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The Gastrointestinal Tract: A Major Site of Antioxidant Action?

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Diets rich in fruits and vegetables delay the onset of many age-related diseases, and contain a complex mixture of antioxidants (including ascorbate, carotenoids, vitamin E and other phenolics such as the flavonoids). However, diet also contains pro-oxidants, including iron, copper, H_2O_2, haem, lipid peroxides and aldehydes. Nitrite is frequently present in diet, leading to generation of reactive nitrogen species in the stomach. In considering the biological importance of dietary antioxidants, attention has usually focussed on those that are absorbed through the gastrointestinal tract into the rest of the body. In the present paper we develop the argument that the high levels of antioxidants present in certain foods (fruits, vegetables, grains) and beverages (e.g. green tea) play an important role in protecting the gastrointestinal tract itself from oxidative damage, and in delaying the development of stomach, colon and rectal cancer. Indeed, carotenoids and flavonoids do not seem to be as well absorbed as vitamins C and E. Hence their concentrations can be much higher in the lumen of the GI tract than are ever achieved in plasma or other body tissues, making an antioxidant action in the GI tract more likely. Additional protective mechanisms of these dietary constituents (e.g. effects on intercellular communication, apoptosis, cyclooxygenases and telomerase) may also be important.

Keywords: Antioxidant, stomach, gastrointestinal tract, ascorbate, iron, haem, vitamin E, flavonoid, catechin, pro-oxidant, carotenoid, β-carotene, lycopene, green tea, vitamin C, nitrite, deamination, colon cancer, gastric cancer, hydroxyl radical, hydrogen peroxide, lipid peroxidation, food

INTRODUCTION

The importance of endogenous and diet-derived antioxidants to the maintenance of human health in the face of continuous assault by reactive oxygen/nitrogen/chlorine species seems indisputable[1]. For the diet-derived antioxidants, particular attention has focussed on vitamin C and on the various tocopherols and tocotrienols that make up vitamin E: both vitamins C and E are in general well-absorbed from the gastrointestinal tract[2,3]. By contrast, carotenoids are absorbed from the human gut only to a limited extent, and some undergo cleavage in the gut, eventually yielding vitamin A[4]. In fact, the only

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established physiological role of carotenoids in humans is as vitamin A precursors[4]. Carotenoids have important antioxidant effects within plants, largely as quenchers of excited chlorophyll states and of singlet O_2 (reviewed in[5]). Carotenoids can scavenge singlet O_2 and several other reactive species *in vitro*[4,6], but the importance of carotenoids as antioxidants after uptake into human tissues is far from established, and may be insignificant since their tissue levels may be too low to exert antioxidant effects[4,7]. If some carotenoids do exert effects that limit the development of certain diseases in humans, a concept for which supportive evidence is growing[8,9], it seems more likely to the authors that they are affecting cell signalling mechanisms, intercellular communication, or gene expression rather than by acting as "bulk" antioxidants[4, 10].

Foods and beverages contain a wide range of antioxidants, many of which are phenolic or polyphenolic compounds that have powerful antioxidant activities *in vitro* (reviewed in[11]). The flavonoids have been a special focus of attention[11], but other phenolics may also important[12]. Evidence for the uptake of significant amounts of phenolic compounds through the gastrointestinal tract in humans has come from measurements of both the compounds themselves and of their metabolites in plasma and urine[11, 13–20]. The levels of phenolics and their metabolites measured in plasma in some studies are theoretically sufficient for them to be able to exert antioxidant actions, in that similar levels of phenolics can scavenge free radicals or prevent peroxidation of low-density lipoproteins (LDL) *in vitro*[11–23]. However, data are conflicting on whether levels of oxidative damage to DNA, lipids or proteins in human tissues, or rates of LDL peroxidation *in vivo*, are affected by dietary flavonoids[19, 21–25].

However, something may be being overlooked in these studies of uptake, urinary excretion and plasma concentration – that the gastrointestinal (GI) tract itself could be a major site of pro-oxidant and antioxidant actions[26]. Compounds that are only present in body fluids at μM levels may be present in the stomach and lumen of the intestines at much greater concentrations. The GI tract is an important site of disease: inflammation (gastritis, *H. pylori* infection, Crohn's disease and ulcerative colitis are examples) and cancer (especially colon and rectum) are major human health problems. All these conditions involve reactive species and oxidative damage during their progression and perhaps even in their origin[1,26–30].

PROBLEMS OF THE GASTROINTESTINAL TRACT

The GI tract has to absorb essential nutrients, prevent the passage of bacteria into the blood, and in general act as a barrier between the gut contents and the rest of the body. Its lining is constantly renewed, and this constant cell proliferation makes it a ready target for genotoxins[29]. One protective mechanism may be the constant shedding of oxidatively-damaged cells. The GI tract is also exposed to pro-oxidants to a degree unprecedented in other body tissues. Some examples –

(1) Ingested food frequently contains copper and iron ions. Iron is usually present as insoluble Fe(III) salts but sometimes as elemental iron, which is used as an iron supplement in certain foods. Gastric acid solubilizes ferric and metallic iron, which can then be reduced by ascorbate and other reductants to Fe^{2+}. Reducing agents such as ascorbate and GSH may be present in the food, but ascorbate is also secreted into gastric juice[30], perhaps in order to facilitate iron uptake by reducing Fe(III) to the more-easily-absorbed Fe^{2+} form, among other reasons. Thus the stomach, duodenum and upper small intestine may be targets for damage by hydroxyl radical ($OH^{\bullet}$) generated by Fenton chemistry from ascorbate/Fe^{2+}mixtures[1, 31, 32], at least until the

Fe^{2+} can be absorbed by the gut and safely sequestered into protein-bound forms incapable[31] of generating $OH^{\bullet}$. Diet-derived copper ions could also generate $OH^{\bullet}$ from ascorbate[33], and multivitamin pills containing iron and copper salts together with ascorbate may deliver a similar pro-oxidant challenge[32–34]. Ascorbate/Fe^{2+} and ascorbate/Cu^{2+} mixtures can generate $OH^{\bullet}$ without it being necessary to add H_2O_2, but addition of H_2O_2 enhances $OH^{\bullet}$ generation[31]. Hydrogen peroxide has been detected in several foods (reviewed in[35]), and high levels (sometimes over 100 μM) are found in certain beverages, including teas, ground coffees and (especially) instant coffees[36]. Indeed, $OH^{\bullet}$ is generated within instant coffee, and the conversion of caffeine to 8-hydroxycaffeine has been used to monitor this[37].

(2) Release of haem from haem proteins, especially in meat-rich diets, may be another source of pro-oxidants since haem and haem proteins are powerful stimulators of lipid peroxidation[1,38]. Mixtures of haem and haem proteins with H_2O_2 and other peroxides are powerful pro-oxidants (reviewed in[1,35]), and unabsorbed haem may contribute to pro-oxidant effects in the colon[39,40].

(3) Lipids in ingested foods are frequently oxidized to some degree, both by the action of lipoxygenase enzymes (e.g. in fish and some plants) and by thermally-induced and/or transition metal ion-catalyzed non-enzymic peroxidation (reviewed in[35, 41, 42]). There is evidence that some oxidized lipids can be absorbed[41–45]. Thermal and/or metal ion-catalyzed decomposition of lipid peroxides can generate cytotoxic aldehydes, including malondialdehyde and 4-hydroxy-2-*trans* nonenal. These can damage proteins and DNA, some of the products of their reactions with DNA being mutagenic[46,47]. Transition metal ions and haem compounds decompose peroxides to peroxyl and alkoxyl radicals, which are propagators of the chain reaction of lipid peroxidation[1] and can also damage DNA and proteins. High levels of cytotoxic aldehydes have been detected in oxidized cooking oils and some other foods, and some of these aldehydes can be absorbed through the gut[48,49]. Lipid peroxides in foods can interact with any free metal ions, haem, and haem proteins present to propagate lipid peroxidation. Although the degree of absorption of oxidized lipids and carbonyls derived from them into the plasma appears limited[41,44,45], the cells of the GI tract will be exposed to the full force of these toxic agents. Indeed, there is considerable evidence that dietary fat is a risk factor for cancer development; the presence of peroxides and their decomposition products could contribute to this by promoting mutation, activation of dietary carcinogens and abnormal cell proliferation[39, 40, 46, 50, 51].

(4) Foods can contain not only peroxides and aldehydes, but also isoprostanes[52]. Some isoprostanes can exert significant biological effects, including vasoconstriction, and several are cytotoxic (reviewed in[53–55]).

(5) Oxidized cholesterol can be present in foods and some cholesterol oxidation products may be absorbed[42,56]. Cholesterol oxidation products are widely reported as cytotoxic[42,56].

(6) Human saliva is rich in nitrite; levels up to, and sometimes over, 100 μM have been reported[57–59]. Nitrite is a widely used food preservative, especially in canned meats and sausages (reviewed in[60]). When nitrite contacts gastric acid, HNO_2 and oxides of nitrogen will be produced[58]. Secondary amines in food can react with HNO_2 and be converted to nitrosamines (reviewed in[61, 62]). Nitrous acid can also deaminate DNA bases, producing mutagenic lesions. Conversion of adenine to hypoxanthine, and of guanine to xanthine and oxanine, are the major reactions (reviewed in[63]). The potential health risks of

dietary nitrites have been debated for many years, but no consensus has emerged, and their powerful abilities to prevent the growth of pathogenic bacteria ensure their continued use[60,61]. Yet nitrite at levels that enter the gastric juice can be highly cytotoxic at low pH, and even at pH 7.4 NO_2^- can cause slow DNA base deamination when added to cells in culture[63, 64].

(7) A large part of the immune system resides in the GI tract, and several of the cells involved can respond to challenge with bacteria or with certain food antigens, to produce reactive species[65–69].

(8) Food can contain many pro-oxidants other than peroxides or transition metals. For example, several phenolic compounds in foods can oxidize to give reactive species, an example being hydroxyhydroquinone in coffee[70]. Autoxidation of phenols is facilitated by transition metal ions. Indeed, shortly after drinking instant coffee the urinary concentration of H_2O_2 increases in humans[71]. Examples of potentially pro-oxidant food additives include the food colourant carminic acid[72], and sulphite, a widely-used preservative[73]. Sulphite itself is essentially a reducing agent[73], but exposure of sulphite to iron or copper ions, to peroxynitrite, or to reactive oxygen species can cause its conversion to highly-oxidizing cytotoxic radicals such as $SO_3^{\bullet-}$ and $SO_5^{\bullet-}$ [69], [74–76].

ANTIOXIDANT DEFENCES OF THE GUT

Like all animal tissues, the gastrointestinal tract contains superoxide dismutase, catalase and glutathione peroxidase enzymes, including an "intestinal form" of glutathione peroxidase[44,77–80]. Some of the SOD and glutathione peroxidase activities of the intestine and colon appear to be located extracellularly, at the external cell surface[78,80]. These extracellular enzymes presumably help to detoxify reactive species reaching the gut surface from the food. Intracellular glutathione peroxidases may remove the bulk of peroxides present in absorbed lipids[44], which accounts for the observation that even highly-peroxidized lipids are not very poisonous to animals, although some deleterious effects are exerted[44,45]. Lymphoid tissues seem particularly affected[45], which makes one wonder how lipid oxidation products might affect the function of the gut immune system. However, some lipid peroxides escape metabolism by the GI tract[41, 43, 44] and enter the circulation. Aldehydes in food are presumably extensively metabolised by conjugation with GSH, catalyzed by glutathione-S-transferases, in the gut[81–83], but again there is evidence that some cytotoxic aldehydes may pass into the circulation[48,49]. Several dietary constituents, including flavonoids and sulphoraphane, can increase levels of GST in the GI tract[83- 85].

TABLE I Possible Interactions Of "Chelating"* Phenolic Compounds With Transition Metals

①	The metal ion is chelated in a redox-inactive form: its reduction potential and/or accessibility is altered so as to disfavour metal ion-catalyzed $OH^{\bullet}$ formation from H_2O_2 and/or metal ion-catalyzed decomposition of lipid and protein peroxides to peroxyl and alkoxyl radicals.
②	The metal ion is chelated in a redox-active form, but on addition of peroxides any reactive radicals generated are scavenged by the phenol and do not escape into free solution to cause damage to other biomolecules.
③	The metal ion undergoes redox interactions with the phenol, being reduced as the phenol is oxidized This can lead to pro-oxidant effects in certain assay systems.

* Those with at least two adjacent electron-donating groups, enabling them to chelate metal ions.

TABLE II IC_{50} Values for Inhibition by Phenolic Compounds of Hypoxanthine/Xanthine Formation in DNA Exposed to Nitrite at pH 3

Phenol	*IC_{50} (μM)*	
	Hypoxanthine	*Xanthine*
Caffeic Acid	383 ± 66	254 ± 168
Catechin	107 ± 24	126 ± 15
Epicatechin	351 ± 184	139 ± 37
Epigallocatechin	384 ± 36	105 ± 7
Epigallocatechin Gallate	187 ± 48	195 ± 6
Quercetin	390 ± 16	398 ± 94

Experiments were carried out as described in the legend to Figure 2.

The intracellular antioxidant defences within the cells of the GI tract cannot offer much protection against external effects of pro-oxidants, such as $OH^{\bullet}$, H_2O_2, lipid peroxides, aldehydes, HNO_2 and oxides of nitrogen. The significance of the extracellular enzymes[78–80] in offering protection requires further investigation. The mucus layer lining the whole GI tract has a high capacity to absorb reactive species (especially $OH^{\bullet}$ and possibly HOCl, HNO_2 and ONOOH)[86,87] and may have a major protective role, stopping many of the most-reactive and damaging species from reaching the cells underneath[86]. Indeed, excess intake of Fe^{2+} appears to cause damage only when taken in sufficient quantities to erode the mucosal layer[88].

A ROLE FOR DIETARY ANTIOXIDANTS?

Foods and beverages deliver a complex mixture of antioxidants and pro-oxidants to the GI tract. It is known that diets rich in fruits and vegetables are associated with decreased risks of gastric, colon and rectal cancer. There are many anti-cancer agents present in plant-derived foods, but considerable attention has focussed on the role played by antioxidants, especially vitamin C, vitamin E, carotenoids and flavonoids. These have often been suggested to be able to delay or prevent cancer development by being absorbed from the GI tract into the plasma and from there into the tissues, where they might decrease levels of oxidative DNA damage. However, evidence that these antioxidants do decrease oxidative DNA damage *in vivo* is sparse (reviewed in[7, 89]). Indeed it is possible to demonstrate decreased oxidative DNA damage after feeding fruits and vegetables to humans under conditions where supplements of vitamins E, C, carotenoids and the flavonoid quercetin have no effect on such damage[7,89].

What has been largely ignored up until now is the potential for *extracellular* antioxidant protective effects within the GI tract. For example, flavonoids and other phenolics are powerful scavengers of $OH^{\bullet}$, peroxyl radicals, HOCl and $ONOO^{-}$ *in vitro*, but many such studies *in vitro* use concentrations much higher than are likely to be achieved in plasma or body tissues (reviewed in[11]). A similar comment may be applied to the ability of carotenoids to scavenge reactive oxygen and nitrogen species[6]. However, when foods rich in phenolics and carotenoids are eaten, and beverages rich in these substances are drunk, substantial levels of these antioxidants are delivered to the GI tract. The stomach probably encounters the highest levels, but there will be substantial exposure of the rest of the GI tract (Figure 1), and considerable amounts of unabsorbed phenolics and caroten-

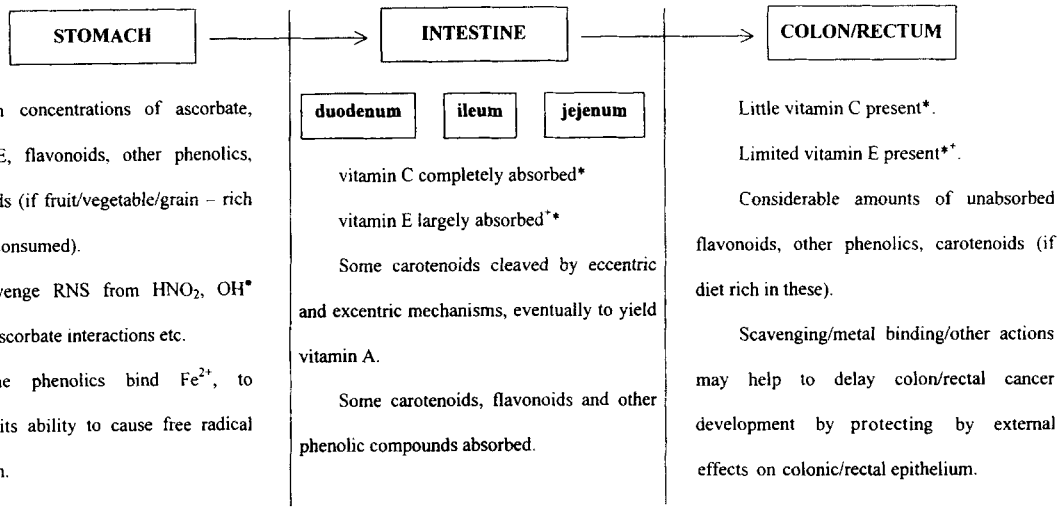
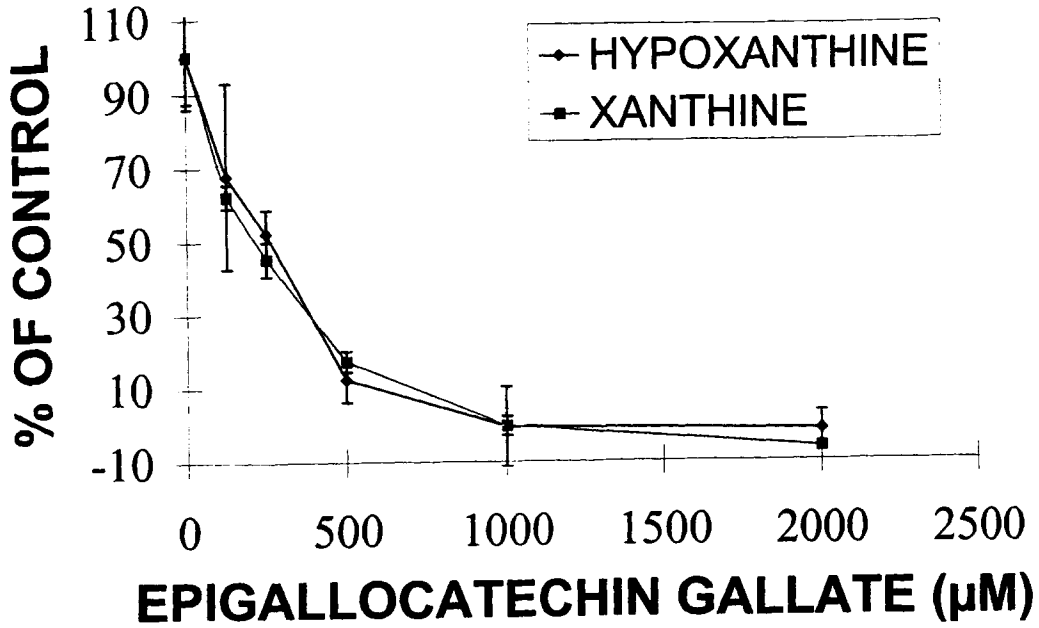


FIGURE 1 Dietary Antioxidants And The Gastrointestinal Tract
* Except when supplements are taken. This diagram refers to normal dietary intake.
+There is considerable inter-subject variability in the efficiency of GI uptake of vitamin E[112]. RNS-reactive nitrogen species

oids will pass into the colon. Hence scavenging of free radicals and other reactive species within the GI tract becomes feasible. Of course, such scavenging might generate oxidized, chlorinated, nitrosylated or nitrated products from the antioxidants[90–94], and these products could themselves have biological effects, an area which remains to be explored. In addition, many phenolic compounds interact with iron, often in complex ways, as summarized in Table I. Overall, the effects are probably usually antioxidant (Chapter 2 in reference[11]). Much unabsorbed dietary iron enters the faeces, where it could represent a pro-oxidant challenge to the colon and rectum[39,95–97]. Indeed, diets rich in fat and low in fibre may aggravate this pro-oxidant effect[97]. Phenolics, by chelating iron, may help to alleviate pro-oxidant actions of colonic iron (Figure 1). If phagocytes in the GI tract become activated, diet-derived antioxidants may help to scavenge such extracellular species as $O_2^{\bullet-}$, H_2O_2, $ONOO^-$ and HOCl that can be produced[11, 90–94], although again the potential biological effects of any oxidized, nitrosylated, nitrated or chlorinated antioxidants so produced must be considered.

Studies have shown that ascorbate can decrease nitrosamine formation from secondary amines, apparently by scavenging reactive nitrogen species[98,99]. Several phenolic compounds found in fruits, vegetables and certain beverages can prevent nitration of guanine in DNA by $ONOO^-$ and deamination of DNA bases by HNO_2[63, 94]. For example, Figure 2 shows that epicatechin and epigallocatechin gallate are powerful inhibitors of DNA base deamination under the pH conditions likely to exist in the human stomach. Inhibition of DNA base deamination at low levels of these phenolics is very variable, as indicated by the size of the error bars on the figures, but becomes clearer at higher concentrations. Most of the phenolic compounds inhibited the deamination almost completely at about 1 mM. With some, such as caffeic acid and epicatechin, complete inhibition of the formation of hypoxanthine was not achieved up to 2 mM[63]. The mechanism of the inhibition may involve oxidation or nitration/nitrosation of the



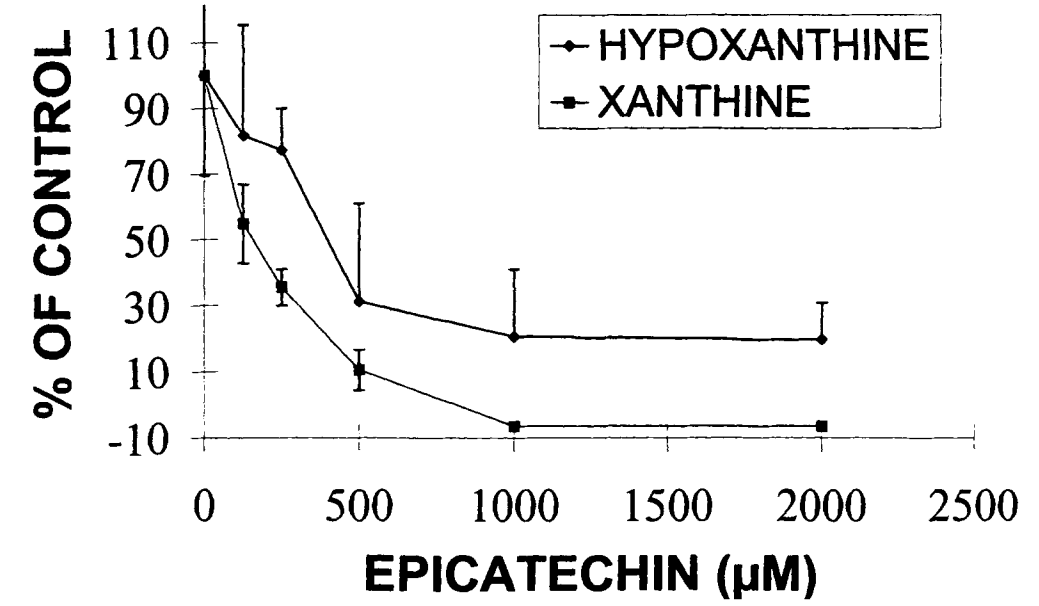


FIGURE 2 Inhibition of DNA base deamination by (A) epigallocatechin gallate and (B) epicatechin. Data are mean±SD, n=3. Calf thymus DNA (0.5 mg/ml) was incubated with sodium nitrite (0.5 mM) at pH 3 (50 mM potassium phosphate buffer) in the presence of various concentrations of phenolic compounds in a total volume of 1 ml. The incubation was carried out at 37°C for 2 hours. The DNA concentration in the reaction mixture was determined following an exhaustive dialysis against water (24 hours with two changes). After freeze-drying, the DNA (100 μg) was hydrolysed in 60% formic acid (0.5 ml) at 145°C for 45 min. The acid was removed by freeze-drying and the residue was dissolved in 1 ml of water for HPLC analysis. The deaminated bases were separated on a Hypersil C18 column (250 × 4.6 mm, 5 μm) by isocratic elution with potassium phosphate buffer (50 mM, pH 3) containing 2 mM triethylamine and 0.05 mM EDTA and assayed as described in[63]

phenolic compounds as a result of scavenging reactive nitrogen species[94, 100–102]. Nitrosated phenolics could also conceivably react with DNA bases, but probably more slowly than does HNO_2. These events could contribute to the large variations in the inhibition of the nitrite-dependent deamination at low levels of phenolics, but high levels of phenolics were clearly protective (Figure 2). Some evidence for binding of several phenolics to DNA was obtained in our studies (data not shown). Table II summarizes IC_{50} values for inhibition of DNA deamination by several dietary phenols. Since many foods and beverages of plant origin are rich in phenolic compounds, the concentrations which achieve protection can easily be achieved in the stomach and probably in the rest of the GI tract after a meal rich in plant products.

CONCLUSION

The essence of our argument in this paper is that, if flavonoids and carotenoids exert antioxidant effects in the human body, the place where they are most likely to do so is within the GI tract. A role for ascorbate and tocopherols as scavengers of reactive species is also feasible in the GI tract, particularly in subjects consuming vitamin E supplements, when considerable amounts of tocopherols may remain unabsorbed to reach the colon[112]. Tocopherols can react not only with reactive oxygen species such as peroxyl radicals, but also with reactive nitrogen species (reviewed in [100]).

Of course, it must not be assumed that all (or even any) of the protective effects of dietary antioxidants in the GI tract are necessarily due to antioxidant actions. Some phenolics induce enzymes that metabolise carcinogens, some inhibit protein kinases, telomerase, angiogenesis, lipoxygenases, cyclooxygenases or the growth of *Helicobacter pylori*, and some might promote apoptosis of malignant cells in the colon[103–111]. For example, COX-2 appears important in the development of colon cancer and its inhibition could be one anti-cancer effect of dietary phenolics[107, 111]. Carotenoids modulate intercellular communication and gene expression[10]. Nevertheless, the powerful antioxidant activities of many of these compounds, the high levels of them that are present in fruits and vegetables and the fact that reactive species are implicated not only in cancer development but also in the progression of most other diseases make it very likely that antioxidant mechanisms are important in maintaining the health of the GI tract.

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