0.8 g/rat/day) diets were significantly different ($P < 0.05$, *t* test). However, both groups exhibited similar gains in body weight (Fig. 3).

We further investigated the effect of 5% dietary quercetin on induction of rat mammary cancer. Five % quercetin in the diet

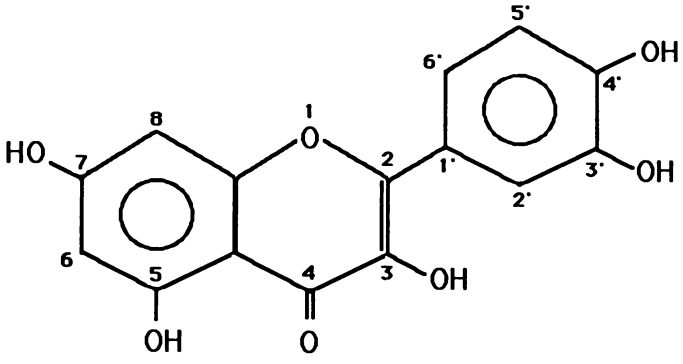
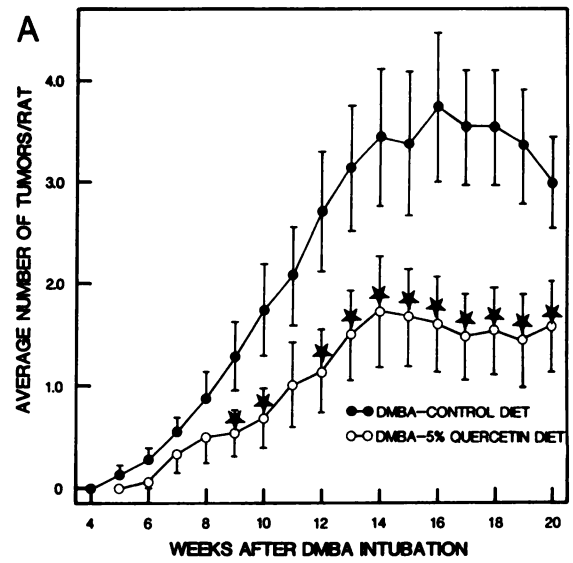


Fig. 1. Chemical structure of quercetin (3,3',4',5,7-pentahydroxyflavone, CAS 117-39-5).



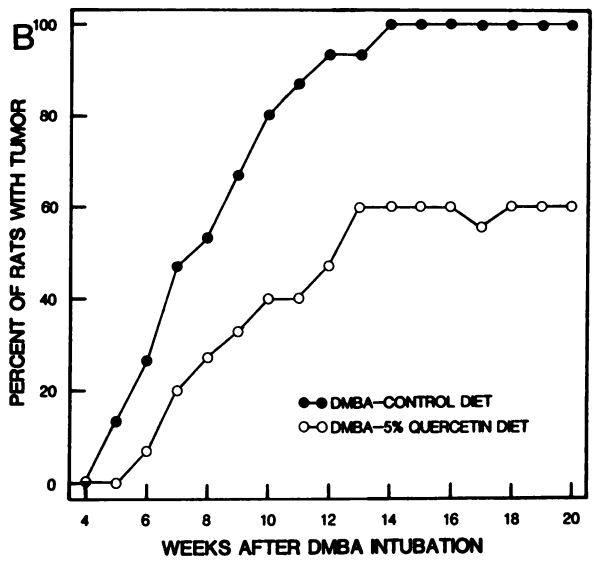


Fig. 2. Effect of a 2% quercetin diet on DMBA-induced rat mammary cancer. The rats were started on control (●) or 2% quercetin diet (○) 1 wk before intragastric instillation of DMBA (65 mg/kg of body weight) and maintained on the diet throughout the length of the experiment. In addition, a group of rats was concurrently fed only a 2% quercetin diet and not intubated with DMBA (□). Tumors were sampled—95% were carcinomas in the control group, and 88% were carcinomas in the quercetin group. *A*, effect of quercetin on the average number of rat mammary tumors per rat. *Points*, mean of the number of tumors/rats surviving; *bars*, SE. *B*, effect of quercetin on the incidence of rat mammary cancer. Rats with huge palpable tumors were killed as shown below. The fraction of animals surviving the 20-wk period was 21 of 30 rats on control and 23 of 30 rats on the quercetin diet. ★, values significantly different from control ($P < 0.05$).

	No. of animals killed after carcinogen administration at the following weeks									
	11	12	13	14	15	16	17	18	19	20
DMBA	0	2	0	1	0	4	0	2	0	0
Quercetin-DMBA	0	0	3	1	0	1	0	2	0	0

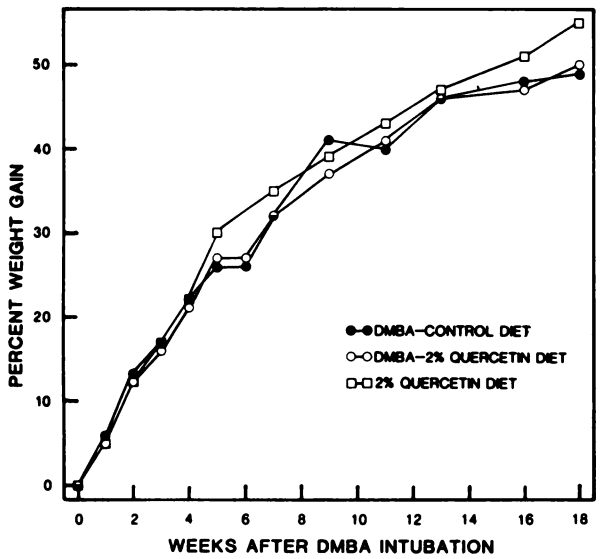
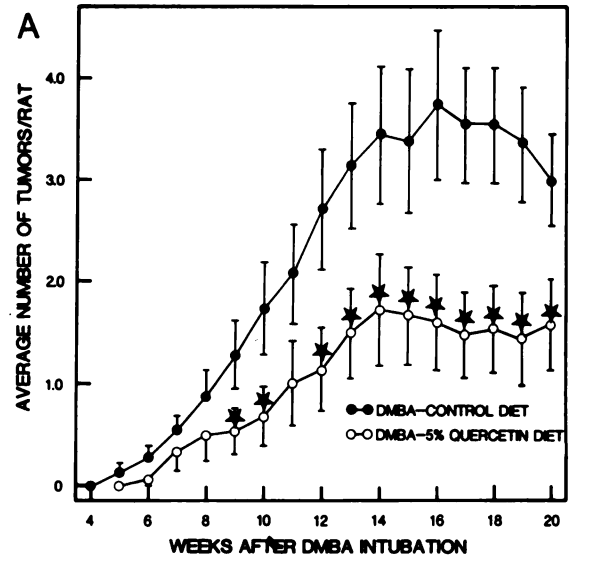


Fig. 3. Effect of dietary quercetin (2%) on the gain in weight. The experiment is described in the legend of Fig. 2. *Points*, percentage of weight gain (mean of group at Wk n − mean of group at Wk 0/mean of group at Wk 0) × 100.



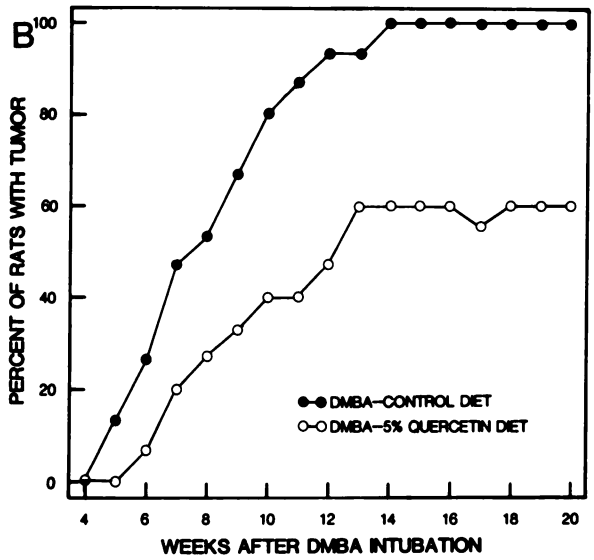


Fig. 4. Effect of dietary quercetin (5%) on the initiation of rat mammary tumors by DMBA. The rats were started on control (●) or 5% quercetin diet (○) 1 wk before intragastric instillation of DMBA (65 mg/kg of body weight) and maintained on the diet throughout the length of the experiment. *A*, effect of quercetin on the average number of rat mammary tumors per rat. *Points*, mean of the number of tumors/rats surviving; *bars*, SE. *B*, effect of quercetin on the incidence of rat mammary cancer. Tumors were sampled—100% were carcinomas in the control, and 92% were tumors in the quercetin group. The rats with huge necrotic palpable tumors were killed as shown below. The fraction of animals surviving the 20-wk period was 11 of 15 rats on control and 14 of 15 rats on the 5% quercetin diet. ★, values significantly different from control ($P < 0.05$).

significantly ($P < 0.0005$, t test) decreased the number of palpable mammary tumors per rat by 48% at 20 wk post-DMBA administration (Fig. 4*A*). Furthermore, there was a greater inhibitory effect on the time to first palpable tumor for animals fed a 5% quercetin diet compared to a 2% diet (Fig. 4*B*). The average consumptions of food by rats on control (17.6 ± 0.3 g/rat/day) and 5% quercetin (18.4 ± 0.6 g/rat/day) diets were not significantly ($P > 0.1$, t test) different, and both groups also demonstrated similar gains in body weight.

Dietary quercetin at a dose of 5% also inhibited the induction of rat mammary tumors by NMU. The extent of inhibition was less than that observed in DMBA-induced tumor formation. An analysis of the time to first palpable tumor indicated a somewhat weaker but significant difference between rats on quercetin and control diets ($P < 0.05$, log-rank test), and in a similar manner to the DMBA experiments, the incidence was reduced throughout the entire length of the experiment (Fig. 5*B*). Furthermore, a comparison of total palpable tumors per rat revealed a significant difference between the control and quercetin-fed rats ($P < 0.05$, t test) at 11, 13, and 14 wk post-NMU injection (Fig. 5*A*). Again, the average consumptions by rats on control (20.1 ± 0.6 g/rat/day) and 5% quercetin-fed (19.2 ± 0.7 g/rat/day) diets were not significantly ($P > 0.1$, t test) different, and both groups also exhibited similar gains in weight.

DISCUSSION

Quercetin, a component of edible plants, is mutagenic in bacterial assay systems (3, 4); however, in the presence of a competent excision repair system, the mutagenic activity is virtually eliminated (3). Generally, quercetin does not induce specific locus mutations in mammalian cells, but if forward mutations were statistically significant, the biological significance is questioned (10, 11). The carcinogenicity of quercetin is controversial and as of yet unresolved (5, 12–20). Quercetin has been shown to inhibit mouse skin carcinogenesis (21). Now, we present that dietary quercetin inhibits both DMBA- and NMU-initiated rat mammary tumor development.

Dietary quercetin, at both 2% and 5% levels, inhibited the incidence (Figs. 2*B*, 4*B*, and 5*B*) and the multiplicity of palpable mammary tumors (Figs. 2*A*, 4*A*, and 5*A*). The diet consumed on a weekly basis per rat was essentially similar in all experiments, and also the gain in body weight was not affected by the consumption of quercetin in the diet (Fig. 3). These results allude to the fact that the inhibition of the induction of rat mammary tumors by quercetin is not attributable to reduced caloric intake (34). The effect of quercetin in the diet on mammary tumor formation initiated by DMBA was dose de-

	No. of animals killed after carcinogen administration at the following weeks									
	11	12	13	14	15	16	17	18	19	20
DMBA	1	0	0	1	0	1	1	0	0	0
Quercetin-DMBA	0	0	0	0	0	0	0	0	1	0

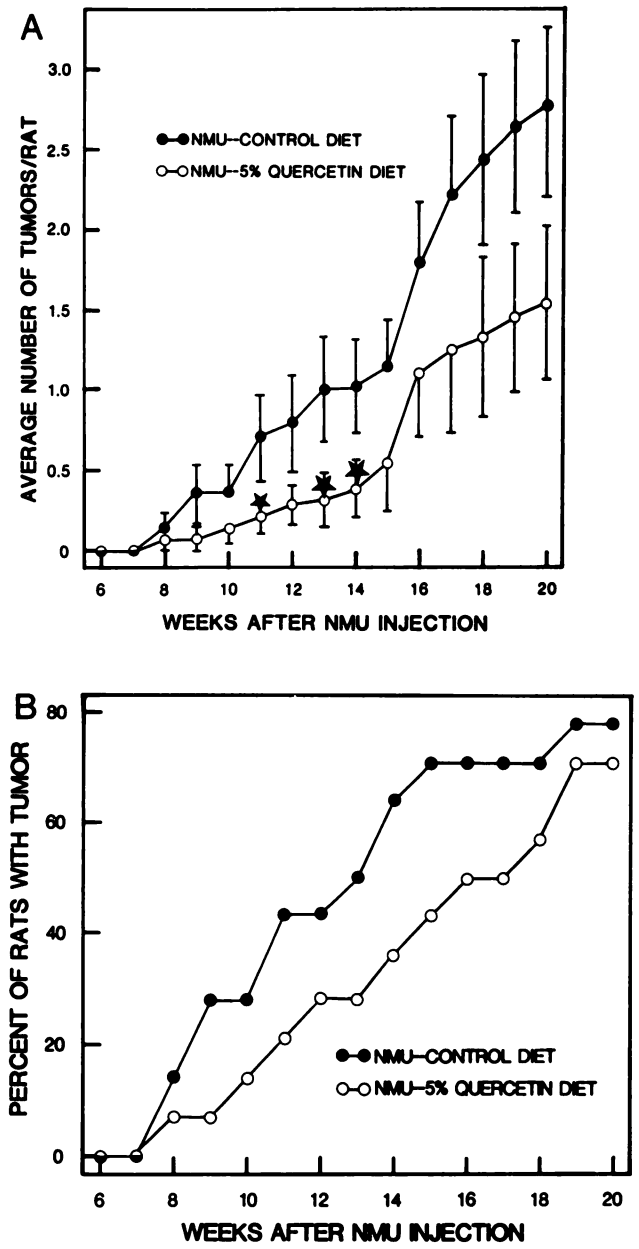


Fig. 5. Effect of dietary quercetin (5%) on the induction of rat mammary tumors by NMU. The rats were started on control (●) or 5% quercetin diet (○) 1 wk prior to i.v. injection of NMU (9.0 mg/rat) and maintained on the diet throughout the length of the experiment. Tumors were sampled—97% were carcinomas in the control, and 93% were carcinomas in the quercetin group. *A*, effect of quercetin on the average number of rat mammary tumors per rat. *Points*, mean of the number of tumors/rats surviving; *bars*, SE. *B*, effect of quercetin on the incidence of rat mammary cancer. The rats with huge palpable tumors were killed as shown below. The fraction of animals surviving the 20-wk period was 14 of 14 rats on control and 11 of 14 on the 5% quercetin diet. ★, values significantly different from control ($P < 0.05$).

	No. of animals killed after carcinogen administration at the following weeks									
	11	12	13	14	15	16	17	18	19	20
DMBA	0	0	0	0	0	0	0	0	0	0
Quercetin-NMU	0	0	1	0	0	1	0	0	1	0

pendent (Figs. 2 and 4). The degree of inhibition of the induction of rat mammary cancer by quercetin was less in the NMU model than the DMBA model, but still significant (Figs. 4 and 5).

The mechanisms involved in the inhibition of the initiation of rat mammary cancer by quercetin are unclear. Quercetin inhibits epidermal cytochrome P-450-dependent monooxygenases (35) and DMBA-DNA adduct formation in SENCAR mice (36). Quercetin has also been shown to inhibit rat and human liver microsome cytochrome P-450-dependent enzymes (27, 28). In the results presented, quercetin diets were started 1 wk prior to direct (NMU) or indirect (DMBA) carcinogen administration. The increased effectiveness of quercetin at inhibiting indirect carcinogen-initiated mammary tumor formation suggests that inhibition of metabolic activation or interaction of the metabolite with DNA could be a possible mechanism of action. Further experimentation would be required to evaluate these hypotheses.

The development of DMBA- and NMU-initiated tumors appears to be hormone dependent (37). There are also limited data on the effects of flavonoids on hormone regulation (38). It is likely that quercetin may be inhibiting the synthesis of the hormone or interfering with hormone-receptor binding. Quercetin could also be blocking some primary events stimulated by the hormone-receptor interaction. Quercetin does appear to inhibit tyrosine protein kinase activity (39, 40) and phosphoinositide phosphorylation (40), both of which have been implicated as necessary for normal mammary growth and development (39, 40).

In summary, we report for the first time that dietary quercetin inhibits the induction of rat mammary cancer induced by either intragastric instillation of DMBA or i.v. injection of NMU. Dietary quercetin inhibited the incidence and multiplicity of rat mammary cancer. In addition, we hypothesize that the natural plant product quercetin may be an inhibitor of cancer induction in other systemic animal models for tissues such as colon, lung, and intestine.

ACKNOWLEDGMENTS

The excellent technical assistance of Shawn W. Laibly is greatly appreciated.

REFERENCES

1. Singleton, V. L. Naturally occurring food toxicants: phenolic substances of plant origin common in risk foods. Adv. Food Res., *27:* 149–242, 1981.
2. International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some food additives and naturally occurring substances. *31:* 33–35, 1983.
3. MacGregor, J. T. Genetic toxicology of dietary flavonoids. Prog. Clin. Biol. Res., *213:* 33–43, 1986.
4. MacGregor, J. T. Genetic and carcinogenic effects of plant flavonoids: an overview. Nutritional and toxicology aspects of food safety. Adv. Exp. Med. Biol., *177:* 497–526, 1900.
5. Stavric, B. Mutagenic food flavonoids. Fed. Proc., *43:* 2454–2458, 1984.
6. Hatcher, J. F., and Bryan, G. T. Factors affecting the mutagenic activity of quercetin for *Salmonella typhimurium* TA98: metal ions, antioxidants, and pH. Mutat. Res., *148:* 13–23, 1985.
7. Bjeldanes, L. F., and Chang, G. W. Mutagenic activity of quercetin and related compounds. Science (Wash. DC), *197:* 577–578, 1977.
8. MacGregor, J. T., and Jurd, L. Mutagenicity of plant flavonoids: structural requirements for mutagenic activity in *Salmonella typhimurium*. Mutat. Res., *54:* 297–309, 1978.
9. Ellinger, C. A., Henika, P. R., and MacGregor, J. T. Mutagenicity of flavones, chromones, and acetophenones in *Salmonella typhimurium*. New structure-activity relationships. Mutat. Res., *135:* 77–86, 1984.
10. Carver, J. H., Carrano, A. V., and MacGregor, J. T. Genetic effects of the flavonols quercetin, kaempferol, and galangin on Chinese hamster ovary cells *in vitro*. Mutat. Res., *113:* 45–60, 1983.
11. van der Hoeven, J. C. M., Bruggeman, I. M., and Debets, F. M. H. Genotoxicity of quercetin in cultured mammalian cells. Mutat. Res., *136:* 9–21, 1984.
12. Pamukcu, A. M., Yalciner, S., Hatcher, J. F., and Bryan, G. T. Quercetin, a rat intestinal and bladder carcinogen present in bracken fern (*Pteridium aquilinum*). Cancer Res., *40:* 3468–3472, 1980.
13. Erturk, E., Hatcher, J. F., Nunoya, T., Pamukcu, A. M., and Bryan, G. T. Hepatic tumors in Sprague-Dawley (SD) and Fischer 344 (F) female rats chronically exposed to quercetin (Q) or its glycoside rutin (R). Proc. Am. Assoc. Cancer Res., *25:* 95, 1984.

14. William, G. M. Food-borne carcinogens. Prog. Clin. Biol. Res., *206:* 73–81, 1986.
15. Saito, F., Shirai, A., Matsushima, T., Sugimura, T., and Hirono, I. Test of carcinogenicity of quercetin, widely distributed mutagen in food. Teratog. Carcinog. Mutagen., *1:* 213–221, 1980.
16. Hosaka, S., and Hirono, I. Carcinogenicity test of quercetin by pulmonary-adenoma bioassay in strain A mice. Gann, *72:* 327–328, 1981.
17. Hirono, I., Ueno, I., Hosaka, S., Takanashi, H., Matsushima, T., Sugimura, T., and Natori, S. Carcinogenicity examination of quercetin and rutin in AC1 rats. Cancer Lett., *13:* 15–21, 1981.
18. Takanashi, H., Aiso, S., Hirono, I., Matsushima, T., and Sugimura, T. Carcinogenicity test of quercetin and kaempferol in rats by oral administration. J. Food Saf., *5:* 55–60, 1983.
19. Aeschbacher, H-U., Meier, H., and Ruch, E. Nonmutagenicity *in vivo* of the food flavonol quercetin. Nutr. Cancer, *4:* 90–98, 1982.
20. Habs, M., Habs, H., Berger, M. R., and Schmahl, D. Negative dose-response study for carcinogenicity of orally administered rutin sulfate in Sprague-Dawley rats. Cancer Lett., *23:* 103–108, 1984.
21. Nakadate, T., Yamamoto, S., Aizu, E., and Kato, R. Effects of flavonoids and antioxidants on 12-*O*-tetradecanoylphorbol-13-acetate caused epidermal ornithine decarboxylase induction and tumor promotion in relation to lipoxygenase inhibition by these compounds. Gann, *75:* 214–222, 1984.
22. Kato, R., Nakadate, T., Yamamoto, S., and Sugimura, T. Inhibition of 12-*O*-tetradecanoyl-phorbol-13-acetate induced tumor promotion and ornithine decarboxylase activity by quercetin: possible involvement of lipoxygenase inhibition. Carcinogenesis (Lond.), *4:* 1301–1305, 1985.
23. Chang, R. L., Huang, M. T., Wood, A. W., Wong, C. Q., Newmark, H. L., Yagi, H., Sayer, J. M., Jerina, D. M., and Conney, A. H. Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of benzo(*a*)pyrene and (±)-7beta,8alpha,-dihydroxy-9alpha, 1alpha-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene on mouse skin and in the newborn mouse. Carcinogenesis (Lond.), *6:* 1127–1133, 1985.
24. Bresnick, E., and Birt, D. Inhibitory effects of the flavonoid, apigenin, on epidermal ornithine decarboxylase and mouse skin tumorigenesis. Proc. Am. Assoc. Cancer Res., *29:* 136, 1988.
25. Nishino, H., Naito, W., Iwashima, A., Tanaka, K., Matsuura, T., Fujiki, H., and Sugimura, T. Interaction between quercetin and Ca^{2+} calmodulin complex: possible mechanism for anti-tumor-promoting action of the flavonoid. Gann, *74:* 311–316, 1984.
26. Huang, M. T., Johnson, E. F., Eberhard, U. M., Koop, D. R., Coon, M. J., and Conney, A. H. Specificity in the activation and inhibition of flavonoids of benzo(*a*)pyrene hydroxylation by cytochrome P-450 enzyme from rabbit liver microsomes. J. Biol. Chem., *256:* 10897–10901, 1981.
27. Buening, M. K., Chang, R. L., Huang, M. T., Chang, R. L., Fortner, J. G., Wood, A. W., and Conney, A. H. Activation and inhibition of benzo(*a*)pyrene and aflatoxin B_1 metabolism in human liver microsomes by naturally occurring flavonoids. Cancer Res., *41:* 67–71, 1981.
28. Lasker, J. M., Huang, M. T., and Conney, A. H. *In vitro* and *in vivo* activation of oxidative drug metabolism by flavonoids. J. Pharm. Exp. Ther., *229:* 162–170, 1984.
29. Wargovich, M. J., Eng, V. W. S., and Newmark, H. L. Inhibition by plant phenols of benzo(*a*)pyrene-induced nuclear aberrations in mammalian intestinal cells: a rapid *in vivo* assessment method. Food Chem. Toxicol., *23:* 47–49, 1985.
30. Huang, M.-T., Wood, A. W., Newmark, H. L., Sayer, J., Yagi, H., Jerina, D. M., and Conney, A. H. Inhibition of the mutagenicity of bay-region diol-epoxides of polycyclic aromatic hydrocarbons by phenolic plant flavonoids. Carcinogenesis (Lond.), *4:* 1631–1637, 1983.
31. Huggins, C. B. Experimental Leukemia and Mammary Cancer. Chicago: University of Chicago Press, 1979.
32. Gullino, P. M., Pettigrew, H. M., and Granthram, F. H. *N*-Nitrosomethylurea as mammary gland carcinogen in rats. J. Natl. Cancer Inst., *54:* 401–414, 1975.
33. Bhatacharyya, G. K., and Johnson, R. A. Statistical Concepts and Methods. New York: Wiley, 1977.
34. Pariza, M. W., and Boutwell, R. K. Historical perspective and energy expenditure in carcinogenesis. Am. J. Clin. Nutr., *45:* 151–156, 1987.
35. Das, M., Mukhtar, H., Bik, D. P., and Bichers, D. R. Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occurring plant phenols. Cancer Res., *47:* 760–766, 1987.
36. Das, M., Khan, W. A., Asokan, O., Bichers, D. R., and Mukhtar, H. Inhibition of polycyclic aromatic hydrocarbon-DNA adduct formation in epidermis and lungs of SENCAR mice by naturally occurring plant phenols. Cancer Res., *47:* 767–773, 1987.
37. Gottardis, M. M., and Jordan, V. C. Animal models to study the therapy of hormone-dependent breast cancer. *In:* V. C. Jordan (ed.), Estrogen/Antiestrogen Action and Breast Cancer Therapy, pp. 283–300. Madison, WI: The University of Wisconsin Press, 1986.
38. Martin, P. M., Horwitz, K. B., Ryan, D. S., and McGuire, W. L. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology, *103:* 1860–1867, 1978.
39. Levy, J., Teuerstein, I., Marbach, M., Radian, S., and Sharoni, Y. Tyrosine kinase activity in the DMBA-induced rat mammary tumor: inhibition by quercetin. Biochem. Biophys. Res. Commun., *123:* 1227–1233, 1984.
40. Sharoni, Y., Teuerstein, I., and Levy, J. Phosphoinositide phosphorylation precedes growth in rat mammary tumors. Biochem. Biophys. Res. Commun., *134:* 876–882, 1986.