Suppression of Intestinal Crypt Cell Proliferation and Aberrant Crypt Foci by Dietary Quercetin in Rats

Jennifer M. Gee, Hiroshi Hara, and Ian T. Johnson

Abstract: Quercetin inhibits proliferation of human gastric and colonic cancer cells in vitro by suppressing mitosis and increasing apoptosis. Quercetin might therefore act as an anticarcinogen in the alimentary tract, but previous findings have been inconsistent. We fed rats quercetin at dietary concentrations of 1, 5, 20, and 50 g/kg. At ≤20 g/kg, we observed a statistically significant reduction in the frequency of crypt cell mitosis in proximal, mid, and distal small intestine and in distal colon, amounting to ~40% of control at 1 g/kg. There was no effect on apoptosis. Quercetin metabolites, but not quercetin aglycone, were detected in plasma of rats fed quercetin at 20 and 50 g/kg. In a second experiment, rats were fed quercetin at 1 g/kg after treatment with 1,2-dimethylhydrazine to induce aberrant crypt foci. In dimethylhydrazine-treated and control rats, crypt cell mitosis was suppressed at 48 h and 42 days after injection, and there was a statistically significant reduction in the number of aberrant crypts and larger aberrant crypt foci (>4 crypts/focus) in the distal colon of treated animals. These findings demonstrate that quercetin can inhibit intestinal crypt cell proliferation in vivo, but the effect diminishes as the level of dietary exposure increases. At low concentrations, dietary quercetin inhibits induction of aberrant crypts by a mechanism that does not involve increased crypt cell apoptosis.

Introduction

The flavonoids are a large and complex group of phenolic compounds found in human foods (1). Collectively, they contribute much to the flavor and color of vegetables and fruits, and they account for most of the dissolved solids in beverages such as tea, coffee, and wine. All flavonoids possess a three-ring structure containing two aromatic centers (A and B rings) and a central oxygenated heterocyclic moiety (C ring). Many of the most common flavonoids in human foods exhibit a range of biological properties, including pro- and antioxidant activities and inhibition of key intracellular enzymes and signaling cascades (2). Quercetin, myricetin, and kaempferol are consumed predominantly in the form of water-soluble glycosides and are partially absorbed from the diet (1). In humans, although flavonoid metabolites are detectable in the circulation soon after consumption, the epithelial tissues of the gastrointestinal tract are likely to be exposed to significantly higher local concentrations (3).

Many phenolic substances exhibit anticarcinogenic activity in the two-stage mouse skin model (4). Quercetin and other flavonoids inhibit proliferation of human gastric cancer cells (5) and colonic carcinoma cells (6–8) often by blocking the G1-to-S transition of the cell cycle and inducing apoptosis. These effects have prompted sustained interest in the possibility that quercetin might act as an anticarcinogen in the alimentary tract. Deschner et al. (9) investigated the effects of dietary supplements of quercetin (1, 5, or 20 g/kg diet) and one of its glycosides, rutin (20 or 40 g/kg diet), on the induction by azoxymethane (AOM) of crypt hyperproliferation, dysplasia, and tumors in mouse colon. There was no observed effect of the flavonoids on the rate or spatial distribution of crypt cell proliferation in the control mice, but in those given AOM, consumption of quercetin (20 g/kg) or rutin (40 g/kg) was associated with the suppression and spatial relocation of crypt cell hyperproliferation induced by the carcinogen and a reduction in tumors. In contrast, Pereira et al. (10) reported a dose-dependent enhancement of tumors by exposure to dietary quercetin (16.8 and 33.6 g/kg diet) in a rat model. Matsukawa et al. (11) explored the ability of quercetin (20 g/kg diet) to reduce induction of aberrant crypt foci (ACF) in rats treated with AOM and subjected to mild restraint stress and noted that induction of ACF was reduced in the stressed and unstressed rats given quercetin compared with their respective controls. Recently, Yang et al. (12) reported that dietary supplementation with quercetin and rutin increased apoptosis in the colonic crypts of mice treated with AOM, and this was associated with a reduction in focal areas of dysplasia. However, quercetin itself induced focal areas of dysplasia in some animals in the absence of AOM. Mahmoud et al. (13) studied the effect of quercetin and rutin on the development of tumors in an APC knockout mouse model but observed no protective effect at a dietary supplementation level of 20 g/kg.
In view of conflicting reports on the effects of quercetin in animal models of intestinal carcinogenesis, coupled with evidence that the compound causes DNA damage in vitro (14) and displays procarcinogenic effects at sites beyond the alimentary tract (15), we sought to clarify the effects of quercetin on crypt cytokinetics and induction of neoplasia in rat intestinal epithelia. In this study, we describe the relationship between dietary quercetin level and its effects on crypt cell proliferation and apoptosis in the small and large intestine. We show that quercetin inhibits crypt cell mitosis at low concentrations, but the effect diminishes as the level of exposure increases. We also confirm that dietary exposure to quercetin, at a level that causes inhibition of crypt cell mitosis, leads to a reduction in the numbers of aberrant crypts and larger ACF induced by treatment with 1,2-dimethylhydrazine (DMH) in the distal rat colon.

**Materials and Methods**

### Animals and Diets

For Experiment 1, 25 male Wistar rats (150–180 g) were obtained from a licensed animal supplier and housed singly in wire-bottomed cages in an environmentally controlled animal facility. All aspects of animal care complied with the ethical guidelines and technical requirements of the UK Home Office. The rats were fed a powdered semisynthetic control diet (Table 1) for 1 wk ad libitum and then randomly assigned to five groups of five animals each, one group remaining on the control diet and the rest being transferred to diets containing 1, 5, 20, or 50 g/kg quercetin (Table 1). Weight gain and food intake were determined daily throughout the feeding period. After 7 days the rats were deeply anesthetized by intraperitoneal injection of sodium barbiturate and killed by cervical dislocation. After laparotomy, a blood sample (2 ml) was taken from the vena cava and mixed with sodium citrate (100 µl, 100 g/l), and the pH of the cecal contents was determined by insertion of a pH microelectrode directly through a small slit in the wall. The entire small and large intestines were then removed, and small segments (1 cm) of proximal (at 10% of total small intestine length from the pylorus), mid (50%), and distal (95%) small bowel, cecum, and mid (50%) and distal (95%) large bowel were placed in fixative (75% ethanol–25% glacial acetic acid) for microscopy. The contents of the cecum and colon were collected and frozen for subsequent extraction and chemical analysis. A plasma fraction was prepared from the blood sample by centrifugation (3,000 g, 10 min) and stored at –20°C for later assay.

Experiment 2 was carried out to determine the effects of dietary quercetin on the induction of ACF. Eighty male Wistar rats were obtained and housed as described above and maintained on the basal diet for 7 days. The rats were then randomly subdivided into 2 groups of 40 rats each. One group received two subcutaneous injections of DMH 7 days apart, and the remainder were given two injections of saline on the same occasions. All rats continued to receive basal diet until the second DMH or saline injection. At 18 h after the second injection, 20 rats from each group were placed on a diet containing 0.1% quercetin, and the other half remained on the basal diet. Ten animals from each group were sacrificed as described previously 30 h later, and segments of proximal small intestine, cecum, and large bowel were collected and transferred to fixative for microscopy, as described for Experiment 1. The remaining rats were killed after a further 6 wk. Segments of proximal small bowel and cecum were collected as described above, and the large bowel was divided into two sections and fixed in its entirety, with care taken to keep the samples as straight as possible.

### Analysis of Crypt Cell Proliferation

The frequencies of mitotic and apoptotic figures within the crypts were assessed using the isolated crypt technique, as previously described (16). Samples of fixed intestine were hydrated in HCl, hydrolyzed, and stained with Feulgen’s reagent, and short strips of whole crypts were dissected out under a low-power dissecting microscope. The dissected crypts were mounted in acetic acid–water (45:55) under coverslips and examined under medium-power bright-field light microscopy for mitoses and apoptotic bodies. All nuclei in prophase, metaphase, anaphase, and telophase were recorded as mitoses. Apoptotic nuclei were identified as single dense spherical or crescent-shaped bodies or as more disperse granular assemblies of dense chromatin (16). The lengths of crypts were measured, and the positions of each mitotic and apoptotic nucleus were recorded by reference to an eyepiece graticule. A total of 10 crypts for mitosis and 30 crypts for apoptosis were counted from samples taken at each site in the gastrointestinal tract, and a mean value was calculated.

The spatial distribution of mitotic events within crypts was expressed as a geometric mean. Using the eyepiece graticule, each mitotic profile was assigned to 1 of 10 equally spaced zones. Data were treated iteratively, dividing the pro-

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**Table 1. Composition of Diets**

<table>
<thead>
<tr>
<th>Component</th>
<th>Control Diet</th>
<th>Quercetin-Containing Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucreose</td>
<td>298</td>
<td>298</td>
</tr>
<tr>
<td>Starch</td>
<td>260</td>
<td>259, 255, 240, or 210</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn oil</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Cellulose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0</td>
<td>1, 5, 20, or 50 g (replacing starch)</td>
</tr>
</tbody>
</table>

*a*: Values are g/kg.

*b*: Concentrations of vitamins and minerals in complete diets have been described previously (45).
files per zone by the total profiles per crypt and multiplying by the zone position counting from the base. Summing these values gave the "mitotic position index," defined as the geometric mean position of maximum frequency of mitosis expressed to two decimal places.

**Aberrant Crypt Foci**

For the enumeration of ACF, the proximal and distal segments of the colon were cut open longitudinally to form a sheet, stained in their entirety with Feulgen’s reagent as described above, and mounted on glass slides under transparent film. The slides were examined under medium-power microscopy with transmitted illumination. Aberrant crypts were identified by their morphological characteristics (16), defined as elevated focal lesions containing one or more distinct lumina, with enlarged openings and thickened epithelial linings. ACF were counted and recorded. In the distal segment, the numbers of individual crypts and larger foci containing more than four crypts and the total area (mm²) occupied by the lesions were evaluated.

**Preparation of Blood Samples and Analysis of Cecal and Colonic Contents**

Quercetin was extracted from plasma samples with 4 vol of acidified ethanol (50 mmol/l HCl in ethanol) according to the method of Manach et al. (17). Plasma (200 µl) and acidic ethanol (800 µl) were mixed in an Eppendorf tube and centrifuged (10,000 g, 2 min). The supernatant extract was immediately stored at −20°C to prevent the degradation of metabolites. Samples of fecal material from the large bowel were placed in preweighed plastic vials, weighed, and stored at −20°C before they were freeze-dried, reweighed, and ground to a powder using a solid plastic rod.

The homogenized fecal powder (30–100 mg) was placed in a screw-cap tube and extracted with hot methanol using a modification of the method of Griffiths (18). Briefly, methanol (4 ml) was added to the powdered dry material and heated in a tightly sealed vial at 60°C for 1 h with vigorous shaking every 10 min. After centrifugation, the supernatant was transferred to a fresh tube, and the solvent was evaporated using a sample concentrator at 60°C under N₂ gas. The dried material was dissolved in 1–2 ml of acidified ethanol (50 mmol/l HCl in ethanol). All solutions were filtered (0.2-µm pore size) before determination of quercetin and tentative identification of metabolites by high-performance liquid chromatography [Prodigy column, 5-µm ODS, 250 × 4.6 mm, Phenomenex; 10 µl loading; room temperature; flow rate 1 ml/min; 98:2:0.1 water-tetrahydrofuran-trifluoroacetic acid (Solvent A), acetonitrile (Solvent B)]. The column was calibrated using quercetin standards (50 and 500 µmol/l in acidic ethanol), and in the case of metabolites, concentrations were expressed as quercetin equivalents after combination of two peaks detected at a wavelength of 370 nm.

**Statistics**

Results are expressed as means ± SE. The significance of differences between means was assessed using one- or two-way analysis of variance, with Tukey’s test or Duncan’s multiple range test for the secondary analysis. All calculations were carried out using Minitab statistical software (State College, PA).

**Results**

**Experiment 1**

**Consumption and assimilation of quercetin:** Consumption of quercetin did not adversely affect body weight gain. For animals (n = 5 per group) fed the control diet or diets containing 0.1%, 0.5%, 2%, and 5% quercetin diet, body weight gains were 4.89 ± 0.48, 5.92 ± 0.31, 5.45 ± 0.64, 4.6 ± 0.35, and 4.94 ± 0.45 (SE) g/day, respectively. Similarly, average daily food intakes were not significantly different at any level of supplementation: 22.7 ± 1.90, 21.9 ± 1.28, 22.1 ± 0.58, 21.5 ± 0.72, and 22.0 ± 0.42 (SE) g/day, respectively. The highest dose of quercetin did, however, increase the wet mass of the contents (Table 2; P < 0.05), and at all except the lowest level of incorporation, it also significantly reduced the pH of the contents (Table 2). There was a positive relationship between the level of quercetin in the diet and its concentration in cecal and colonic contents (Fig. 1, left).

Two principal metabolites were identified in the plasma of rats fed quercetin, and these were tentatively identified as a quercetin diglucuronide and methylhydroxyphenyl acetic acid. The relationship between dietary quercetin and the molar sum of these metabolites in the plasma is shown in Fig. 1, right. No aglycone was detected in the circulation, and no quercetin metabolites were present in the lumen of the large bowel.

**Crypt cell proliferation:** Consumption of quercetin at <50 g/kg reduced the frequency of crypt cell mitosis in the proximal, mid, and distal small intestine (Fig. 2, left) and the distal colon (Fig. 2, right) relative to animals fed control diet. The differences were highly significant at all sites in the small intestine and distal colon (P < 0.001). The lowest level of consumption of quercetin did not adversely affect body weight gain.

Table 2. Effect of Dietary Quercetin on Mass and pH of Large Intestinal Contents

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Wet Mass of Contents, g/100 g body wt</th>
<th>Cecum</th>
<th>Colon</th>
<th>Cecal pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.86 ± 0.023†</td>
<td>0.49 ± 0.052*</td>
<td>7.75 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.1%</td>
<td>0.91 ± 0.041†</td>
<td>0.68 ± 0.039*</td>
<td>7.58 ± 0.05*†</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>0.88 ± 0.028†</td>
<td>0.62 ± 0.028*</td>
<td>7.44 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>1.00 ± 0.071†</td>
<td>0.47 ± 0.034*</td>
<td>7.05 ± 0.06†</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>1.45 ± 0.056*</td>
<td>0.36 ± 0.068*</td>
<td>6.84 ± 0.03†</td>
</tr>
</tbody>
</table>

a: Values are means ± SE (n = 5). Rats were fed test diets for 1 wk. b: Mean values not sharing a common superscript (*, †, ‡) within a vertical column differ significantly (P < 0.001).
quercetin (1 g/kg) was associated with the greatest reduction in mitoses per crypt. In contrast, there was a tendency for mitosis in the cecum to increase with dietary levels of quercetin ($P = 0.056$). There were no significant intergroup differences in crypt length at these sites (data not shown).

**Apoptosis:** There was no overall effect of quercetin intake on apoptosis at any site in the small or large intestine, apart from a small but significant increase above control values in the distal colon in rats fed 20 g/kg quercetin: $2.4 \pm 0.75$ compared with $0.4 \pm 0.24$ at 50 g/kg and undetectable levels in all other groups ($P < 0.01$).

**Experiment 2**

**Food intake and body weight:** Neither food intake nor body weight was significantly affected by DMH treatment or dietary intervention. Final body weights of rats in-

![Figure 1](image1.png)

**Figure 1.** Effect of increasing concentrations of dietary quercetin (0–50 g/kg) on quercetin concentration in cecal (open squares) and colonic (filled squares) contents (left) and sum of the 2 principal metabolites of quercetin detected in plasma (right). Values are means ± SE for 5 rats. *, Significantly higher than colonic ($P < 0.05$).

![Figure 2](image2.png)

**Figure 2.** Relationship between dietary quercetin (0–50 g/kg) and crypt cell mitosis in proximal, mid, and distal small intestine (SI, left) and cecum, proximal colon, and distal colon (right). Values are means ± SE for 5 rats. For each intestinal location, mean values not sharing a common superscript (a, b, c) differ significantly ($P < 0.05$).
jected with DMH and killed 48 h later were 263.5 ± 4.54 and
267.5 ± 4.57 (SE) g for the control and test diets groups, re-
spectively. Similarly, animals receiving a sham treatment
and consuming control and test diets for the same period
weighed 275.4 ± 5.69 and 271.2 ± 5.25 g, respectively. The
average body mass of animals sacrificed at the end of the 6-
wk feeding period was 467.2 ± 6.89 and 455.6 ± 4.96 g for
DMH-treated rats and 461.6 ± 13.44 and 457.8 ± 8.49 for the
saline-injected group.

Crypt cell proliferation: As in Experiment 1, crypt cell
proliferation was significantly lower in animals consuming
quercetin at 1 g/kg than in animals fed the control diet. This
difference was evident in the DMH-treated rats and saline-
treated controls after only 30 h of exposure to the test diet
(Fig. 3, left). At 6 wk after the second DMH injection, the
lower levels of crypt cell mitosis were still evident at all sites
other than the cecum, where there was no difference between
the groups (Fig. 3, right).

In animals treated with DMH, the spatial distribution of
mitoses along the crypt axis appeared shifted toward the
crypt orifice in the mid and distal colon (Fig. 4, left; data for
mid colon not shown), and this was confirmed by the mitotic
cell position indexes, which were significantly higher in
DMH-treated animals than in controls (P < 0.001). Dietary
intervention with quercetin had no significant effect on this
parameter. There were no significant differences in the spa-
tial distribution of mitoses in the cecum (Fig. 4, right).

Apoptosis: The frequencies of apoptosis induced by the
DMH treatment in the mid and distal colons of rats fed
quercetin and control diets were not significantly different
(Fig. 5).

Aberrant crypt foci: After 42 days, aberrant crypts
were detected throughout colons of all DMH-treated rats but
were absent from control animals treated with saline. These
lesions were much less frequent in the proximal than in the
distal segment of the colon (Table 3), and a full analysis of the
total numbers and areas of aberrant crypts and foci was im-
practical. In the distal colon, however, the numbers of single
aberrant crypts (P < 0.05), the total area of ACF (P < 0.05),
and the numbers of larger ACF (>4 crypts/focus) were signif-
icantly lower in animals fed quercetin than in control animals
(Table 3). The total number of ACF was also lower, but the
difference was not quite statistically significant at the 5%
level (P < 0.063).

Discussion

In the present study, consumption of quercetin at ~25 mg/
day was associated with a reduction in crypt cell prolifera-
tion of ~50% in the small intestine and distal colon of
healthy rats. This suppression of normal cell turnover began
within 30 h of administration and was still detectable 42
days later. Quercetin has previously been shown to suppress
mitosis in vitro and in vivo (19), but our findings provide ev-
idence for a large and sustained suppression of crypt cell
proliferation throughout much of the alimentary tract. One
explanation for the previous inconsistencies in published ac-
counts of the effects of quercetin on colorectal carcino-
genesis is provided by our observation that the antiproliferative
effects of quercetin were dose related and that the sup-
pressed rate of crypt cell mitosis tended to return to control
levels as the level of dietary supplementation was increased.
We observed a progressive decrease in pH in animals fed
quercetin, and those fed the highest levels showed a signifi-
cant increase in the mass of contents. These findings suggest that unabsorbed quercetin was utilized by the cecal microflora, yielding metabolites that were probably available for uptake into the circulation (17).

The antimitotic effects of quercetin and other flavonoids in vitro are well established and have been demonstrated for cell lines derived from a range of tissues, including those of the alimentary tract (20–22), as well as melanoma cells (23) and mammary (24) and prostate tissues (25). There are, however, relatively few reports describing suppression of cell proliferation by quercetin in vivo. One example is the report by Makita et al. (26), who described the effects of orally administered chalcone, 3-hydroxychalcone, and quercetin on oral carcinogenesis induced with 4-nitroquinoline-1-oxide in a rat model. Treatment with all three of these flavonoids induced a reduction in the bromodeoxyuridine labeling index of the tongue squamous epithelium, and these antiproliferative effects were associated with a significant reduction in carcinomas of the tongue.

Although there are some notable dissenters (27), it is generally believed that a high rate of mitosis in an otherwise healthy tissue increases the risk of accumulation of somatic mutations (28,29). In rapidly proliferating cell populations, less time is available for DNA repair (30,31),

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>Control diet</th>
<th>Quercetin diet</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACF</td>
<td>1.8 ± 0.39</td>
<td>0.78 ± 0.28</td>
<td>0.051</td>
</tr>
<tr>
<td>Distal segment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>227 ± 37.7</td>
<td>132 ± 35.6</td>
<td>0.034</td>
</tr>
<tr>
<td>ACF</td>
<td>89.2 ± 14.9</td>
<td>55.3 ± 14.6</td>
<td>0.063</td>
</tr>
<tr>
<td>ACF with &gt;4 AC</td>
<td>20.4 ± 3.68</td>
<td>11.6 ± 3.63</td>
<td>0.041</td>
</tr>
<tr>
<td>ACF area, mm$^2$</td>
<td>3.43 ± 0.656</td>
<td>1.77 ± 0.595</td>
<td>0.025</td>
</tr>
</tbody>
</table>

$^{a}$ Values are means ± SE ($n = 10$) as nontransformed data. Dietary quercetin was administered at 1 g/kg for 6 wk. $P$ values were evaluated after logarithmic transformation. AC, aberrant crypt; ACF, AC foci; DMH, dimethylhydrazine.
and there is a greater probability of somatic recombination, causing cells heterozygous for a tumorigenic mutation to become homozygous (32). Conversely, a more quiescent intestinal mucosa may be less susceptible to the progression of carcinogenesis after chemical induction with DMH. The reduction in numbers of aberrant crypts and larger ACF in animals treated with quercetin, which was observed in Experiment 2, supports this hypothesis. The relatively rapid metabolism and the timing of dietary supplementation with quercetin 18 h after the second injection ensured that there could be no possibility of any effects due to differences in carcinogen metabolism or a difference in proliferation during the formation of DNA adducts. Our results suggest that the suppression of mitosis by quercetin may have inhibited the development of ACF during the long period after induction of damage by DMH, during which the tissue recovers its physical integrity and rates of mitosis and apoptosis are stabilized. The mechanism of aberrant crypt formation during this period is poorly understood, but the relative rates of mitosis and apoptosis may be critical to the development of these lesions.

The relative importance of crypt cell proliferation and apoptosis was addressed directly by Pereira (33), who explored the effects of several different classes of compounds on tumors, ACF, cell proliferation, and apoptosis in the AOM-rat model for colorectal carcinogenesis. Some compounds appeared to reduce ACF numbers by inhibiting mitosis, while others acted by inducing apoptosis. Compounds that induced an increased rate of crypt cell apoptosis were the most effective at suppressing the induction of malignant tumors. This is consistent with the work of Chang et al. (34), who explored the anticarcinogenic effects of fish oil in the AOM model and concluded that the most important mechanism of action was induction of apoptosis and differentiation in the crypt epithelium. However, in the present study, we were unable to detect any consistent evidence of increased apoptosis in the intestinal crypts of animals fed quercetin at any level and, therefore, conclude that the suppression of mitosis was the major factor in reducing the incidence of preneoplastic lesions.

A complex network of signal transduction pathways, several of which may be modulated by quercetin, mediates promotion of neoplasia after exposure to a carcinogen. There is, for example, substantial evidence to show that phenolic compounds can inhibit protein kinase C, which is involved in the upstream regulation of mitosis (35). Quercetin has also been shown to specifically inhibit a class of cAMP-sensitive casein kinases also associated with the control of cell proliferation in uterine tissue (36) and colonic mucosa (37). In addition, quercetin has been shown to downregulate other enzymes associated with control of cell division in mammalian tissues, including mitogen-activated protein kinase and cyclin D1 (38) and oncogenic proline-directed protein kinase FA (39). The quercetin-sensitive casein kinase of the rat colon is particularly interesting, because it shows a spatial distribution along the crypt that is consistent with a role in normal cell proliferation (37). Casein kinase II is upregulated in various transformed cell lines (40) and in human colorectal tumors in vivo (41).

It is difficult to extrapolate directly from such in vitro evidence to feeding studies, because, in addition to quercetin aglycone absorbed from the lumen, intestinal epithelial cells are exposed to a complex range of quercetin metabolites derived from transformations mediated by mucosal and hepatic enzymes (42). When isolated intestinal tissue is exposed to quercetin in vitro, the free aglycone does not appear on the serosal side of the intestinal wall, but several metabolites are detectable, principally quercetin 3- and 7-glucuronides, together with smaller quantities of other glucuronides and sulfates (43). A similar pattern of metabolites is observed in the mucosal tissue under these circumstances, together with some free quercetin. In the present study, we detected quercetin metabolites in the circulation of rats fed the compound at the higher concentrations. We did not attempt to isolate quercetin or its metabolites from the mucosal tissue, but it is reasonable to conclude that, after uptake of the free aglycone, intestinal epithelial cells are exposed to a cocktail of metabolites, some of which will be derived from hepatic metabolism and transported to the mucosa via the vascular bed. The cells lining the gastrointestinal tract are also exposed to endogenous metabolites derived from resecretion into the bile and, more distally, to increasing concentrations of bacterial metabolites. Relatively little is known about the biological activities of quercetin glucuronides and other metabolites, but it has been shown, for example, that some retain the ability to inhibit lipooxygenase activity in vitro (44). Perhaps increasing exposure of the epithelium to biologically active quercetin metabolites circulating in the vascular bed causes the biphasic effect of quercetin consumption on crypt cell proliferation that we have observed in the present study. Further dose-response studies on the complex variety of compounds derived from mucosal, hepatic, and bacterial metabolism of flavonoids are required before the nutritional and pharmacological effects of the parent compounds can be properly understood.

**Acknowledgments and Notes**

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