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Effect of Grape Seed Proanthocyanidins on Colon Aberrant Crypts and Breast Tumors in a Rat Dual-Organ Tumor Model

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Abstract: *Cancers of the colon and breast are two of the most prevalent cancers in developed countries. The present experiments were conducted to determine the influence of several dietary doses of grape seed proanthocyanidins on 7,12-dimethylbenz[*a*]anthracene-induced mammary tumorigenesis and azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) formation in a dual-organ tumor model. In addition, the effects of the grape seed proanthocyanidins on liver cytochrome* P-*450 1A and 2E1 and glutathione* S-*transferase activities and on colonic ornithine decarboxylase activity were examined to determine possible mechanisms of action. Feeding female rats diets containing 0.1–1.0% grape seed proanthocyanidins was associated with a significant 72–88% inhibition of AOM-induced aberrant crypt foci formation and a 20–56% inhibition of ornithine decarboxylase activity in the distal third of the colon. Feeding the grape proanthocyanidins resulted in no significant effect on the activity of liver cytochrome* P-*450 2E1. There was no effect of feeding these doses of proanthocyanidins on 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumorigenesis. This lack of action on mammary tumorigenesis in part may be due to lack of effect of dietary proanthocyanidins on the liver carcinogen-metabolizing enzymes cytochrome* P-*450 1A and glutathione* S-*transferase. These results indicate that grape polyphenolics warrant further evaluation as potential colon cancer chemopreventive agents.*

Introduction

Breast and colon cancers are two of the most prevalent cancers in developed societies, with considerable research attention being focused on their prevention (1). It has been estimated that a considerable portion of risk for these two cancers is related to diet or nutritional factors. In particular, increased fruit and vegetable consumption has been reported to be associated with a reduction in risk for numerous cancers including colon and breast cancer (1–3). There is, as well, great interest in identifying and testing synthetic and naturally occurring compounds that could be used as cancer chemopreventive agents (4,5). Oligomeric proanthocyanidins are naturally occurring antioxidants, present in fruits, vegetables, nuts, and seeds, that possess a variety of biological activities (6). In grapes, these proanthocyanidins are found in greatest concentrations in the lignified portions of grape clusters, particularly in the seed, and are known to contribute to the organopeptic properties of wines (7). Recently, it was reported that a proanthocyanidin fraction from grape seeds was effective as an inhibitor of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced and 12-*O*-tetradecanoylphorbol 13-acetate-promoted skin tumorigenesis in mice (8). The action of this grape fraction in inhibiting skin tumorigenesis was determined to be due, in part, to its effectiveness as a suppressor of ornithine decarboxylase (ODC) and myeloperoxidase activities, suggestive of an antiproliferative and anti-inflammatory mechanism of action. Its action as an inhibitor of ODC activity was associated with a prior inhibition of epidermal protein kinase C activity (9). Grape and similar proanthocyanidins exhibit not only anticancer but also cardioprotective actions, which are attributed to their capacity to inhibit oxidation, modulate signal transduction pathways, and alter activities of carcinogen-metabolizing enzymes (10–25). Thus these naturally occurring compounds exhibit a variety of biological actions that make them attractive candidates for chronic disease prevention strategies, including cancer chemoprevention.

To further characterize the anticancer efficacy of this proanthocyanidin fraction from grape seeds in breast and colon cancers, studies were performed using a dual-organ model for mammary carcinogenesis and colon preneoplasia (26,27). This dual-organ model was developed by others to provide a short-term carcinogenesis assay for screening chemopreventive agents for their relative ability to inhibit the development of mammary and/or colon tumors (26). The model has been characterized (26) and used to examine the influence of dietary fat on the two cancers (27). The present studies were conducted to determine the effectiveness of dietary grape seed proanthocyanidins (GSP) in inhibiting DMBA-induced rat mammary tumorigenesis and the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF). The initiation of rat mammary tumorigenesis by

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DMBA is a widely used model to study mammary carcinogenesis and its modulation by dietary agents (28). ACF, which can be induced by dimethylhydrazine (DMH) or AOM, a metabolite of DMH, are reported to be preneoplastic lesions that have the potential to form colon tumors in the rat (29–31). Foci with multiple aberrant crypts (ACM), in particular, have been determined to correlate well with the development of colorectal tumors, especially those in more advanced stages (32). Therefore, for the present experiments, the influence of several dietary doses of this grape seed fraction on DMBA-induced mammary tumorigenesis and AOM-induced colonic ACF formation was determined. In addition, the effects of this fraction on liver cytochrome *P*-450 1A and 2E1 activities, on glutathione *S*-transferase (GST) activities, and on colonic ODC activity were examined to determine possible mechanisms of action.

Materials and Methods

Animals and Diets

Female Sprague-Dawley rats at 30 days of age were purchased from Harlan (Indianapolis, IN) and housed separately in wire-bottom, stainless-steel cages in rooms with controlled temperature and lighting. Animals were acclimated to the control AIN-93G diet for six days. This control diet consisted of (% by wt) 20 vitamin-free casein, 3 L-cystine, 10 sucrose, 13.2 maltodextrin, 7 soybean oil, 5 cellulose, 1 AIN-93G vitamin mix, 3.5 AIN-93G mineral mix, and 37.3 cornstarch. All diet ingredients were purchased from Harlan-Teklad (Madison, WI). GSP fraction (Traconol) was obtained from Traco Labs (Champaign, IL). As determined by high-performance liquid chromatography (unpublished observations), essentially 98% of the GSP has been identified as a mixture of monomers (3.8%, as catechin and epicatechin), oligomers of two to seven units (58.9%), and polymers of eight or more units (37.3%). GSP was added to the diets at the expense of cornstarch.

Experiment 1: For Experiment 1, the effect of feeding GSP on DMBA-induced rat mammary tumorigenesis and on the formation of AOM-induced rat colon ACF formation was examined. At 36 days of age, animals were randomized into four groups (n = 25/group) and fed diets containing GSP at 0%, 0.1%, 0.5%, or 1.0% (by wt). At 50 days of age, animals were administered DMBA (37 mg/kg body wt ig in corn oil). Subsequently, AOM was administered to the rats (15 mg/kg ip in acidified water) at 57 and 64 days of age. Beginning five weeks after DMBA treatment, animals were palpated weekly. The study was terminated at 25 weeks after DMBA administration. Mammary tumors were removed and classified histopathologically (34). Colons were removed, fixed in formalin, and examined for the presence of ACF as previously described (29). Livers also were removed at termination of the study, and samples were prepared for enzyme analyses as described below.

Four additional animals per group were fed the diets supplemented with 0%, 0.1%, 0.5%, and 1.0% GSP from 36 to 50 days of age and administered DMBA as described above. At 51 days of age, the livers from these rats were removed for determination of the effect of GSP feeding for two weeks on liver enzymes involved in carcinogen activation (cytochromes *P*-450 1A and 2E1) and detoxification (GST).

Experiment 2: For Experiment 2, rats at 36 days of age (n = 10/group) were fed 0%, 0.1%, 0.5%, and 1.0% GSP and dosed with DMBA and AOM according to the tumor study dosing protocol described for Experiment 1. At two weeks after the last AOM dose, colonic mucosa and livers were removed. This study was performed to determine the effect of GSP feeding on liver carcinogen-metabolizing enzyme activities (cytochromes *P*-450 1A and 2E1 and GST) and colonic ODC activity.

Enzyme Assays

Liver samples were homogenized in 0.15 M KCl, pH 7.5, and centrifuged at 9,000 *g*. The resulting supernatant was centrifuged at 100,000 *g*, and the subsequent cytosolic and microsomal fractions were stored at −80°C until use. Cytosolic GST activity was determined according to the method of Habig and co-workers (35). Microsomal cytochrome *P*-450 1A activity was determined as ethoxyresorufin *O*-deethylase (EROD) activity using the procedure of Pohl and Fouts (36). Microsomal cytochrome *P*-450 2E1 activity was determined as *p*-nitrophenol hydroxylase activity according to the procedures of Reinke and Moyer (37). For colonic ODC assays, mucosa samples were removed by scraping, then homogenized in 50 mM sodium phosphate (pH 7.2) containing 5 mM dithiothreitol, 1 mM EDTA, and 0.4 mM pyridoxal-5′-phosphate, and subsequently centrifuged at 30,000 *g* for 30 minutes. ODC activity was determined using the supernatant by measuring the release of CO_2 from L-[1-^{14}C]ornithine as described by Gendimenico and colleagues (38).

Statistical Methods

Means among three or more groups were evaluated for statistical significance by analysis of variance using the least significant difference test for post hoc comparisons ($p < 0.05$). Tumor incidence curves of treatment groups were evaluated for statistically significant differences compared with controls by life-table survivor analysis.

Results

For Experiment 1, the feeding of GSP at up to 1% in the diet did not result in significant differences in weight gain among groups at the end of the tumor study (Table 1). There were also no significant differences in animal weights among treatment groups for the subset of rats sacrificed at 51

Table 1. Effect of Feeding GSP on Rat Growth in Experiment 1[a,b]

Group	Animal Weight, g				
	36 days	51 days[c]	57 days[d]	64 days[e]	225 days[f]
Control	l22.5 ± 1.8	177.5 ± 2.2	193.7 ± 2.3	208.3 ± 3.2	302.4 ± 3.2
GSP					
0.1%	122.5 ± 1.6	178.4 ± 2.4	194.2 ± 2.6	212.7 ± 2.9	311.4 ± 3.4
0.5%	124.0 ± 2.9	175.3 ± 1.9	195.5 ± 1.9	195.5 ± 2.2	300.5 ± 2.9
1.0%	123.4 ± 1.3	172.3 ± 2.5	191.9 ± 2.5	214.0 ± 3.1	308.7 ± 3.3

a: Values are means ± SE. GSP, grape seed proanthocyanidins.
b: Values are not significantly different ($p > 0.05$) among treatment groups.
c: One day after dimethylbenz[*a*]anthracene dosing.
d: First azoxymethane dose.
e: Second azoxymethane dose.
f: Termination of study.

days of age in Experiment 1, in animal weights for Experiment 2, or in liver weights for all studies (data not shown).

For Experiment 1, dietary concentrations of GSP up to 1% were not associated with significant changes in DMBA-induced palpable mammary tumor incidence, in adenocarcinoma multiplicity, or in tumor mass (Figure 1, Table 2). In contrast, provision of dietary GSP at all levels was associated with significant decreases in the number of colonic ACF as well as the number of ACF with multiple crypts (>4), henceforth, referred to as ACM (Table 3). Total colon ACF incidence was 93%, 78%, 69%, and 69% for animals fed 0%, 0.1%, 0.5%, and 1.0% GSP, respectively. Total colon ACF multiplicity was decreased by 62%, 67%, and 77% for animals fed the diets supplemented with 0.1%, 0.5%, and 1.0% GSP, respectively, compared with controls ($p < 0.05$ for all treatments compared with controls). Likewise, total colon ACM numbers decreased significantly ($p < 0.05$) by 65%, 65%, and 78% for the 0.1%, 0.5%, and 1.0% GSP-supplemented diets, respectively, compared with controls. Most of the ACF-inhibitory action of the GSP in the colon was due to the inhibition of ACF and ACM formation in the distal third of the colon. In the distal third of the colon, ACF formation decreased 76%, 72%, and 88%, and ACM formation decreased 94%, 78%, and 89%, for animals fed 0.1%, 0.5%, and 1.0% GSP in the diet, respectively, compared with controls (all treatments significantly different at $p < 0.05$ compared with controls).

AOM is an intermediate metabolite of the colon carcinogen DMH and is metabolized by cytochrome *P*-450 2E1 and, possibly, *P*-450 1A as well as by the phase II carcinogen detoxifying enzyme GST (39,40). To determine whether the inhibition of AOM-induced ACF formation in Experiment 1 might be due to altered metabolism of AOM to its ultimate carcinogenic form, cytochrome *P*-450 2E1 activity (measured as *p*-nitrophenol hydroxylase activity) and cytochrome *P*-450 1A activity (measured as EROD activity) were determined in three sets of livers. These liver samples were obtained from animals fed the GSP-supplemented diets for 2 weeks (from a subset of animals sacrificed at 51 days of age in Experiment 1), 8 weeks (from animals sacrificed 2 wk after AOM dosing in Experiment 2), and 27 weeks (from animals at termination of Experiment 1). GSP feeding for 27 weeks was associated with significant 53%, 49%, and 37% decreases in liver *p*-nitrophenol hydroxylase activities for animals fed the 0.1%, 0.5%, and 1.0% GSP diets, respectively, compared with the control value of 6.2 ± 0.5 (SE) nmol *p*-nitrocatechol/mg protein. However, GSP feeding had no effect on liver *p*-nitrophenol hydroxylase activities for animals fed the diets for two or eight weeks. For these latter two feeding periods, liver *p*-nitrophenol hydroxylase activities for animals fed the GSP-supplemented diets changed on average ±20% compared with controls ($p > 0.05$). There also was no significant effect of feeding GSP on liver EROD activity for any of the time periods from 2 to 27 weeks in duration. In general, EROD activity was changed on average by ±17% for animals fed GSP-supplemented diets compared with controls ($p > 0.05$). With respect to phase II enzyme activity, liver GST activity was increased by GSP feeding, although this was dependent on duration of feeding. Animals fed diets containing 0%, 0.1%, 0.5%, and 1.0% GSP for two weeks (from Experiment 1) exhibited liver GST activities of 279.7 ± 54.1, 233.1 ± 56.9, 241.2 ± 15.7, and 455.2 ± 38.8 nmol/min/mg protein, respectively (each group different from 1.0% group at $p < 0.05$). However, for animals fed the diets for 8 or 27 weeks, no significant influence on liver GST activities was observed. In general, liver GST activities in rats fed the GSP-supplemented diets for 8 or 27 weeks changed on average by ±63% among controls and treatment groups ($p > 0.05$).

To determine whether GSP feeding might be acting to inhibit proliferative events important for ACF formation, the ODC activities of colonic mucosa from animals in Experiment 2 were determined (Table 4). For extracts prepared from the proximal and middle thirds of the colons, there was a trend toward decreased ODC activity as dietary concentration of GSP was increased, although the differences were not significant. However, for the distal colon, ODC activity was significantly decreased for all levels of dietary GSP compared with controls. ODC activity decreased significantly ($p < 0.05$) by 20%, 26%, and 56% for animals fed the 0.1%,

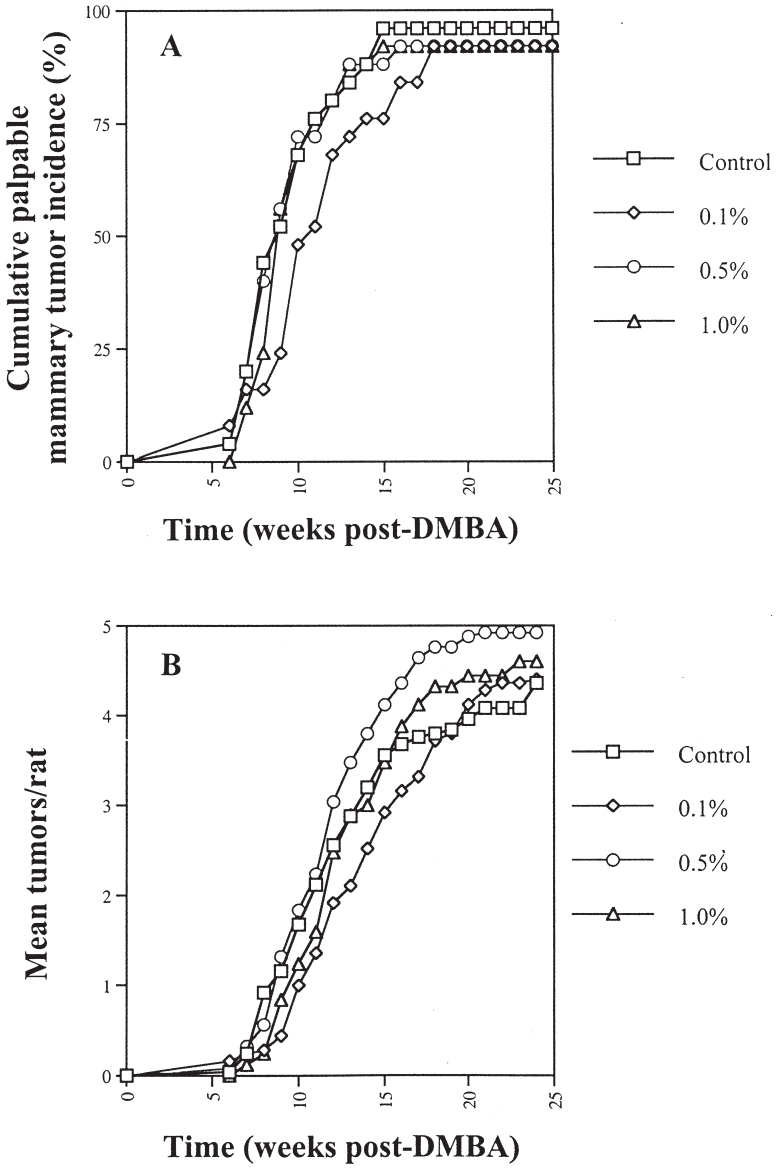

Figure 1. Effect of feeding grape seed proanthocyanidins at various concentrations (% by wt) on dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumor incidence (A) and multiplicity (B).

Table 2. Effect of Feeding GSP on Final Mammary Tumor Incidence, Multiplicity, and Mass[a]

Group	Final Incidence, %	Adenocarcinomas per Rat[b]	Fibroadenomas and Adenomas per Rat[b]	Tumor Mass,[b] g
Control	96	5.6 ± 0.7	0.6 ± 0.2	20.7 ± 5.6
GSP				
0.1%	92	4.4 ± 0.6	0.5 ± 0.2	23.9 ± 6.7
0.5%	92	5.2 ± 0.7	0.6 ± 0.2	25.8 ± 6.3
1.0%	92	5.4 ± 0.8	0.4 ± 0.2	21.4 ± 5.8

a: Values are means ± SE at termination.
b: Values are not significantly different ($p > 0.05$) among treatment groups.

Table 3. Effect of Feeding GSP on Colonic Aberrant Crypt Formation[a,b]

Group	*n*	Proximal/Mid Colon		Distal Colon		Total	
		ACF	ACF > 4	ACF	ACF > 4	ACF	ACF > 4
Control	15	4.3 ± 1.4*	1.5 ± 0.6*	9.8 ± 2.7*	3.6 ± 1.4*	14.1 ± 3.1*	5.1 ± 1.6*
GSP							
0.1%	18	3.0 ± 1.0*	1.6 ± 0.9*	2.4 ± 0.7†	0.2 ± 0.1†	5.4 ± 1.6†	1.8 ± 1.0†
0.5%	13	1.9 ± 0.9*	1.0 ± 0.8*	2.7 ± 1.8†	0.8 ± 0.5†	4.6 ± 2.6†	1.8 ± 1.3†
1.0%	16	2.1 ± 0.9*	0.7 ± 0.4*	1.2 ± 0.5†	0.4 ± 0.3†	3.3 ± 1.2†	1.1 ± 0.5†

a: Values are means ± SE. ACF, aberrant crypt foci.
b: Values among treatment groups sharing different symbols (*, †) are significantly different at $p < 0.05$.

0.5%, and 1.0% GSP diets, respectively, compared with controls.

Discussion

The objective of this study was to determine the effects of feeding diets supplemented with 0.1%, 0.5%, and 1.0% GSP on DMBA-induced rat mammary carcinogenesis and on AOM-induced ACF formation. The results indicate that diets supplemented with GSP did not significantly inhibit mammary carcinogenesis. The reason for no effect of GSP feeding on DMBA-induced mammary tumorigenesis is not known. It is possible that the GSP constituents are not readily absorbed and, thus, are not present in sufficient amounts at the mammary gland to modulate tumorigenesis. However, Bagchi and co-workers (6) reported that an oligomeric GSP extract is bioavailable. In addition, a report by Manach and colleagues (41) indicated that significant concentrations of dietary flavonoid metabolites were detected in the blood of rats. Yet, consistent with our data, others (42–44) have determined that catechins and other flavonoids had only a weak or no inhibitory effect on DMBA-induced mammary carcinogenesis. Our data demonstrating a lack of inhibition of liver EROD activity in rats fed the GSP-containing diets for two weeks before DMBA dosing indicate that GSP is unlikely to be substantially decreasing DMBA bioactivation by cytochrome *P*-450 1A. The effect of feeding plant phenolics on cytochrome *P*-450 1A activity has been observed by others to be variable and to depend on the specific flavonoid examined (45). We also observed that feeding the GSP-supplemented diets for two weeks had no significant effect on liver GST activity, except at the highest GSP level of 1.0%. Others (46,47) have reported variable responses in liver GST activity after feeding plant phenolics to rodents, which may be explained in part by differences in specific flavonoid, dose, and duration of feeding. Thus, for our studies, although the constituents present in our GSP might be bioavailable, they may be inactive in the mammary gland or liver in blocking formation of DNA-binding DMBA metabolites and mammary tumor initiation or inactive in the DMBA-treated mammary gland and mammary tumors in suppressing the process of tumor promotion.

Table 4. Effect of Feeding GSP on ODC Activity in Colonic Mucosa[a,b]

Group	ODC Activity	
	Proximal/mid colon	Distal colon
Control	3.0 ± 0.2*	6.6 ± 0.3*
GSP		
0.1%	3.0 ± 0.1*	5.3 ± 0.4†
0.5%	1.8 ± 0.6*	4.9 ± 0.3†
1.0%	1.9 ± 0.5*	2.9 ± 0.3‡

a: Values are means ± SE expressed as nmol $^{14}CO_2$ produced/h/mg protein. ODC, ornithine decarboxylase.
b: Values among treatment groups sharing different symbols (*, †, ‡) are significantly different at $p < 0.05$.

In contrast to our findings with mammary gland tumorigenesis, our data clearly indicate that feeding diets supplemented with GSP at levels as low as 0.1% is associated with a significant inhibition of ACF formation in the distal colon. We are not aware of other reports assessing GSP feeding in the AOM-induced ACF model. However, it has been demonstrated that plant phenolics such as quercetin, diosmin, and hesperidin are effective inhibitors of AOM-induced colonic ACF formation or of colon tumorigenesis (48,49), although quercetin also has been reported to stimulate colon tumorigenesis induced by AOM (42). For our study, animals were fed the GSP-containing diets through the initiation and promotion stages of AOM-induced ACF formation. Although inhibition of cytochrome *P*-450 2E1 at the time of AOM dosing could lead to inhibition of ACF formation, we observed no inhibition by dietary GSP of liver cytochrome *P*-450 2E1 or 1A activity after two or eight weeks of feeding. Liver GST activity also increased significantly only for rats fed the 1.0% diets at the end of Experiment 1, making it unlikely that increased GST activity could explain the decrease in ACF formation at the lower doses of GSP. It is possible that feeding the GSP-supplemented diets is altering the activities of cytochrome *P*-450 2E1 and 1A and GST in the colonic mucosa. This was not determined in the present studies. In contrast to these enzyme activities, we did observe that all dietary GSP concentrations were associated with a significant inhibition of ODC activity in the distal third of the colon. Although the effect of feeding GSP on colon ODC activity has not been reported, feeding other plant phenolics has been reported to affect colon ODC activity

(49). Proanthocyanidins have been reported to inhibit chemically induced ODC activity in mouse skin (8,9,50) as well. ODC is important in cell growth and differentiation and has been suggested to play an important role in tumor promotion (51). Thus, in the present report, the inhibition of AOM-induced ACF and ACM formation for animals consuming GSP in part is explained by the inhibition of ODC activity in the colonic mucosa.

In summary, feeding female rats diets containing 0.1–1.0% GSP was associated with a significant inhibition of AOM-induced ACF formation and ODC activity in the distal third of the colon. No effect of feeding these doses of proanthocyanidins on DMBA-induced rat mammary tumorigenesis was observed, which may be explained in part by lack of effect of feeding GSP on liver carcinogen-metabolizing enzymes. These results indicate that GSP warrants further evaluation as a potential colon cancer chemopreventive agent.

Acknowledgments and Notes

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References

1. World Cancer Research Fund: *Food Nutrition and the Prevention of Cancer: A Global Perspective*. Washington, DC: Am Inst Cancer Res, 1997.
2. Gandini, S, Merzenich, H, Robertson, C, and Boyle, P: Meta-analysis of studies of breast cancer risk and diet: the role of fruit and vegetable consumption and intake of associated micronutrients. *Eur J Cancer* **36,** 636–646, 2000.
3. Franceschi, S, Parpinel, M, LaVecchia, C, Favero, A, Talamini, R, et al.: Role of different types of vegetables and fruits in the prevention of cancer of the colon, rectum and breast. *Epidemiology* **9,** 338–341, 1998.
4. Singletary, K: Diet, natural products and cancer chemoprevention. *J Nutr* **130,** 465S–466S, 2000.
5. Kelloff, G, Crowell, J, Steele, V, Lubet, R, Malone, W, et al.: Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J Nutr* **130,** 467S–471S, 2000.
6. Bagchi, D, Bagchi, M, Stohs, S, Das, D, Ray, S, et al.: Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* **148,** 187–197, 2000.
7. Kovac, V, Alonso, E, and Revila, E: The effect of adding supplementary quantities of seeds during fermentation on the phenolic composition of wines. *Am J Enol Viticul* **46,** 363–367, 1995.
8. Bomser, J, Singletary, K, Wallig, M, and Smith, M: Inhibition of TPA-induced tumor promotion in CD-1 mouse epidermis by a polyphenolic fraction from grape seeds. *Cancer Lett* **135,** 151–157, 1999.
9. Bomser, J, Singletary, K, and Meline, B: Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin ornithine decarboxylase and protein kinase C by polyphenolics from grapes. *Chem Biol Int* **127,** 43–59, 2000.
10. Gali, H, Perchellet, E, Gao, X, Karchesy, J, and Perchellet, J: Comparison of the inhibitory effects of monomeric, dimeric and trimeric procyanidins on the biochemical markers of skin tumor promotion in mouse epidermis *in vivo*. *Planta Med* **60,** 235–239, 1993.
11. Tebib, K, Rouanet, J, and Besançon, P: Effect of grape seed tannins on the activities of some rat intestinal enzyme activities. *Enzyme Protein* **48,** 51–60, 1994.
12. Gao, X, Perchellet, E, Gali, H, Rodriguez, L, Hemingway, R, et al.: Antitumor-promoting activity of oligomeric proanthocyanidins in mouse epidermis *in vivo*. *Int J Oncol* **5,** 285–292, 1994.
13. Lanninghamfoster, L, Chen, C, Chance, D, and Loo, G: Grape extract inhibits lipid peroxidation of human low-density lipoproteins. *Biol Pharm Bull* **18,** 1347–1351, 1995.
14. Polya, G, Wang, B, and Foo, L: Inhibition of signal-regulated protein kinases by plant-derived hydrolysable tannins. *Phytochemistry* **38,** 307–314, 1995.
15. Tabib, K, Rouanet, J, and Besançon, P: Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chem* **59,** 135–141, 1996.
16. Clifford, A, Ebeler, S, Ebeler, J, Bills, N, Hinrichs, S, et al.: Delayed tumor onset in transgenic mice fed an amino acid-based diet supplemented with red wine solids. *Am J Clin Nutr* **64,** 748–756, 1996.
17. Koide, T, Kamei, H, Hashimoto, Y, Kojima, T, and Hasegawa, M: Antitumor effect of hydrolyzed anthocyanin from grape rind and red rice. *Cancer Biother Radiophar* **11,** 273–277, 1996.
18. Castonguay, A, Gali, H, Perchellet, E, Gao, X, Boukharta, M, et al.: Antitumorigenic and antipromoting activities of ellagic acid, ellagitannins and oligomeric anthocyanin and procyanidin. *Int J Oncol* **105,** 367–373, 1997.
19. Bagchi, D, Garg, A, Krohn, R, Bagchi, M, Tran, M, et al.: Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. *Res Commun Mol Pathol Pharmacol* **95,** 179–189, 1997.
20. Huynh, H, and Teel, R: Effects of pycnogenol on the microsomal metabolism of the tobacco-specific nitrosamine NNK as a function of age. *Cancer Lett* **132,** 135–139, 1998.
21. Fitzpatrick, D, Bing, B, and Rohdewald, F: Endothelium-dependent vascular effects of pycnogenol. *J Cardiovasc Pharmacol* **32,** 509–515, 1998.
22. Zhao, J, Wang, J, Chen, Y, and Agarwal, R: Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3′-gallate as the most effective antioxidant constituent. *Carcinogenesis* **20,** 1737–1745, 1999.
23. Ye, X, Krohn, R, Liu, W, Joshi, S, Kuszynski, C, et al.: The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. *Mol Cell Biochem* **196,** 99–108, 1999.
24. Joshi, S, Kuszynski, C, Benner, E, Bagchi, M, and Bagchi, D: Amelioration of the cytotoxic effects of chemotherapeutic agents by grape seed proanthocyanidin extract. *Antioxid Redox Signal* **1,** 563–570, 1999.
25. Ray, S, Kumar, M, and Bagchi, D: A novel proanthocyanidin IH636 grape seed extract increases *in vivo* Bcl-X_L expression and prevents acetaminophen cell death in mouse liver. *Arch Biochem Biophys* **369,** 42–58, 1999.
26. Skivapurkur, N, Tang, Z, Frost, A, and Alabaster, O: A rapid dual-organ rat carcinogenesis bioassay for evaluating the chemoprevention of breast and colon cancer. *Cancer Lett* **100,** 169–179, 1996.
27. Tang, Z, Shivapurkur, N, Frost, A, and Alabaster, O: The effect of dietary fat on the promotion of mammary and colon cancer in a dual-organ rat carcinogenesis model. *Nutr Cancer* **25,** 151–159, 1995.
28. Welsch, C: Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res* **45,** 3415–3443, 1985.
29. Bird, R: Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett* **37,** 147–151, 1987.
30. Pretlow, T, O'Riordan, M, Pretlow, T, and Stellato, T: Aberrant crypts in human colonic mucosa: putative preneoplastic lesions. *J Cell Biochem* **16G,** 55–62, 1992.

31. Wargovich, M, Chen, C, Jiminez, A, Steele, V, Velasco, M, et al.: Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. *Cancer Epidemiol Biomarkers Prev* **5,** 355–360, 1996.
32. McLellin, E, Medline, A, and Bird, R: Sequential analysis of the growth and morphological characteristics of aberrant crypt foci: putative neoplastic lesions. *Cancer Res* **51,** 5270–5274, 1991.
34. Baker, H, Lindsey, J, and Weisbroth (eds): *The Laboratory Rat: Biology and Diseases*. New York: Academic, 1979, vol 1, pp 361–363.
35. Habig, W, Pabst, M, and Jakoby, W: Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249,** 7130–7139, 1974.
36. Pohl, R, and Fouts, J: A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal Biochem* **107,** 150–155, 1980.
37. Reinke, L, and Moyer, M: *p*-Nitrophenol hydroxylation. A microsomal oxidation which is highly inducible by ethanol. *Drug Metab Dispos* **13,** 548–552, 1985.
38. Gendimenico, G, Capetola, R, Rosenthale, M, McGuire, J, and Mezick, J: Retinoid modulation of phorbol ester effects in skin. *Methods Enzymol* **190,** 346–352, 1990.
39. Sohn, O, Ishazaki, H, Yang, C, and Fiala, E: Metabolism of azoxymethane, methylazoxymethanol, and *N*-nitrosodimethylamine by cytochrome P450IIE1. *Carcinogenesis* **12,** 127–131, 1991.
40. Delker, D, Bammler, T, and Rosenberg, D: A comparative study of hepatic and colonic metabolizing enzymes in inbred mouse lines before and after treatment with the colon carcinogen, 1,2-dimethylhydrazine. *Drug Metab Dispos* **24,** 408–413, 1996.
41. Manach, C, Texier, O, Morand, C, Crespy, V, Regerat, F, et al.: Comparison of the bioavailability of quercetin and catechin in rats. *Free Radic Biol Med* **27,** 1259–1266, 1999.
42. Pereira, M, Grubbs, C, Barnes, L, Li, H, Olson, G, et al.: Effects of phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[*a*]anthracene-induced mammary cancer in rats. *Carcinogenesis* **17,** 1305–1311, 1996.
43. Rogers, A, Hafer, L, Iskander, Y, and Yang, S: Black tea and mammary gland carcinogenesis by 7,12-dimethylbenz[*a*]anthracene in rats fed control or high fat diets. *Carcinogenesis* **19,** 1269–1273, 1998.
44. Tanaka, H, Hirose, M, Kawabe, M, Sato, M, Takesada, Y, et al.: Post-initiation inhibitory effects of green tea catechins on 7,12-dimethylbenz[*a*]anthracene-induced mammary gland carcinogenesis in female Sprague-Dawley rats. *Cancer Lett* **116,** 47–52, 1997.
45. Canivenc-Lavier, M, Verevault, M, Totis, M, Siess, M, Magdalou, J, et al.: Comparative effects of flavonoids and model inducers on drug-metabolizing enzymes in rat liver. *Toxicology* **114,** 19–27, 1996.
46. Breinholt, V, Lauridsen, S, and Dragsted, L: Differential effects of dietary flavonoids on drug metabolizing and antioxidant enzymes in female rats. *Xenobiotica* **29,** 1227–1240, 1999.
47. Kawabata, K, Tanaka, T, Honjo, S, Kakumoto, M, Hara, A, et al.: Chemopreventive effect of dietary flavonoid morin on chemically-induced rat tongue carcinogenesis. *Int J Cancer* **83,** 381–386, 1999.
48. Yamane, T, Hagiwara, N, Tateishi, M, Akachi, S, Kim, M, et al.: Inhibition of azoxymethane-induced colon carcinogenesis in rats by green tea polyphenol fraction. *Jpn J Cancer Res* **84,** 1007–1009, 1993.
49. Tanaka, T, Makita, H, Kawabata, K, Mori, H, Kakumoto, M, et al.: Chemoprevention of azoxymethane-induced rat colon carcinogenesis by the naturally-occurring flavonoids, diosmin and hesperidin. *Carcinogenesis* **18,** 957–965, 1997.
50. Chen, G, Perchellet, E, Gao, X, Newell, S, Heminway, R, et al.: Ability of *m*-chloroperoxybenzoic acid to induce the ornithine decarboxylase marker of skin tumor promotion and inhibition of this response by gallotannins, oligomeric proanthocyanidins, and their monomeric units in mouse epidermis *in vivo*. *Anticancer Res* **15,** 1183–1189, 1995.
51. Sharma, S, Stutzman, J, Kelloff, G, and Steele, V: Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer Res* **54,** 5848–5855, 1994.