Procyanidin from Black Beans (*Phaseolus vulgaris*) Inhibits Nutrient and Electrolyte Absorption in Isolated Rat Ileum and Induces Secretion of Chloride Ion$^{1,2}$

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ABSTRACT Dietary tannins are reported to impair the absorption of nutrients and minerals in whole animals and in semi-isolated intestinal preparations. The present studies investigated the effect of purified procyanidin from black beans (*Phaseolus vulgaris*) on tissue electrical parameters, isotopic Na$^+$ and Cl$^-$ fluxes and Na$^+$-dependent absorption of labeled glucose by isolated rat ileum. In short-circuited ileal preparations, 0.5–2 g/L procyanidin (PC) inhibited Na$^+$ and Cl$^-$ absorption and stimulated Cl$^-$ secretion, with consequent increases in short circuit current ($I_{sc}$), total tissue conductance and transepithelial voltage. The effect was not blocked by indomethacin (20 μmol/L). Also, PC significantly inhibited the glucose-dependent and phlorizin-sensitive component of $I_{sc}$; a similar result was obtained for the alanine-dependent fraction of $I_{sc}$. In everted ileal sacs PC inhibited Na$^+$-dependent uptake of labeled glucose, but not passive uptake, by a noncompetitive mechanism. The effects of PC are reminiscent of those of recognized intestinal secretagogues and suggest that the antinutrient effects of condensed tannins involve stimulation of intestinal secretion at the expense of absorption. The results argue against use of black bean broth or cooking liquor in rehydration media for treatment of secretory diarrhea. J. Nutr. 126: 1688–1695, 1996.

INDEXING KEY WORDS:

• tannins • legumes • antinutrients • rats • glucose absorption

Beans and legumes are consumed throughout the world and constitute a major source of protein in many developing countries. The food value of these staples may be affected by the presence of several types of antinutrients in the seed coat, including tannins. Tannins are defined as water-soluble phenolic compounds with the ability to complex proteins (Swain and Bate-Smith 1962). A legume-based diet may deliver as much as 2.5 g tannin · person$^{-1}$ · d$^{-1}$ (Rao and Prabhavathi 1982).

To date, the effects of tannins on gut function and somatic growth have been attributed mainly to their recognized inhibition of digestive enzymes (Griffiths and Moseley 1980, Horigome et al. 1988, Tamir and Alumot 1969) or reduction of protein digestibility by complexation and denaturation (Aw and Swanson 1985, Elias et al. 1979). Of most relevance to the present studies is a report that crude or partly purified extracts of seed coats of *Phaseolus vulgaris* and *Vicia faba*, containing mainly polyphenols, inhibit sugar absorption in rat jejunum perfused in situ (Barcina et al. 1984, Motilva et al. 1983). Inhibition of sugar absorption has important implications for the inclusion of black and colored beans in the diets of children in developing countries.

The present studies focus on the polyphenol procyanidin (PC)$^5$, which may account for as much as 4% of the dry mass of the seed coat of black beans (Elias et al. 1979) and which concentrates in bean broth, a common weaning food in Guatemala that has been suggested as an oral rehydration agent to treat diarrhea (Bressani et al. 1985).


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$^5$Abbreviations used: $G_{t}$, total tissue conductance; $I_{sc}$, short-circuit current; PC, procyanidin; $V_{oc}$, open circuit voltage.
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![Graph of electric current (Iec) over time](image)

**FIGURE 1** (A) short circuit current (Iec), (B) open circuit current (Voc) and (C) total tissue conductance (Gt) in control and procyandin (PC)-treated tissue pairs from the same rat. Ileal tissues were mounted in two Ussing chambers, and 2 g/L PC was added at 6 min. Electrical parameters were measured for 30 min. Data are means ± SEM for tissue pairs from 10 animals. Procyandin-treated tissue electrical parameters were significantly different from control at 10, 20 and 30 min.

In the normal ileum, sodium and chloride are absorbed while bicarbonate is secreted [Field 1979]. Both absorptive and secretory processes go on simultaneously, but normally, absorption overbalances secretion. Secretory diarrhea, defined as an excessive loss of fluid and electrolytes [Binder 1979, Hamilton 1985] in stool occurs when solute and water secretion overbalance absorption. Known agents causing net secretion include bacterial enterotoxins such as cholera toxin and *Escherichia coli* enterotoxin and are major contributors to the epidemic of diarrheal disease. Diarrhea is associated with slowed growth rate, decreased weight for age [Mata et al. 1977] and increased death rate among children [Jelliffe 1968]. The resulting dehydration is a major cause of death among children in developing countries [Jelliffe 1968].

Oral rehydration formulas to treat dehydration in-clude electrolytes and glucose and act by stimulating absorptive processes [Jelliffe 1968]. When glucose and other nutrients are actively absorbed, the coupled sodium absorption draws fluid from the intestinal lumen to the serosa (blood side). Any substance that interferes with nutrient absorption, or even causes net secretion, may antagonize rehydration and exacerbate diarrhea.

The results indicate that in isolated rat ileal sheets mounted in Ussing chambers or as everted sacs, PC both inhibits Na⁺-dependent nutrient absorption and electrolyte absorption and induces electrolyte secretion. These effects could contribute to the general anti-nutritional effect of bean seed coats. Also, these effects suggest that dietary PC could exacerbate secretory diarrheas. Therefore, the use of beans or bean broth, or other tannin-containing foods, in oral rehydration therapy for diarrhea may require further consideration.

**MATERIALS AND METHODS**

**Procyandin extraction and purification.** Procyandin was extracted from the hulls of *Phaseolus vulgaris* (cv. Black Beauty) obtained from CENEX (Othello, WA) using the method of Arzt [1984]. Bean hulls were extracted three times in acetone:water [80:20, 15 min with stirring]. The extract was centrifuged for 5 min at 2000 × g. The supernatant was rotoevaporated at 30°C to remove the acetone, freeze-dried and solubilized in ~50 mL of 95% (v/v) ethanol and then layered on a column of Sephadex LH-20 (Sigma Chemical, St. Louis, MO). Smaller molecular weight phenols were eluted with 95% (v/v) ethanol and discarded. Procyandin were eluted with acetone:water (70:30). The eluate was rotoevaporated and freeze-dried. The yield was 98% as determined by vanillin-HCl assay [Burns 1971] using catechin as the standard. The purified PC was stored frozen and desiccated until the day of use.

**Gut preparations.** Sprague-Dawley rats (150–300 g) were obtained from the Washington State University Lab Animal Resource Center and cared for under National Research Council guidelines. Rats of either sex were used; no differences were noted in the results from males vs. females. For experiments, fed rats were anesthetized lightly with ether and injected intraperitoneally with pentobarbital (50 mg/kg). The terminal ileum (2–15 mm from the cecum) was removed and rinsed with bicarbonate Ringer's solution at room temperature. Ileum segments 1–2 cm long and free of Peyer's patches were opened along the mesentery and mounted as flat, unstripped sheets between washers coated with silicone grease and containing pins to maintain tissue alignment. The washer and tissue assemblies were mounted between Ussing chambers (Schultz and Zalusky 1964), which are used to study electrolyte transport in isolated tissues. Bathing solution was recircu-
were time the (Isc) made to both final solution below. Stock (Sigma)

\[
\text{Na}^{+}\text{MS} \quad \text{Na}^{+}\text{SM} \quad \text{Na}^{+}\text{Net} \\
\begin{array}{ccc}
\mu A/cm^2 \\
\text{Control}^3 & 402.0 & 332.4 & 69.7 \\
\text{PC-treated}^4 & 312.3 & 310.9 & 21.4 \\
\end{array}
\]

\[
\begin{array}{ccc}
\text{Cl}^{-}\text{MS} & \text{Cl}^{-}\text{SM} & \text{Cl}^{-}\text{Net} \\
\mu A/cm^2 \\
\text{Control} & 343.0 & 289.4 & 53.6 \\
\text{PC-treated} & 316.2 & 332.3 & -18.8 \\
\end{array}
\]

\[
\begin{array}{c}
\text{Isc} \\
\text{Conductance} \\
\text{Residual} \\
\mu A/cm^2 \\
\text{flux}^2 \\
\text{ms/cm}^2 \\
\end{array}
\]

\[
\begin{array}{c}
45.8 \\
\pm 3.7 \\
8.0 \\
\pm 10.7 \\
\end{array}
\]

1 Values are means ± SEM, n = 8.
2 Residual flux is defined as I_{sc} = |J_{Na}^{Net} - J_{Cl}^{Net}| and represents the net secretion of anion and/or the net absorption of cation.
3 Control solutions were Ringer's with 9 mmol/L of mannitol on the mucosal side and 9 mmol/L of glucose on the serosal side. A control flux rate was determined a 30-min tissue stabilization period and a 20-min isotope equilibration period.
4 The PC-treated flux rate was determined 15 min after introduction of 2 g/L PC in mannitol-Ringer's solution to the mucosal solution.
5 The P-values were obtained by paired t-test between control and PC-treated flux periods in the same tissue. NS = P > 0.05.

Bathing solutions. The basic bathing solution [bicarbonate Ringer's] consisted of [mmol/L] Na^+, 140; K^+, 5.2; Ca^{2+}, 1.2; Mg^{2+}, 1.2; Cl^−, 119.8; HCO_3^−, 25; HPO_4^{2−}, 2.4; H_2PO_4^−, 0.4 [pH 7.4]. In addition, the mucosal side reservoir contained 9 mmol/L of glucose and the serosal side 9 mmol/L of mannitol, except as noted below. Procyanidin dissolved in mannitol Ringer's solution was added to the mucosal half-chamber to the final concentrations indicated, control tissues received Ringer's solution. In some experiments, indomethacin (Sigma Chemical) dissolved in 95% ethanol was added to both sides of the tissue to a final concentration of 20 μmol/L; control chambers received ethanol only. Stock solutions of procyanidin and indomethacin were made up immediately before use.

**TABLE 1** Short circuit current (I_{sc}), total tissue conductance and sodium, chloride and residual fluxes in rat ileum in the presence and absence of 2 g/L procyanidin (PC)^1

**FIGURE 2** The effect of indomethacin pretreatment on procyanidin (PC)-stimulated increase in short circuit current (I_{sc}) in control and procyanidin (PC)-treated tissue pairs from the same rat. Procyanidin (2 g/L) was added to tissues pretreated for 25 min with indomethacin (20 mmol/L) in ethanol carrier or ethanol carrier alone (control). Data are means ± SEM for tissue pairs from three animals. Treatment means were not significantly different from control means at any time point.

**FIGURE 3** A representative experiment showing the method of measuring the effect of procyanidin (PC) on glucose-stimulated, phlorizin-sensitive short circuit current (I_{sc}) in control and procyanidin (PC)-treated tissue pairs from the same rat. Either PC in ethanol carrier (to a final concentration of 2 g/L in this experiment; concentrations used in the series ranged from 0.1 to 2 g/L) or ethanol carrier alone was added to tissue pairs at time zero (A, arrow). At 30 min, 3 mmol/L glucose was added to the mucosal reservoir and 3 mmol/L mannitol to the serosal reservoir of each tissue (B, arrow). When stimulation of I_{sc} by glucose peaked, phlorizin (0.5 mmol/L) was added to the mucosal reservoir (C, arrow). Data from the complete series of PC doses are shown in Figure 4.
**Transepithelial Na\(^+\) and Cl\(^-\) fluxes.** Lumen-to-blood and blood-to-lumen unidirectional fluxes of Na\(^+\) or Cl\(^-\) were measured simultaneously using the methods of Schultz and Zalusky (1964) in two sets of half-chambers using adjacent pieces of intestine from the same animal. Briefly, at 30 min after mounting the tissue pairs, appropriate quantities of either \(^{22}\)Na or \(^{36}\)Cl (New England Nuclear, Boston, MA) were added to the mucosal or serosal side of the mounted tissues, and 100-mL samples were taken from the opposite half-chamber at 15-min intervals thereafter. After the control flux interval, PC (2 g/L) was added to the mucosal side and a second flux interval collected. In analyzing the data, 20–30 min were allowed for isotopic equilibration and at least 15 min were allowed for \(I_{sc}\) to reach a new steady state after addition of PC.

The effect of 2 g/L PC on glucose-dependent components of the transepithelial fluxes of Na\(^+\) or Cl\(^-\) was measured by first determining the flux rates over an interval of 15–30 min in Ringer's solution with 2 g/L PC, but without glucose or mannitol. Subsequently, glucose was added to the mucosal side and mannitol to the serosal side to final concentrations of 9 mmol/L, and a second flux interval of 10–25 min was collected. Preliminary experiments showed that these substrate concentrations are \(\approx 3\) times greater than those necessary to achieve maximal stimulation of \(I_{sc}\). Of 16 flux experiments, two were discarded because of unstable \(I_{sc}\), an indication of tissue damage or poor mounting.

**Glucose uptake.** Glucose uptake rates were measured using the everted sleeve technique (Karason and Diamond 1983). D-glucose was selected in preference to glucalogues to avoid possible differences in transport kinetics (Syme and Levin 1980). Before removal, the ileum was rinsed in situ with 10 mL of ice-cold Ringer's solution gassed with 95% \(\text{O}_2\):5% \(\text{CO}_2\). The ileum was everted on a glass rod and cut into 1.5-mm-long segments. These were mounted on plexiglas rods 5 mm in diameter and 20 cm in length and with grooves cut at 1 mm and 11 mm from the tip. Six such preparations were obtained from each rat and held in gassed, ice-cold Ringer's solution.

Uptake measurements were conducted in the 2nd h after anesthetization of the rat. Each tissue was placed in a 16-mm test tube held at 37°C and gassed with 95% \(\text{O}_2\):5% \(\text{CO}_2\) and stirred with a magnetic stirring bar at 2000 \(\times\) g. First, the tissues were preincubated for 15 min in glucose Ringer's solution with or without 1 g/L PC. Then the tissues were transferred for the 2-min uptake interval to medium containing \(\Delta\)-[\(^{14}\)C][U]lucose as a marker for total glucose uptake and L-[\(^{3}\)H][N]glucose as a marker for nonspecific passive uptake and adhering solution. Immediately after beginning the uptake interval, 100 mL of medium was taken for determination of initial tracer activities.

At the end of the uptake interval, tissues were rinsed for 20 sec in ice-cold Ringer's solution with stirring, blotted on filter paper, cut from the rods and placed in

\[\text{FIGURE 4} \text{ Dose-response curves for the effect of procyan} \]

\[\text{idin (PC)} \text{ concentration on glucose-stimulated, phlorizin-sensitive tissue electrical parameters in control and PC-treated tissues pairs from the same rat. The increment in electrical parameter values after 3 mmol/L of glucose addition to PC-treated tissues represents the magnitude of active glucose transport remaining. Increments were normalized by dividing the change in electrical parameters after addition of glucose to PC-treated tissues by the baseline value of the electrical parameter and taken as a percent of control increment. The actual increments of \(I_{sc}\), \(V_{oc}\) and total tissue conductance for controls were 115.2 ± 1.9 \(\mu\text{A/cm}^2\), 1.12 ± 0.01 mV and 15 ± 1.75 ms/cm\(^2\). Each point represents means ± SEM of at least five experiments.}\]
TABLE 2
Short-circuit current (Isc), total tissue conductance and sodium, chloride and residual fluxes in rat ileum in the presence of 2 g/L of procyanidin (PC) and 9 mmol/L of glucose

<table>
<thead>
<tr>
<th>Flux</th>
<th>Na</th>
<th>Cl</th>
<th>Conductance</th>
<th>Residual flux²</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>JNaMS</td>
<td>JNaSM</td>
<td>JNaNet</td>
<td>JClMS</td>
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<tr>
<td>Na</td>
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<td>Cl</td>
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1 Values are means ± SEM, n = 4.
2 Residual flux is defined as Isc = [JNaNet − JClNet] and represents the net secretion of anion and/or the net absorption of cation.
3 Control solutions were Ringer’s with 9 mmol/L of manitol on the mucosal side and 9 mmol/L of glucose on the serosal side. A control flux rate was determined in the presence and absence of 2 g/L PC after a 30-min tissue stabilization period and a 20-min isotope equilibration period.
4 The effect of glucose on unidirectional flux was determined by adding 9 mmol/L of manitol on the serosal side and 9 mmol/L of glucose on the mucosal side in control and PC-treated tissues. A second flux rate was determined 10–25 min after glucose addition.
5 The P-values were obtained by paired t-test for flux periods in the same tissue. NS = P > 0.05.

scintillation vials containing scintillation cocktail (10 mL) with tissue solubilizer [New England Nuclear, Boston, MA]. After overnight storage in the dark to minimize chemiluminescence, the vials were counted in a Packard Tricard 1500 counter [Packard Instrument, Downers Grove, IL]. Samples were corrected for quench using the transformed spectral index (tSIE). In preliminary experiments, the rate of active glucose uptake was found to be linear within at least the first 8 min of the experiment with a D-glucose concentration of 1 mmol/L and for at least the first 2 min at 50 mmol/L on this basis an incubation time of 2 min was chosen for the present studies.

Statistics. Student’s two-tailed t-test for control and treated pairs from the same rat or unpaired variants was performed using StatsView [Version 1, Abacus Concepts, Berkeley, CA]. Values that differed with P < 0.05 were considered significant. Curves were fit to glucose uptake data using the method of Duggleby (1981).

RESULTS

Effects on basal electrical parameters and electrolyte fluxes. In 10 experiments, addition of 2 g/L PC to the mucosal medium caused the Isc and Gt [Fig. 1A and C, Table 1] to increase, whereas that of control tissues remained steady, resulting in significant differences due to PC treatment. Also, PC almost aboliished the decline in Vc typical of control tissues [Fig. 1B]. These stimulatory effects of PC were not reversed after emptying the chambers and refilling with PC-free solution. A similar stimulation of Isc was obtained in three experiments [Fig. 2] in which the tissues were preincubated in 20 mmol/L of indomethacin; in these experiments there was no significant difference in the response to PC of the indomethacin-treated tissues and the controls that received the ethanol carrier.

For the eight tissue pairs for which results are given in Table 1, net absorption of Na⁺ and Cl⁻ occurred during the control interval. Procyanidin significantly inhibited the unidirectional mucosal-to-serosal Na⁺ flux [JNaMS], causing a significantly lower net Na⁺ absorption [JNaNet]. The net Cl⁻ flux [JClNet] was apparently reversed [i.e., net secretion occurred], although statistical analysis did not reveal a significant effect at the level of the unidirectional fluxes [JClMS and JClSM]. The mean residual fluxes [that fraction of Isc not accounted for by the sum of JNaNet and JClNet] of treatment and control intervals were not significantly different, nor was the mean residual flux significantly different from zero.

Effects on Na⁺-glucose and Na⁺-alanine cotransport. In Ussing chamber preparations, PC inhibited Na⁺-coupled glucose transport as indicated by reductions in both the rise in Isc with glucose addition and the subsequent decrease in Isc with phlorizin addition.
Controlled plots of reaction, by ride response increments components was PC; conductance. 

**FIGURE 5** (A) concentration dependence of active glucose uptake in everted sacs in tissues from the same rat treated with 1 g/L procyanidin or carrier only. The smooth curves are fit to the points using a nonlinear regression program [Duggleby 1981]. (B) Lineweaver-Burke plot of the same data indicating noncompetitive inhibition. Procyanidin decreased the $V_{\text{max}}$ from $1.34 \pm 0.1 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{mg wet wt}^{-1}$ to $0.66 \pm 0.2 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{mg wet wt}^{-1}$ ($P < 0.02$), whereas the $K_m$ was unaffected ($3.79 \pm 1.1 \mu\text{mol}/L$ for 1 g/L PC vs. $3.70 \pm 0.4 \mu\text{mol}/L$ for the controls). Each point represents means $\pm$ SEM of five animals for the experimental group and four animals for the control group.

(Fig. 3), with parallel effects on the corresponding components of transepithelial voltage and total tissue conductance. The effect was dose dependent and became significant at 0.6 g/L PC. The results for glucose stimulation of $I_{\text{sc}}$ are shown in Figure 4, and means for control data are given in the legend; the results using phlorizin were similar. Alamine transport was also inhibited by PC; in five experiments using 2 g/L procyanidin, the increments in $I_{\text{sc}}$, $V_{\text{oc}}$ and $G_T$ with addition of 9 mmol/L alanine were $10.9 \pm 7.7\%$, $4.8 \pm 6.2\%$ and $15.4 \pm 9.3\%$, respectively, of controls.

As expected, addition of glucose to the mucosal side significantly increased $J^{\text{Na}+}_{\text{MS}}$ in control tissues. This response was inhibited by PC [Table 2]. In contrast, chloride and residual fluxes were not significantly affected by glucose in the presence or absence of PC [Table 2].

For simplicity, we treated the glucose uptake of everted sac preparations (Fig. 5A) as a single-substrate reaction, even though Na+ binding is also required for glucose uptake. As estimated from Lineweaver-Burke plots of the data (Fig. 5B), 1 g/L PC significantly decreased the $V_{\text{max}}$ from $1.34 \pm 0.1 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{mg wet wt}^{-1}$ to $0.66 \pm 0.2 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{mg wet wt}^{-1}$ ($P < 0.02$), whereas the $K_m$ was unaffected ($3.79 \pm 1.1 \mu\text{mol}/L$ for 1 g/L PC vs. $3.70 \pm 0.4 \mu\text{mol}/L$ for the controls).

Assuming that net uptake of D-glucose mainly represented passive entry of glucose into the tissue, with a negligible contribution from unabsorbed glucose in the adhering solution [Karavos and Diamond 1983], we can conclude that passive glucose uptake was linear ($r^2 = 1.00$) over the range 0.5–50 mmol/L glucose and was not significantly affected by PC [data not shown].

**DISCUSSION**

The present studies add to the evidence that dietary tannins may impair intestinal absorption and provide new evidence that this effect also involves stimulation of electrolyte secretion. The effect of PC on baseline electrolyte transport in glucose-free medium was reminiscent of cAMP-dependent intestinal secretagogues such as enterotoxins and prostaglandins [Field 1979, Fondicaro 1986] because it decreased Na+ absorption while stimulating Cl− secretion (Table 1). The failure of indomethacin, an inhibitor of prostaglandin biosynthesis, to block the effect of PC on tissue electrical parameters and electrolyte fluxes is evidence against involvement of prostaglandin in the PC effect. Also, both cyclic nucleotides and prostaglandins are reported to reduce total tissue conductance [Powell et al. 1974, Simpson et al. 1981, Smith et al. 1981], whereas PC increased it (Fig. 1C). These considerations argue against the possibility that the effects of PC are by way of a nonspecific irritation that stimulated secretion via endogenous prostaglandin formation.

Effects on baseline electrolyte absorption and Cl− secretion similar to those reported here were obtained for a phytohemagglutinin from red kidney bean [Dobbins et al. 1986]. The effects were correlated with binding of the agent to the surface of villus cells. However, in contrast to our findings, the phytohemagglutinin did not inhibit Na+–coupled glucose or amino acid absorption.

The PC preparation used in these studies contained catechin and its high molecular weight polymers (500–3000 Da). Polyphenols such as PC can exhibit both hydrophilic and hydrophobic interaction with membrane proteins [Artz et al. 1986, 1987, Butler et al. 1984, Spencer et al. 1988]. The hydrophobic aspects of the molecule also allow it to interact with membrane lipids [Cousin and Motais 1978]. Catechin, the monomeric unit of PC, is a close congener of the flavanone phloretin, the aglycone of phlorizin. Although the present studies were not designed to identify a molecular mechanism of PC action on the glucose and alanine transporters, it is useful to consider whether any parallels can be drawn between the effects of PC and flavanones.

First, phlorizin is a fully competitive, highly specific
inhibitor of the glucose transporter of enterocytes and is so used in the present studies. In contrast, phloretin is also reported to affect the activity of a number of membrane transport proteins and ion channels (Antonenko and Bulychev 1991, Klusemann and Meves 1992, Koh et al. 1994, Wang et al. 1993); the effect is frequently attributed to its effect on the dipole moment of the lipid membrane (Deuticke et al. 1991, Deves and Krupa, 1990) rather than a direct effect on membrane protein. In addition, phloretin was reported to have slow-onset secondary effects on cellular metabolism of enterocytes that disable the Na⁺-K⁺ ATPase, reducing the transapical Na⁺ gradient (Kimmich and Randles 1978) and leading ultimately to inhibition of Na⁺-dependent glucose uptake. Procyanidin acts like phlorizin because it inhibits Na⁺-dependent glucose absorption, but like phloretin, its action is nonspecific because it affects at least one other transport pathway (alanine).

Catechin and 17 flavanones and flavanone analogues were reported to inhibit specifically the non-Na⁺-dependent influx of 3-O-methyl glucose in enterocytes, but with less potency than phloretin (Kimmich and Randles 1978). The distinction is physiologically important; the Na⁺-dependent pathway is the route of glucose absorption, whereas the Na⁺-independent pathway acts in vivo as a leak that opposes glucose accumulation by enterocytes. In the same studies, flavanones were found not to inhibit valine influx. In the present study, PC clearly inhibited Na⁺-dependent uptake of both glucose and alanine; this result is not readily attributable to a general effect on cellular energetics because at the same time Iₖ increased. In summary, although the effects of PC are not easily accounted for by invoking the effects of its subunit flavanone catechin, a general effect on membrane proteins or lipids cannot be ruled out.

The actual impact of tannin consumption on human nutrition is presently unclear. A recent study that attempted to extrapolate typical human tannin consumption to the equivalent for a 200-g rat found no detrimental effect on growth or protein balance (Chang et al. 1994). The highest tannin levels used in that study were 0.57 g/kg diet, and its authors suggested that antinutrient effects reported in other animal studies were an artifact of tannin administration at levels higher than those normally consumed by humans. The approach to the question through animal feeding studies is complicated by the protective role of salivary tannin-binding proteins, the effectiveness of which varies considerably from species to species (Salunkhe et al. 1990). Salivary and dietary tannin-binding proteins would influence the concentration of PC available to react with the intestine, potentially eliminating any physiologically adverse effects of PC in the diet.

In the present studies, the concentrations of PC used (i.e., 1-2 g/L) are what would be expected in the gut if a meal of dry beans was extracted into ~10 times its weight of water as the aggregate of cooking liquid, salivary and gastrointestinal secretion, and so forth. These levels seem realistic, especially if the cooking liquor of black beans was used as a rehydration agent in treatment of diarrhea in children (Bressani et al. 1985). Indeed, the effects we report here would be expected to exacerbate diarrhea and oppose the beneficial effects of the fluid, Na⁺ and nutrients in oral rehydration solutions and food-based rehydration media. However, shorter diarrheal episodes in Guatemalan children were reported to correlate with consumption of 50 g or more of black bean broth per day (Birch 1985), suggesting that such media can have a net benefit in spite of the presence of tannins. Pending more comprehensive studies of human subjects, the present studies suggest that even better performance might be obtained from bean-based rehydration media if the seed coats were eliminated, or alternatively, if cultivars with unpigmented seed coats were used.

LITERATURE CITED


