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Forum Review

Bioavailability of Flavan-3-ols and Procyanidins: Gastrointestinal Tract Influences and Their Relevance to Bioactive Forms *In Vivo*

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ABSTRACT

There is considerable interest in the bioavailability of flavan-3-ols such as tea catechins and cocoa-derived procyanidin components of the diet and their bioactivity *in vivo*. Their hydrogen-donating abilities and their propensity for nitration make these compounds powerful scavengers of reactive oxygen and nitrogen species. In addition, recent evidence has suggested that these compounds may interact with redox-sensitive cell signaling pathways. However, their bioactivity *in vivo* will be dependent on the absorption and metabolism of these compounds after ingestion and the reducing properties of resulting metabolites. Many cell, animal, and human studies have shown that flavanol monomers, such as epicatechin, are extensively metabolised to *O*-methylated forms and/or conjugated to glucuronides and sulphates during absorption into the circulation. The cleavage of higher procyanidin oligomers to mixtures of monomer and dimer in the stomach may act to enhance the potential for their absorption in the small intestine as higher oligomers have very limited absorption. Studies suggest that the major bioactive forms of flavanol monomers and procyanidins *in vivo* are likely to be metabolites and/or conjugates of epicatechin. One such metabolite, 3′-*O*-methylepicatechin, has been shown to exert protective effects against oxidative stress-induced cell death. Future studies will continue to concentrate on the exact mechanism of action of the bioactive forms of flavan-3-ols *in vivo*. Antioxid. Redox Signal. 3, 1023–1039.

INTRODUCTION

FLAVAN-3-OLS, such as catechin and epicatechin (Fig. 1A), are polyphenolic phytochemicals, found mainly in green and black teas and red wine (49). Common dietary flavanols also include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Fig. 1A), which are present in tea. Procyanidins, oligomeric chains of catechins and ECs (Fig. 1B), are constituents of cocoa, apples, grapes, tea, wine, and strawberries (53). Cocoa beans (*Theobroma cacao*) are extremely rich in polyphenols, in the form of procyanidin oligomers of epicatechin/catechin flavanols, comprising 12–48% of dry weight of the whole bean (5).

EC and polyphenolic extracts of cocoa display potent antioxidant properties *in vitro* (50, 52). Procyanidins are potent inhibitors of lipid peroxidation (67) and polyphenols, including procyanidin oligomers, which suppress peroxynitrite-induced nitration of tyrosine *in vitro* (2, 41, 42). Chocolate polyphenols have also been suggested to display immunoregulatory effects

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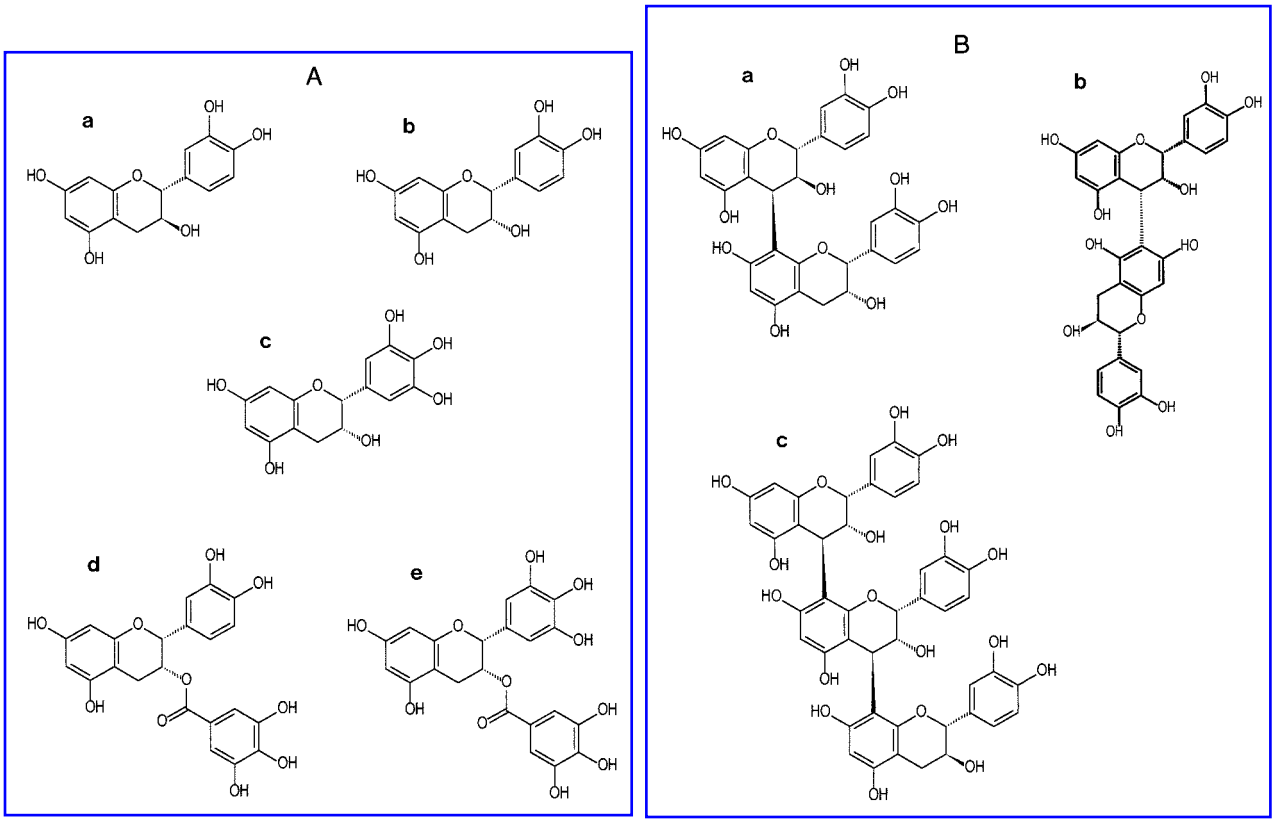

FIG. 1. (**A**) Structures of flavan-3-ols: (a) catechin; (b) epicatechin (EC); (c) epigallocatechin (EGC); (d) epicatechin gallate (ECG); and (e) epigallocatechin gallate (EGCG). (**B**) Structure of procyanidin oligomers: (a) procyanidin dimer B4 [catechin-(4β-8)-epicatechin]; (b) dimer B5 [catechin-(4α-6)-epicatechin]; and (c) trimer [epicatechin-(4β-8)-epicatechin-(4β-8)-catechin].

(51). However, many studies have concentrated on the antioxidant potential of catechins and procyanidins, a nature that resides in their structure-dependent hydrogen-donating abilities (4, 47) and their ability to bind transition metal ions (4). Both of these abilities are mainly determined by the B-ring, specifically the 3′, 4′-dihydroxy functional system that these compounds possess.

Epidemiological studies based on assessment of intake and clinical endpoints have implicated a role for polyphenolic compounds in reducing the risk of myocardial infarction and stroke (17, 21, 24). Despite the observed negative correlation between disease risk and intake, the question of whether these compounds exert their beneficial action *in vivo* by acting as antioxidants or by some other mechanism is still unclear. The extent of their antioxidant potential *in vivo* will be dependent on the absorption, metabolism, distribution, and excretion of these compounds within the body after ingestion and the reducing properties of the resulting metabolites (Fig. 2). An understanding of the absorption, distribution, and metabolism of polyphenols is essential for determining their significance and bioactivities *in vivo*.

PREABSORPTION EVENTS

Preabsorption events are important as they will determine the forms of the compounds presented for absorption in the small and large intestine. Two principal biological fluids are encountered before the small intestine is entered: saliva and gastric juice. Studies of the effects of saliva on green tea catechins suggest that there is little or no modification of these compounds (62), and extraction of phenolics from saliva after mouth rinsing with catechins resulted in high mean recoveries of 86–99%. However, evidence exists for the degalloylation of the flavanol gallate esters, such as

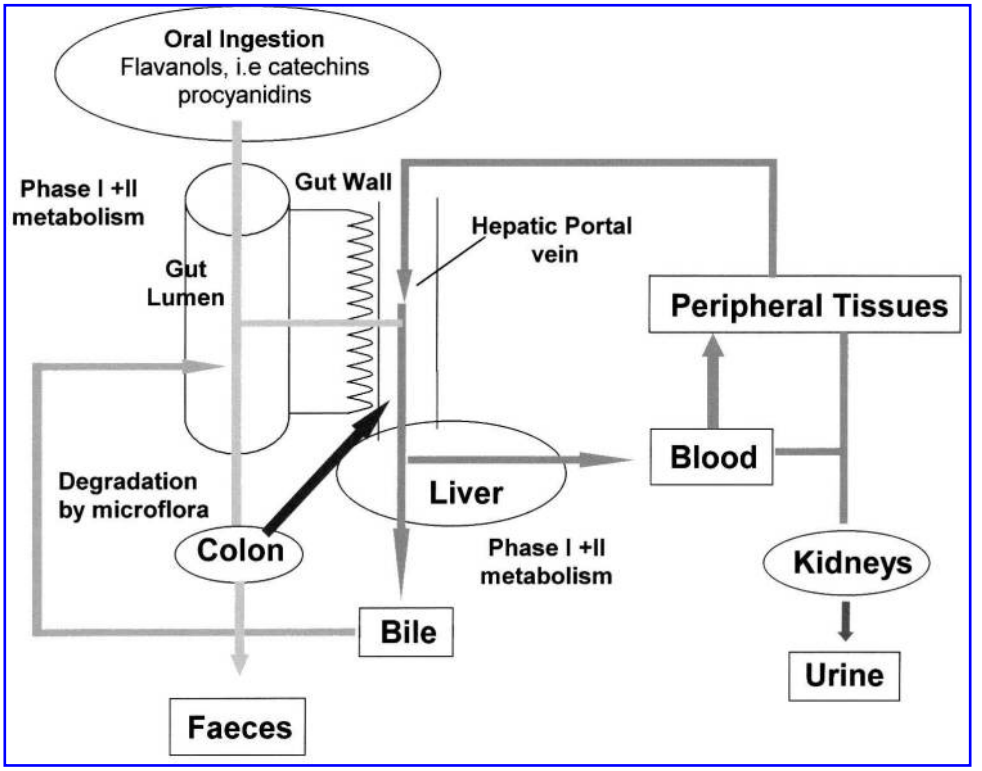

FIG. 2. Diagrammatic representation of the fate of dietary polyphenols *in vivo*.

EGCG, in human saliva (69). Incubation of procyanidins, dimer–hexamer, in human saliva for up to 30 min did not result in any modification of the compounds and, after extraction and analysis, yielded recoveries >99% (unpublished observation of authors).

There has been interest in whether such polyphenol complexes are stable in the acidic environment of the gastric lumen after consumption, prior to absorption. The stability of the larger oligomers at low pH will ultimately control the forms of such compounds that will be presented to the jejunum and ileum of the small intestine. This in turn will have important implications for the nature of the potentially bioactive components of procyanidins *in vivo*. The decomposition of procyanidins in mild acid environments has formed the basis of an assay to detect procyanidins (Fig. 3). Studies examining the effects of simulated gastric juice (a pH environment similar to that encountered in the stomach) on the stability of procyanidin oligomers ranging from a dimer to decamer (isolated from *Theobroma cacao*), as well as on the monomer EC, show that procyanidin oligomers are unstable under these conditions of acidity and decompose essentially to EC monomeric and dimeric units, but also to other oligomeric units (57). During incubation in acid, other oligomeric units, such as trimer and tetramer, are also formed and degraded and larger oligomers are formed at longer time points (Fig. 4). The decomposition is rapid with almost complete loss of oligomer after ~3 h, and furthermore the higher the polymerisation index of the monomer, the more readily the components are cleaved. The time of cleavage (0–3 h) and the pH conditions (pH <2) indicate that these processes are likely to occur in the stomach, where the average residence time ranges from 0.5 to 3.0 h and the pH usually varies from 1 to 3. Thus, absorption of flavanols and procyanidins, for example after consumption of chocolate or cocoa, are likely to be influenced by preabsorption events in the gastric lumen within the residence time, unless the food matrix influences these effects on the pure compounds. Thus, the decomposition products in the forms of procyanidin dimers and monomers would be the major compo-

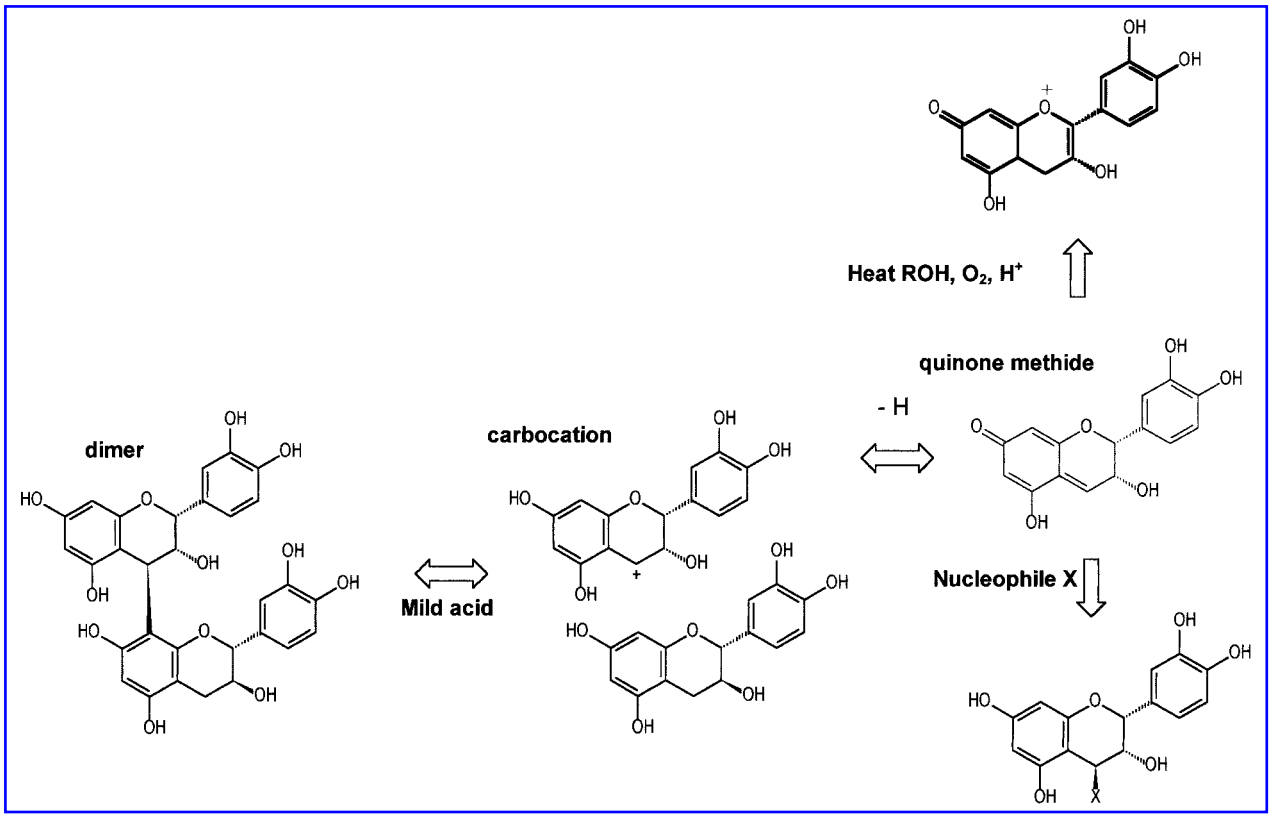

FIG. 3. Decomposition of procyanidins in mild acidic environments (dimer as example). Procyanidins are readily cleaved in mild acid solutions to form flavan-3-ol and quinone methide, which is in equilibrium with a carbocation in stronger acidic conditions. The carbocation is converted to an anthocyanin after heating in alcoholic solutions. The quinone methide may be captured with a suitable nucleophile (X). Figure adapted from reference 71.

nents for consideration for absorption via the small intestine or entering the colon. However, consideration needs to be given to the food matrix and its influence on the pH of the procyanidins and their decomposition.

SMALL INTESTINAL ABSORPTION AND METABOLISM

There are many factors that influence the extent and rate of absorption of ingested compounds by the small intestine (30). These include physiochemical factors such as molecular size, lipophilicity, solubility, and p*K*a, and biological factors including gastric and intestinal transit time, lumen pH, membrane permeability, and first-pass metabolism (18, 19). Clearly, it is vital to determine to what extent and in what forms flavanols appear in the blood after crossing the gastrointestinal tract, in particular the jejunum and ileum of the small intestine. There are many considerations including transformation of compounds in intestinal fluid, uptake into enterocytes, metabolism or biotransformation in the enterocyte, and transport into hepatic portal vein on the serosal side.

Intestinal fluid

On transfer from the stomach to the jejunum, the top two thirds of the small intestine, the pH rises from ~2.0 to 8.5. It is well known that polyphenolic compounds such as those with catechol structures rapidly oxidise at alkaline pH environments. Flavanols, including the procyanidins, have a catechol structure (3′,4′-dihydroxylation) in the B-ring and might be expected to oxidise in intestinal fluid. Indeed, studies incubating EGCG with intestinal fluid (pH 6.5) for only 5 min resulted in an 81.6% decrease in the amount of EGCG (70), whereas a similar incubation in plasma (pH 7.4) resulted in only a 29.3% decrease in amount. Oxidation

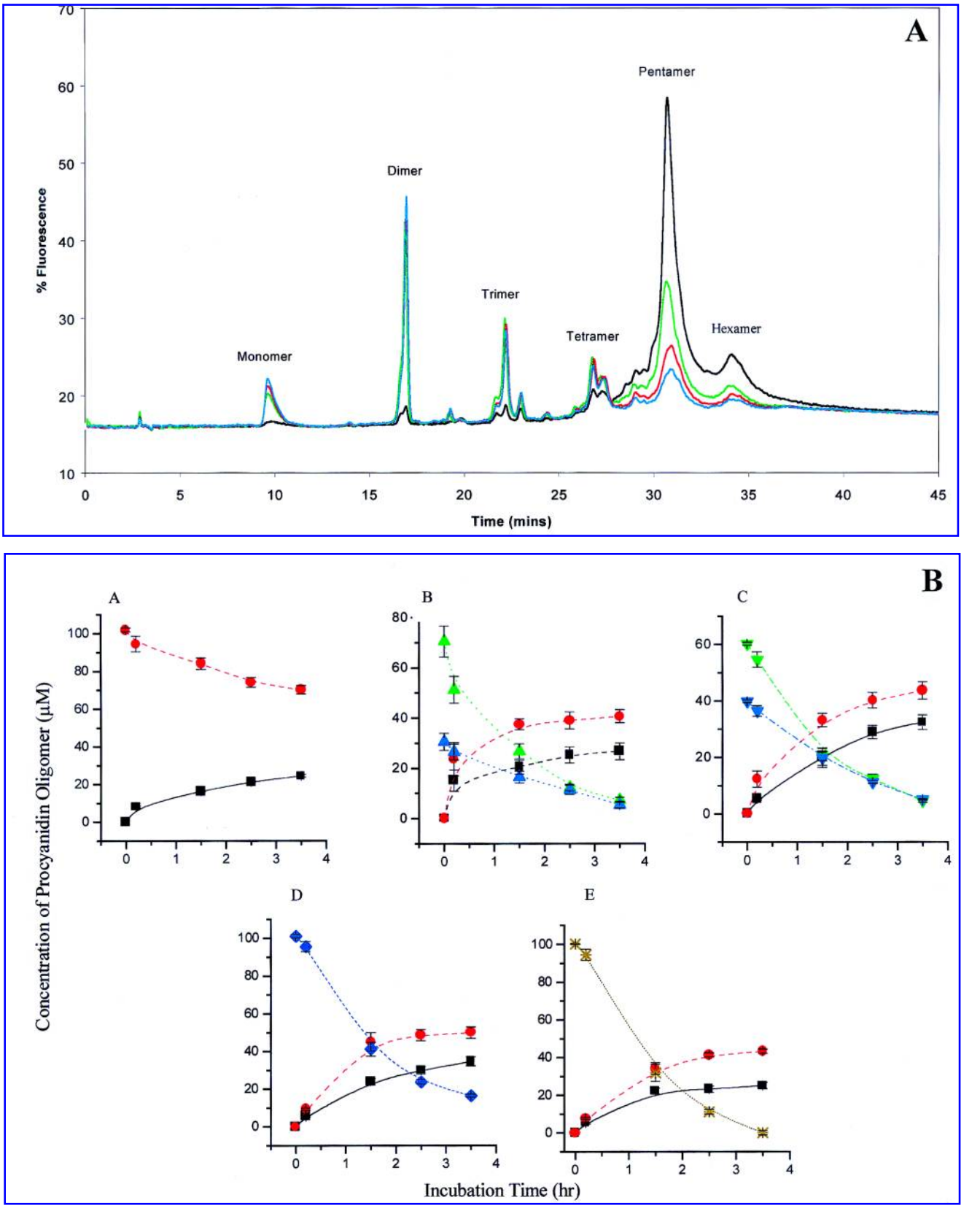

FIG. 4. (**A**) Representative HPLC chromatogram of the decomposition of the pentamer over time during incubation in simulated gastric juice (pH ~2.0). Each line represents a time point of incubation: 0, 1.5, 3.5 and 6 h. The pentamer peak is observed to decrease in area over time, whereas monomer, dimer, trimer, tetramer and hexamer peaks increase (20). (—) 0 h; (—) 1.5 h; (—) 3.5 h; (—) 6 h. (**B**) Decomposition of procyanidin oligomers (100 μM) during incubation in simulated gastric juice (pH 2.0) for up to 3.5 h. (A) dimer; (B) trimer; (C) tetramer; (D) pentamer; (E) hexamer. Data are plotted as means ± SD of three separate experiments each analysed twice by HPLC (20). (■) Monomer (EC); (●) dimer; (▲) trimer; (▼) tetramer; (◆) pentamer; (★) hexamer.

under the alkaline conditions resulted in dehydrogenation and decarboxylation of EGCG to form theasinensin A and a dimerised product. Interestingly, the dimerised product was observed to possess greater superoxide radical scavenging activity than EGCG, and all products were found to be more powerful iron chelators than EGCG, indicating that the catechol structure remains intact (70). However, it is worth bearing in mind that in a more complex food matrix the pH changes are likely to be buffered on the inside for long periods of time (up to 5 h), and therefore oxidation of the flavanols may occur only to a limited extent during intestinal transit. In addition, it has been suggested that ascorbate significantly increases the stability of flavanols incubated in intestinal fluid (7), and therefore the presence of ascorbate *in vivo* may stabilise the polyphenols in the neutral or alkaline environment of the small intestine.

Absorption in the jejunum and ileum

Certain classes of polyphenols, such as the flavonols and flavones, are usually present in the plant as glycosides, whereas flavanols are almost always present in the nonglycosylated form. This will have implications for the solubility and partitioning of flavanols. The removal of the glycoside is thought necessary if passive diffusion of the flavonoid is to occur across the intestine brush border. However, the mechanism of uptake of these compounds is still unknown to a large extent, and although removal of the sugar may still be important for transport by proteins such as lactate phloridzin hydrolase (9, 54), it may not be a prerequisite for all glycosides. The acylation of flavanols with gallic acid changes the partition coefficient of the native flavanol, but does not greatly influence their bioavailability; however, there is some evidence to suggest that flavanols can pass through biological membranes and be absorbed without deconjugation or hydrolysis (54).

Absorption in the small intestine has been studied primarily in two ways: by using isolated preparations of small intestine, such as that from the rat, and by using a Caco-2 cell line. In most cases, although the models are very different and each suffers from its own problems, the two often yield similar results. For example, studies investigating the transport of chrysin using the human intestinal Caco-2 cell line found that although the flavone has favourable properties for membrane penetration, its overall bioavailablity is limited due to extensive metabolism during transfer, primarily to glucuronide and sulphate conjugates (66). In support of this, a wide range of flavonoids were found to be extensively glucuronidated during transfer across isolated sections of rat jejunum and ileum (56). In particular, the more highly reducing flavonoids, *i.e.*, those with 3,4-dihydroxylation of the B-ring, such as luteolin, were found to be more susceptible to glucuronidation during transfer than monophenolic B-ring flavonoids, such as hesperetin.

Caco-2 cell model. In recent years, studies with Caco-2 cells have helped to unravel the complex processes involved in the absorption and metabolism of dietary polyphenols. Caco-2 cells are derived from human colon adenocarcinoma cells. After culture in Dulbecco's modified Eagle's medium supplemented with foetal calf serum (10%) and glutamate (20 m*M*) for ~21 days (37°C, 5% CO_2) on transwell plates, the confluent monolayer undergoes differentiation and exhibits characteristics of small intestinal cells. It is important to consider the enzymatic profile of the cells, which may not be identical to normal human small intestinal cells. Transformed varieties of Caco-2 cells exist, *e.g.*, the Caco-2 TC7 cell line has been transformed to express certain P450 enzymes present in normal human small intestinal cells.

Absorption studies with flavanols and procyanidins using Caco-2 cells are few; however, the system has been used to study the effect of flavonoids on the induction of phase I and II enzymes. Galijatovic *et al.* (13) observed that chrysin and quercetin induced UDP-glucuronosyltransferase in Caco-2 cells. Exposure of the cells to chrysin (50 μM, 2 h) resulted in a 3.8-fold increase in chrysin glucuronidation and a 38% decrease in sulphation in intact cells. Induction of phase II enzymes, such as UDP-glucuronosyltransferase, may be important for

the bioavailability of carcinogens and other toxic chemicals by increasing the ease and rate at which they are excreted from the body. Wahlgren *et al.* (65) studied the absorption of the predominant dietary form of quercetin, quercetin-4′-β-glucoside. Although quercetin itself was not transferred, they found that the transport of the glucoside involved apical multidrug resistance-associated protein-2, suggesting that the transfer of glycosides may occur in the small intestine. Experiments with normal Caco-2 cells and radiolabeled procyanidins suggested that dimer and trimer were transferred to the same extent as the EC monomer, whereas oligomers with an average degree of polymerisation of 7 were not (10). In contrast, we observed no transfer of the dimer across Caco-2 cells, which was in agreement with studies in an isolated rat small intestine model (see below).

Isolated rat gut model. The advantage of the isolated preparation (Fig. 5) to assess intestinal transfer of dietary polyphenols (and their glycosides) is that serial measurements of the rate of absorption from the lumen can be made using defined intestinal segments at short intervals. In addition, the solute under study appears on the serosal surface in the same form as if it were transferred to the mesenteric circulation. Therefore, enterocyte metabolism of flavanols and procyanidins, as well as their rate of transfer across specific gut regions, may be studied. Tissue viability is assessed by measurement of glucose transfer (12), and viability is confirmed by the finding that fluid transfer continues at a constant rate for the 90-min collection period and that glucose concentration in the absorbed fluid is over double that initially present in the perfused buffer (65.4 ± 3.7 m*M* versus 28 m*M*) (56). This indicates the ability for active solute transport.

Absorption studies, utilising this model, with a wide range of flavonoids, their glycosides, and hydroxycinnamates, show that there was in almost all cases extensive metabolism of the polyphenol in the enterocyte during transfer from the mucosal to the serosal side (56).

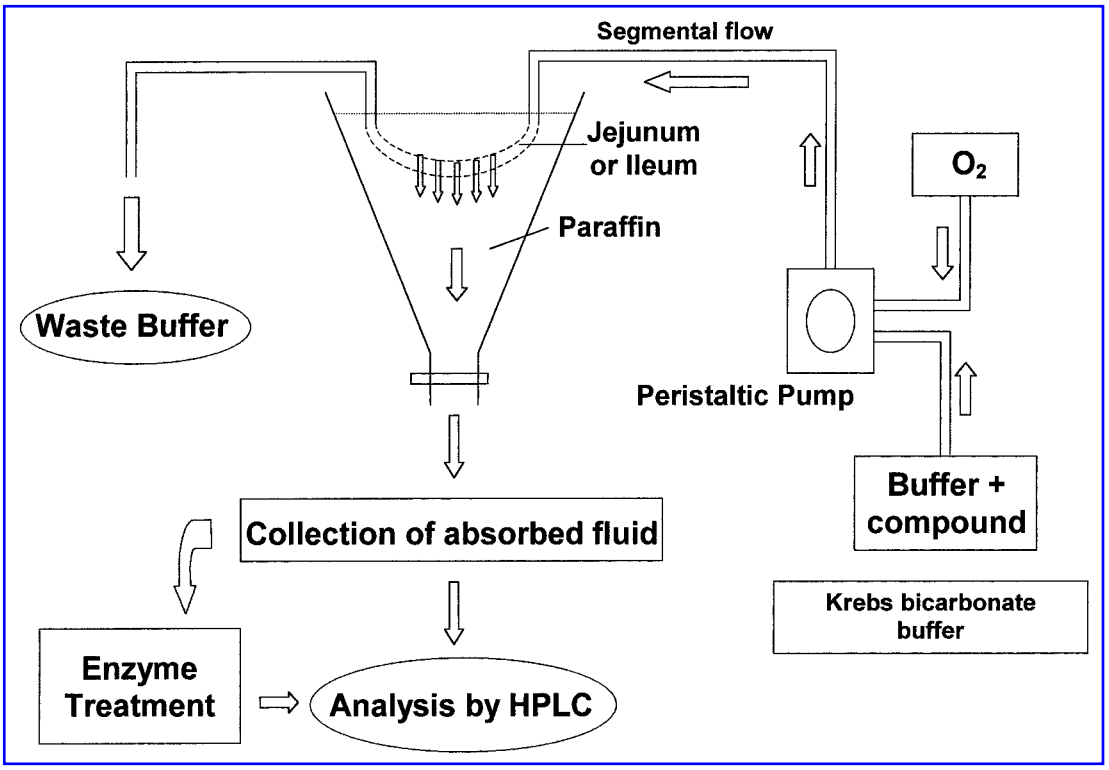

FIG. 5. Diagramatic representation of the isolated rat small intestine perfusion model. Buffers and compound are perfused for up to 90 min at a rate of 8 ml/min at 37°C using a peristaltic pump. Serosal fluid was collected from the bottom of the paraffin chamber and analysed by HPLC.

The flavonoid glycosides, luteolin-7-glucoside, kaempferol-3-glucoside, and quercetin-3-glucoside, were cleaved by rat jejunal or ileal mucosa, suggesting the presence of a glucosidase before efflux into the serosal fluid. It had traditionally been assumed that the glycosides could not be absorbed from the small intestine and cleavage of the β-glycosidic bond will not occur until the compound reaches the microflora of the large intestine (15). However, this study shows that transfer of these compounds is possible and occurs to different extents depending on the compounds' structure. The data of Spencer *et al.* (56) suggested that the major products (90–100%) transferred across the small intestinal epithelium were glucuronides of the parent aglycone or of the hydrolysed glycoside, although *O*-methylated metabolites were also observed. The extent of glucuronidation seemed dependent on the structure in that the monophenolic hydroxycinnamates and flavonoids with a substituted hydroxyl group on the B-ring (ferulic acid, *p*-coumaric acid, and hesperetin, respectively) were less predisposed to glucuronidation, whereas the flavonoids containing a 3′,4′-*ortho*-dihydroxy (or catechol) B-ring were transferred predominantly as glucuronides.

Similar studies with the flavanols catechin and EC yielded slightly different results. Although the major metabolites on the serosal side after perfusion of the jejunum with catechin or EC were always glucuronidated, there were also high levels of both *O*-methylated and *O*-methylglucuronidated forms (27) (Fig. 6). These *O*-methylated and *O*-methylglucuronidated catechins were the predominant metabolites detected in the serosal fluid (~50%), suggesting these as the most bioavailable forms (Fig. 7). In addition, the total percentage of flavanol transfer across the jejunum was lower (about sixfold) than across the ileum, which was due primarily to greater transfer of unmetabolised flavanol (27). The greater susceptibility to methylation of flavanols over other flavonoids in the jejunum may reside in the specificity of catechol-*O*-methyltransferase for these compounds (32). It is of interest, however, to note that cytochrome P450 has the potential to demethylate flavonols such as quercetin at the 4′ position, but not at the 3′ position (38). Methylation of the tea catechins, EGC, ECG, and EGCG, has been observed *in vitro* in the presence of rat liver homogenates and *S*-adenosyl-L-methionine (38). Structural analysis of the end products of the reaction by mass spectrometry (MS) and NMR showed that

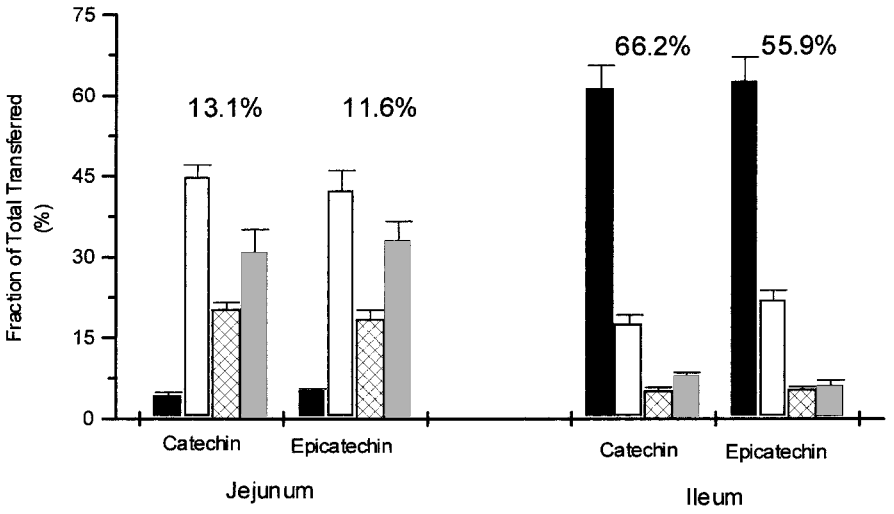

FIG. 6. Cumulative absorption of catechin and EC and their conjugates and metabolites after 90 min of perfusion through the isolated rat jejunum. The *y*-axis shows fraction of the total percent transferred. The numbers above columns indicate total percentage of flavanol-related compounds transferred in 90 min. Data are plotted as the means ± SEM of at least three separate experiments each analysed twice in triplicate. (■) Perfused compound; (□) glucuronide; (▩) *O*-methylated glucuronide; (▨) 3′-*O*-methylated and 4′-*O*-methylated.

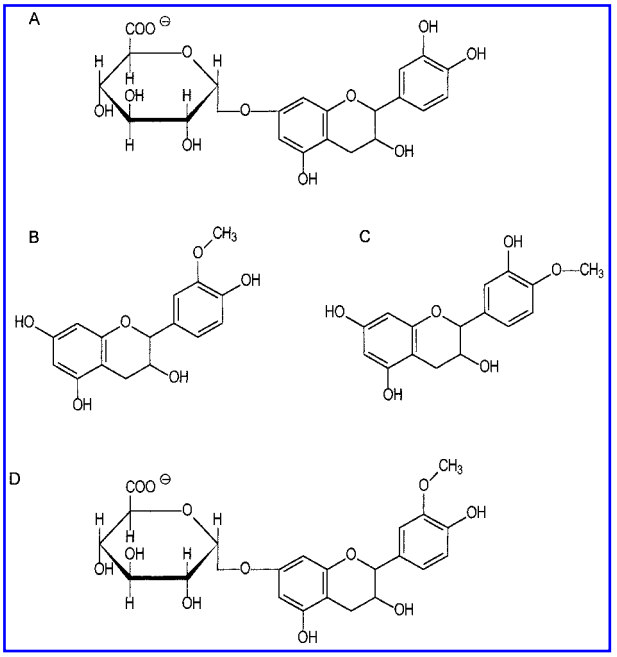

FIG. 7. Structures of conjugates and metabolites of EC. (A) Epicatechin-7-glucuronide; (B) 3′-*O*-methylepicatechin; (C) 4′-*O*-methylepicatechin; (D) 3′-*O*-methyl-(7-glucuronide)-epicatechin.

4′-*O*-methylated products were preferred in the case of the flavanols tested. This study indicates that any unmetabolised flavanols in the portal vein, after transport across the small intestine, may still undergo extensive metabolism in the liver.

The transfer and metabolism of the procyanidins are still unclear. Procyanidin oligomers greater than dimer are unlikely to be absorbed at the level of the small intestine in their native forms on the basis of their molecular size and as a consequence of their exposure to the acidic environment of the gastric lumen. The authors have found that there is no absorption of B2 and B5 procyanidin dimers across either the jejunum or ileum of the rat small intestine. However, perfusion of jejunum with dimer for 90 min resulted in recovery of both EC and 3′-*O*-methylcatechin in the serosal fluid, but no EC conjugates. These data suggest that enterocytes may be capable of cleaving the dimer to EC monomer units during transfer, and that these monomers are methylated by the catechol-*O*-methyltransferases before reaching the serosal side, but not glucuronidated. Higher procyanidin oligomers appear to be absorbed to a smaller extent in the small intestine, although no metabolites have been detected, suggesting that paracellular transport of these high molecular weight polyphenols may be occurring. With studies of this nature, the ultimate transfer and metabolism of such compounds may be affected by the extent to which they may bind both to proteins in the food matrix and also importantly to cell-surface proteins. The latter might be expected to cause possible damage to, or loss of viability of, small intestinal cells involved in the uptake of the polyphenols, which could complicate the interpretation of amounts of absorption.

METABOLISM AND ABSORPTION IN THE COLON

The role of the large intestine in the metabolism of polyphenols prior to subsequent absorption is increasingly considered to be important, as the total absorption in the small intestine is low. Studies have suggested that the extent of absorption of dietary polyphenols in the small intestine is ~10–20% (27,28,56), the majority reaching the large intestine. Enzymatic degradation of polyphenolics by colonic microflora results in a plethora of new metabolites. Bacterial enzymes may catalyse many reactions, including hydrolysis, dehydroxylation, demethylation, ring cleavage, and decarboxylation. The 5,7,3,3′,4′-hydroxylation pattern that catechin and EC possess is believed to enhance ring opening after hydrolysis (55). Metabolism of flavanols by enzymes of the microflora of the large intestine results in many metabolites: 3,4-dihydrophenylacetic acid, 3-hydroxyphenylacetic acid, homovanillic acid, and their conjugates derived from the B-ring (55) and phenolic acids from the C-ring. Flavanols, because of their structures (no C-4 carbonyl group), can also degrade to the specific metabolites phenylvalerolactones. Phenylpropionic acids (which may undergo further metabolism to benzoic acids) may also be the products of flavanol metabolism in animal studies, which demonstrates fission of the A-ring (55).

Such metabolites of flavanols have been detected in human plasma and urine after a single ingestion of green tea (29), which suggests

that there may be significant metabolism by gut microflora in the colon. The two metabolites, (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, were identified in urine by both liquid chromatography-MS/MS and NMR, appearing 7.5–13.5 h after ingestion (after a 3-h lag time), whereas EC and EGC peaked at 2 h. As well as their late excretion profiles, the amounts of metabolite excreted were 8–25-fold greater than that of EC and EGC excretion and accounted for 6–39% of the EC and EGC ingested. The late excretion and high levels of these metabolites would suggest that they are generated from the precursors EC and EGC by the intestinal microorganisms (Fig. 8).

Other specific metabolites have been observed in urine after consumption of a variety of phenolics. The glycine conjugate of benzoic acid, hippuric acid, is derived primarily from plant phenolics and aromatic amino acids through the action of intestinal bacteria. Consequently, the level of hippuric acid would be expected to increase in the urine of individuals consuming diets rich in flavanols or polyphenols in general. In human studies on black tea consumption (8) and on the application of a crude extract from *Equisetum arvense* (14), an association between the polyphenol intake and excreted amounts of hippuric acid was found. Hippuric acid could possibly derive from other sources such as quinic acid (43) or in quantitative terms, more importantly from the aromatic amino acids tryptophan, tyrosine, and phenyl-

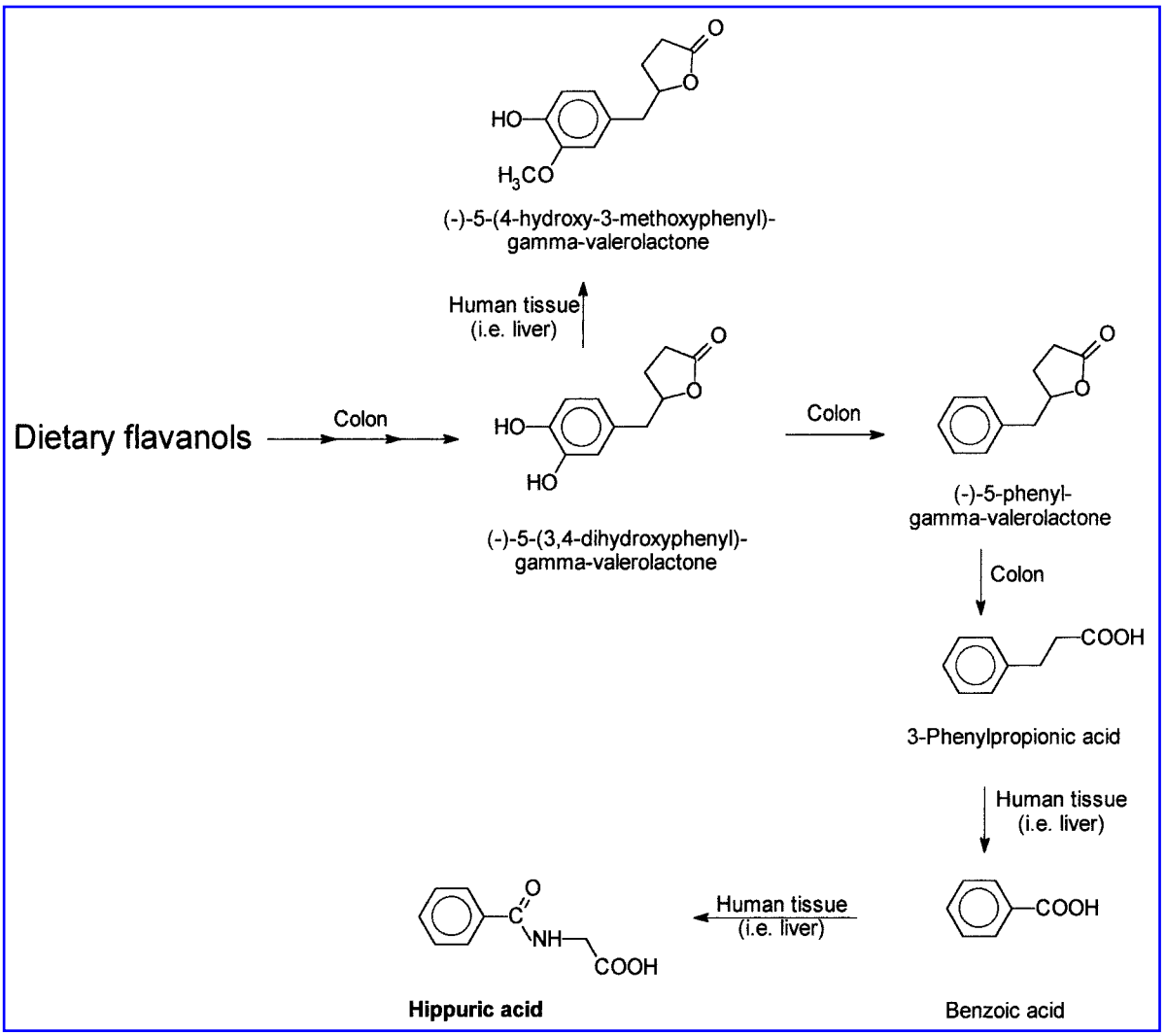

FIG. 8. Possible pathway of the formation of hippuric acid in humans.

alanine, as well as from the use of benzoic acid as a food preservative. The formation of hippuric acid and hydroxyhippuric acids seems to be a possible central metabolic pathway for dietary flavonoids, in which the colon microflora and the liver are active metabolic sites (55). A possible mechanism for the formation of hippuric acid from flavanols is suggested in Fig. 8. Other hydroxybenzoic acid glycine derivatives such as 4-hydroxyhippuric acid, vanilloylglycine, and isovanilloylglycine might also appear in reasonable amounts in urine after polyphenol consumption in general.

BIOAVAILABILITY AND POTENTIAL BIOACTIVITY

Bioavailability

Oral absorption and bioavailability of flavanols and procyanidins have been studied in both animals (in particular the rat) (6, 33, 35, 36, 43, 60, 63, 71) and humans (23, 34, 68) in terms of uptake and distribution. Zhu *et al*. (71) studied the absorption characteristics of three common tea catechins in rats that received either an intravenous (50 mg/kg) or oral (5,000 mg/kg) dose of a decaffeinated mixture containing EC (5%), ECG (50%), and EGCG (13%). Results showed low systemic availability of the catechin, which is presumably the result of slow absorption, high first-pass metabolism, and a wide tissue distribution. To highlight this, urinary recoveries of the catechins were low ranging between 0.17–4.72% after oral dosing and 2.11 and 14.20% after intravenous dosing. Absorption of EGCG into both the rat portal blood (36) and the circulation (63) has been observed in rats following relatively high intakes (50 mg) of the flavanol, indicating that it is absorbed unmetabolised to a small extent, reaching its highest level ~60 min after intake (63). Blood and urine levels of flavanols in humans were observed to increase after consumption of 1.5, 3.0, and 4.5 g of green tea solids (68). An increase in the dose from 1.5 to 3.0 g resulted in an approximate threefold increase in plasma concentrations of EGCG and EGC. However, as the dose was increased further, a saturation effect was observed. The pharmacokinetic parameters of excretion indicated that there was also no dose–response relationship (68). Tea catechins were observed to be increased in the protein-rich fraction of plasma (60%) and also in high-density lipoprotein (23%) after consumption of eight cups of tea a day (every 2 h) (64). However, this did not significantly enhance the resistance of the low-density lipoprotein to oxidation *ex vivo* (64).

More recent studies have concentrated on the detection of flavanol metabolites, in addition to the native compounds (3, 11, 16, 22, 31, 37, 44, 61). Work by Okushio *et al.* (37) reported that both methylated and glucuronidated metabolites could be detected in rat urine and plasma after oral administration of EC, but not in the bile. Time-course analysis indicated that the predominant metabolites in plasma and urine after EC consumption were conjugated forms of EC, such as a glucuronide, and 3′-*O*-methylepicatechin. The cumulative urinary excretion of the metabolites in a 24-h period amounted to 8% of the administered EC (37). Examination of bile from rats after oral administration of EGCG showed that six *O*-methylated metabolites of EGCG were present conjugated either to sulphate or to a glucuronide (22). Other studies detected epicatechin-5-*O*-β-glucuronide and catechin-5-*O*-β-glucuronide in plasma, bile, and urine of rats after consumption of EC and catechin, respectively (16), and these conjugates retained a similar ability to act as superoxide anion scavengers as their parent compounds. Piskula and Terao (44) have proposed that the mechanism of flavanol metabolism involves glucuronidation in the small intestine as the first detoxification step followed by *O*-methylation in the liver and kidney. Increases in 3′-*O*-methylcatechin, sulphate, and glucuronide metabolites in plasma have been noted after consumption of either red wine or dealcoholised red wine, both of which contained an ~35-mg dose of catechin (11). Plasma levels of catechin and all measured metabolites increased from <2 nmol/L to 91 ± 14 nmol/L after red wine consumption and 81 ± 11 nmol/L after consumption of dealcoholised wine. Plasma analysed 60 minutes after consumption demonstrated that ~21% of these metabolites were methylated and <2% of

catechin and 3′-*O*-methylcatechin were unconjugated, for example by glucuronidation (11).

Recently, Rein *et al.* (45) found that consumption of 80 g of semisweet chocolate, which is rich in procyanidins, resulted in 12-fold increases in plasma levels of EC 2 h after ingestion. In addition to the rise in EC levels from baseline levels of 22 nmol/L to 257 nmol/L, in the same time period there was also a significant increase in plasma Trolox equivalent antioxidant capacity (31%) and a significant decrease (40%) in thiobarbituric acid reactive substances (45). In support of these findings, Richelle *et al.* (48) also observed an increase in plasma levels of EC after ingestion of black chocolate (80 g) with levels peaking at 0.7 μmol/L between 2 and 3 h.

Bioactivity

The ability of 3′-*O*-methylepicatechin and EC to protect against apoptotic cell death induced by hydrogen peroxide or oxidised low-density lipoprotein has been investigated (58). Both the EC and its *O*-methylated metabolite exhibited the same degree of protection against apoptosis, highlighting the possibility that the hydrogen-donating ability of these compounds is not necessarily the significant factor in protection, but that both compounds may act on a molecular level by influencing the cell signaling pathway. However, the demethylation of the 3′-*O*-methylepicatechin may be a feature of its intracellular mechanism of action, allowing the regeneration of antioxidant activity. Other studies have demonstrated that oral administration of catechin to gerbils protected against ischaemia–reperfusion-induced death of hippocampal CA1 cells (20). The superoxide radical-scavenging activity of brains isolated from animals that received the catechin supplementation was significantly higher than in those that received control, suggesting that orally administered catechin is absorbed and is able to

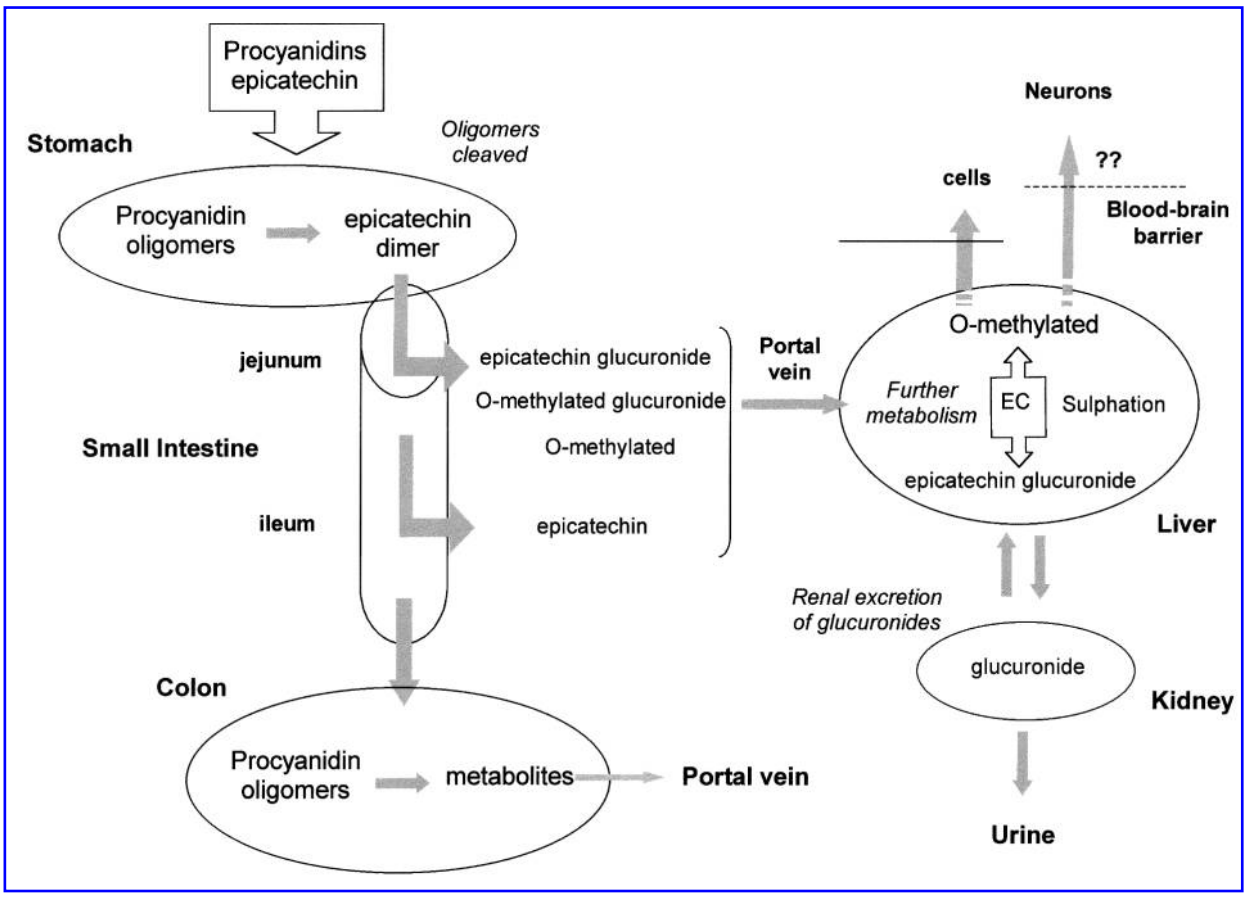

FIG. 9. Summary of the formation of metabolites and conjugates of flavanols in humans.

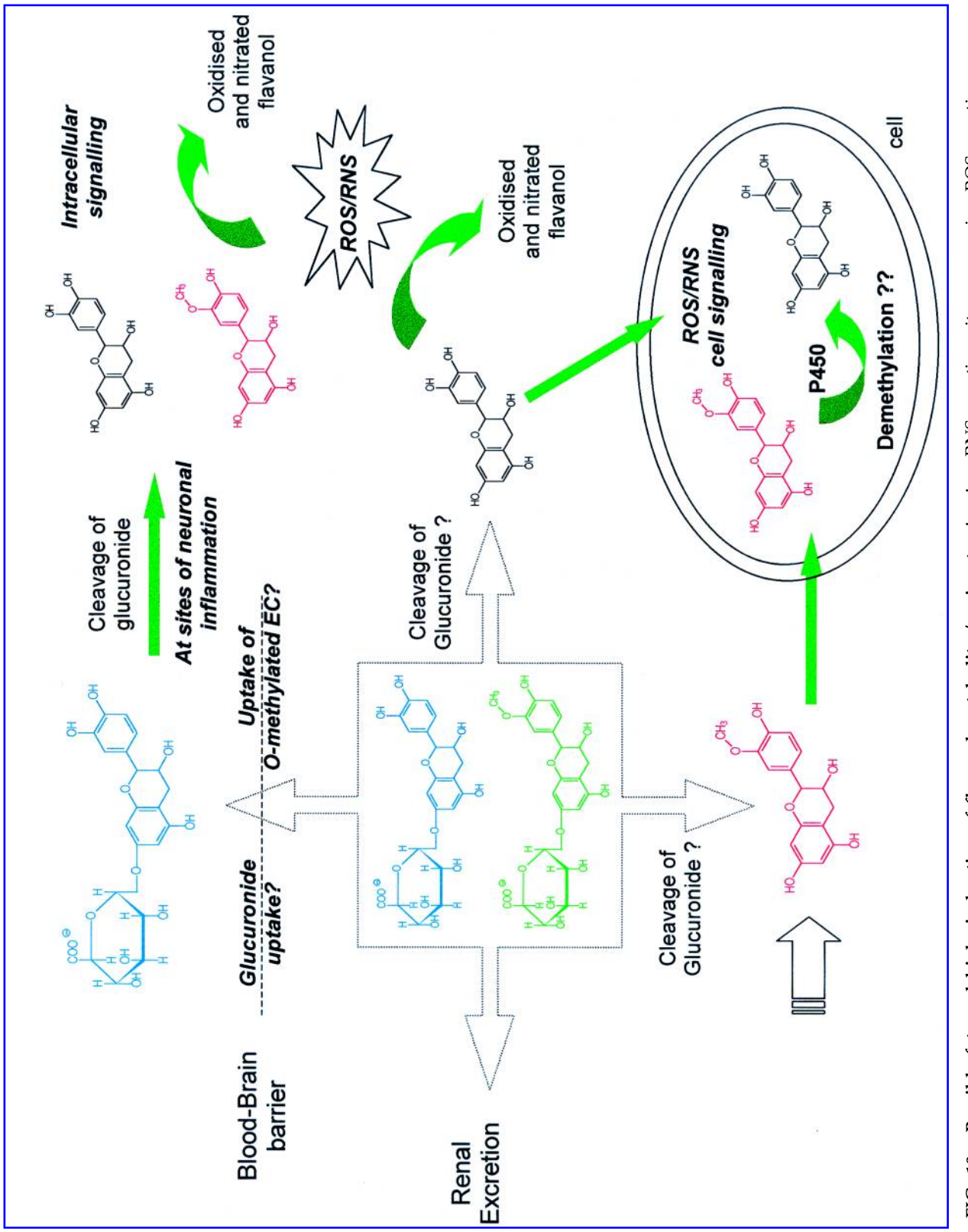

FIG. 10. **Possible fate and biological action of flavanol metabolites/conjugates *in vivo*.** RNS, reactive nitrogen species; ROS, reactive oxygen species.

cross the blood–brain barrier (20). The nature of the functional form of the flavanol and its mechanism of action in scavenging the superoxide radical is unclear. Recently, cocoa consumption has been shown to be active in suppression of ADP- or epinephrine-stimulated platelet activation and microparticle formation (45).

SUMMARY

Figure 9 summarises the absorption and metabolism of EC and procyanidin oligomers *in vivo*. Procyanidin oligomers are cleaved in the stomach into monomer and dimer units. Transfer across enterocytes in the small intestine results in extensive metabolism of EC to glucuronides, *O*-methylglucuronides, and *O*-methyl conjugates/metabolites. Any native EC entering the portal vein would be further metabolised/conjugated in the liver. Glucuronides of phenolic compounds have generally been assumed to be rapidly excreted *in vivo* and to be pharmacologically inactive. However, several studies are demonstrating that some drug glucuronides may be pharmacologically active (26, 59). For example, the ability of morphine glucuronide to cross the blood–brain barrier (1) and the analgesic action of morphine-6-glucuronide have been reported (39). If glucuronides are major circulating forms of flavanols, the question arises as to whether they are bioactive *in vivo*. β-Glucuronidases have been found in a variety of organs and body fluids, such as macrophages, blood cells, liver, lung, and serum (40, 59). Consequently, it is conceivable that glucuronides of flavanols could be cleaved back to the aglycone *in vivo* via the action of β-glucuronidases present in a variety of different cell populations, for example, macrophages at sites of inflammation or by other cells under oxidative stress (Fig. 10).

There is little doubt that catechins and procyanidins are powerful scavengers of reactive oxygen and reactive nitrogen species *in vitro* (2, 25, 41, 42, 50, 52, 67), however, it is not clear what influence the extent and positions of conjugation/metabolism (glucuronidation, sulphation, or *O*-methylation) might have on the antioxidant activities. It can be predicted that conjugation of the A-ring will not adversely influence antioxidant action, whereas modulation of the B-ring might limit antioxidant properties. However, the latter mechanism would also prevent oxidation of the flavanol and quinone formation, which would have deleterious effects.

Arteel and Sies (2) demonstrate that EC oligomers found in cocoa and chocolate powder, especially the tetramer, are effective inhibitors of peroxynitrite-induced tyrosine nitration, implying their role as potent dietary sources for defence against reactive nitrogen species. The bioactivities of these polyphenols *in vivo* will be independent of their oligomeric forms *in vitro*, and the metabolism of their monomeric and dimeric units is the significant feature. The affect of acidic gastric lumen on dietary phenols may have implications for the absorption of procyanidins from cocoa, apples, edible plant material (such as pinebark extract), and other forms of larger molecules derived from catechins, such as the theaflavins and thearubigins, chemically formed aggregates of catechin flavanols, in black tea. As oligomeric procyanidins are observed to liberate monomer and dimer units on incubation with acidic gastric contents, this would suggest that even though oligomers are not likely to cross the enterocytes of the small intestine, they may release large quantities of EC that can be absorbed. This has important implications for the action of procyanidins *in vivo*.

Recently, Spencer *et al.* (58) have shown that the *in vivo* EC metabolite, 3′-*O*-methylepicatechin, inhibits cell death induced by hydrogen peroxide and that the mechanism involves suppression of caspase-3 activity. Furthermore, the protection elicited by 3′-*O*-methylepicatechin is not significantly different from that of EC, suggesting that antioxidant activity is not the prime mechanism of protection. This would suggest a nonredox mechanism of action of flavonoids, which might be relevant *in vivo*. Although the native flavonoid is efficacious in scavenging reactive oxygen and reactive nitrogen species *in vitro*, on absorption *in vivo* it is subject to extensive metabolism and conjugation, both in the small intestine and in the liver, which may affect the redox potential and therefore the ability to act as a classical hydrogen-

donating antioxidant. These findings suggest that the ability of flavonoids, such as EC, to provide health benefits may not necessarily be dependent on the ability of the native compound to act as a scavenger of free radicals or reactive oxygen and reactive nitrogen species, but on the ability of their metabolites to interact with cell signaling cascades, to influence the cell at a transcriptional level, or to down-regulate pathways leading to apoptosis. Consequently, the redox potential *per se* may not be the fundamental feature when determining the ability of specific phenolics to protect against either oxidative or other cell insults.

ABBREVIATIONS

EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; MS, mass spectrometry.

REFERENCES

1. Aasmundstad TA, Morland J, and Paulsen RE. Distribution of morphine-6-glucuronide and morphine across the blood–brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. *J Pharmacol Exp Ther* 275: 435–441, 1995.
2. Arteel GE and Sies H. Protection against peroxynitrite by cocoa polyphenol oligomers. *FEBS Lett* 462: 167–170, 1999.
3. Bell JR, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, and Kasim-Karakas SE. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. *Am J Clin Nutr* 71: 103–108, 2000.
4. Bors W, Heller W, Michel C, and Saran M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. *Methods Enzymol* 186: 343–355, 1990.
5. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr Rev* 56: 317–333, 1998.
6. Chen L, Lee MJ, and Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab Dispos* 25: 1045 1050, 1997.
7. Chen ZY, Zhu QY, Wong YF, Zhang ZS, and Chung HY. Stabilising effect of ascorbic acid on green tea catechins. *J Agric Food Chem* 46: 2512–2516, 1998.
8. Clifford MN, Copeland EL, Bloxsidge JP, and Mitchell LA Hippuric acid as a major excretion product associated with black tea consumption. *Xenobiotica* 50: 317–326, 2000.
9. Day AJ, DuPont MS, Ridley S, Rhodes MJC, Morgan MRA, and Williamson G. Deglycosylation of the flavonoid and its isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett* 436: 71–75, 1998.
10. Deprez S (Ed). *Biomarquage de Tannins Condenses et Etude de leur Biodisponibilite dans l'Organisme Humain.* Paris, France: Institut National Agronomique Paris-Grignon, 1999.
11. Donovan JL, Bell JR, Kasim-Karakas S, German JB, Walzem RL, Hansen RJ, and Waterhouse AL. Catechin is present as metabolites in human plasma after consumption of red wine. *J Nutr* 129: 1662–1668, 1999.
12. Fisher RB and Gardner MLG. A kinetic approach to the study of absorption of solutes by isolated perfused small intestine. *J Physiol* (*Lond*) 241: 211–234, 1974.
13. Galijatovic A, Walle UK, and Walle T. Induction of UDP-glucuronosyltransferase by the flavonoids chrysin and quercetin in caco-2 cells. *Pharm Res* 17: 21–26, 2000.
14. Graefe EU and Veit M. Urinary metabolites of flavonoids and hydroxycinnamic acids in humans after application of a crude extract from *Equisetum arvense*. *Phytomedicine* 6: 239–246, 1999.
15. Griffiths LA and Barrow A. Metabolism of flavonoid compounds in germ-free rats. *Biochem J* 130:1161–1162, 1972.
16. Harada M, Kan Y, Naoki H, Fukui Y, Kageyama N, Nakai M, Miki W, and Kiso Y. Identification of the major antioxidative metabolites in biological fluids of the rat with ingested (+)-catechin and (−)-epicatechin. *Biosci Biotechnol Biochem* 63: 973–977, 1999.
17. Hertog MG, Feskens EJ, Hollman PC, Katan MB, and Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 342: 1007–1011, 1993.
18. Higuchi WI, Ho NF, Park JY, and Komiya I. Rate-limiting steps and factors in drug absorption. In: *Drug Absorption,* edited by Prescott LF and Nimno WS. New York: ADIS Press, 1981, pp. 35–60.
19. Ho NF, Park JY, Ni PF, and Higuchi WI. Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. In: *Animal Models for Oral Drug Delivery. In Situ and In Vivo Approaches,* edited by Crouthamel W and Sarapu AC. Washington, D.C.: American Pharmaceutics Association, 1983, pp. 27–106.
20. Inanami O, Watanabe Y, Syuto B, Nakano M, Tsuji M, and Kuwabara M. Oral administration of (−)-catechin protects against ischemia–reperfusion-induced neuronal dearth in the gerbil. *Free Rad Biol Med* 29: 359–365, 1998.
21. Keli SO, Hertog MGL, Feskens EJM, and Kromhaut D. Flavonoids, antioxidant vitamins and the risk of stoke. *Arch Intern Med* 154: 637–642, 1996.
22. Kida K, Suzuki M, Matsumoto N, Nanjo F, and Hara Y. Identification of biliary metabolites of (−)-epicatechin gallate in rats. *J Agric Food Chem* 48: 4151–4155, 2000.
23. Kim S, Lee MJ, Hong J, Li C, Smith TJ, Yang GY, Seril DN, and Yang CS. Plasma and tissue levels of tea cat-

echins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* 37: 41–48, 2000.
24. Knekt P, Jarvinen R, Reunanen A, and Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ* 312: 478–481, 1996.
25. Kondo K, Hiran R, Matsumoto A, Igarishi O, and Itakure H. Inhibition of LDL oxidation by cocoa. *Lancet* 348: 1514, 1996.
26. Kroemer HK and Klotz U. Glucuronidation of drugs: a re-evaluation of the pharmacological significance of the conjugates and modulating factors. *Clin Pharmacokinet* 23: 292–310, 1992.
27. Kuhnle G, Spencer JPE, Schroeter H, Shenoy B, Debnam E, Srai SK, Rice-Evans C, and Hahn U. Epicatechin and catechin are *O*-methylated and glucuronidated in the small intestine. *Biochem Biophys Res Commun* 227: 507–512, 2000.
28. Kuhnle G, Spencer JPE, Schroeter H, Srai SK, Debnam ES, Rice-Evans C, and Hahn U. Absorption and metabolism of resveratrol in the small intestine. Implications for resveratrol *in vivo*. *Biochem Biophys Res Commun* 272: 212–217, 2000.
29. Li C, Lee MJ, Sheng SQ, Meng XF, Prabhu S, Winnik B, Huang BM, Chung JY, Yan SQ, Ho CT, and Yang CS. Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem Res Toxicol* 13: 177–184, 2000.
30. Lin JH, Chiba M, and Baillie TA. Is the role of the small intestine in first-pass metabolism over-emphasised? *Pharmacol Rev* 51: 135–157, 1999.
31. Manach C, Texier O, Morand C, Regerat F, Demigne C, and Remesy C. Comparison of the bioavailability of quercetin and catechin in rats. *Free Radic Biol Med* 27: 1259–1266, 1999.
32. Mannisto PT and Kaakkola S. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 51: 593–628, 1999.
33. Nakagawa K and Miyazawa T. Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. *J Nutr Sci Vitaminol* 43: 679–684, 1997.
34. Nakagawa K, Okuda S, and Miyazawa T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. *Biosci Biotechnol Biochem* 61: 1981–1985, 1997.
35. Okushio K, Matsumoto N, Suzuki M, Nanjo F, and Hara Y. Absorption of (−)-epigallocatechin gallate into rat portal vein. *Biol Pharm Bull* 18: 190–191, 1995.
36. Okushio K, Matsumoto N, Kohri T, Suzuki M, Nanjo F, and Hara Y. Absorption of tea catechins into rat portal vein. *Biol Pharm Bull* 19: 326–329, 1996.
37. Okushio K, Suzuki M, Matsumoto N, Nanjo F, and Hara Y. Identification of (−)-epicatechin metabolites and their metabolic fate in the rat. *Drug Metab Dispos* 27: 309–316, 1999.
38. Okushio K, Suzuki M, Matsumoto N, Nanjo F, and Hara Y. Methylation of tea catechins by rat liver homogenates. *Biosci Biotechnol Biochem* 63: 430–432, 1999.
39. Osbourne RJ, Joel SP, and Slevin ML. Analgesic action of morphine-6-glucuronide. *Lancet* 1: 828, 1988.
40. Paigen K. Mammalian β-glucuronidase: genetics, molecular biology and cell biology. *Prog Nucleic Acid Res Mol Biol* 37: 155–205, 1989.
41. Pannala AS, Rice-Evans CA, Halliwell B, and Singh S. Inhibition of peroxynitrite-mediated tyrosine nitration by catechin polyphenols. *Biochem Biophys Res Commun* 232: 164–168, 1997.
42. Pannala AS, Razaq R, Halliwell B, Singh S, and Rice-Evans CA. Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation. *Free Radic Biol Med* 24: 594–606, 1998.
43. Phibbs AN, Stewart J, Wright B, and Wilson ID. Effect of diet on the urinary excretion of hippuric acid and other dietary-derived aromatics in rat. A complex interaction between diet, gut microflora and substrate specifity. *Xenobiotica* 28: 527–537, 1998.
44. Piskula MK and Terao J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *J Nutr* 128: 1172–1178, 1998.
45. Rein D, Lotito S, Holt RR, Keen CL, Schmitz HH, and Fraga CG. Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma antioxidant status. *J Nutr* 130: 2109S–2114S, 2000.
46. Rein D, Paglieroni T, Wun T, Pearson D, Schmitz HH, Gosselin R, and Keen CL. Cocoa inhibits platelet activation and function. *Am J Clin Nutr* 72: 30–35, 2000.
47. Rice-Evans CA, Miller NJ, and Paganga G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20: 933–956, 1996.
48. Richelle M, Tavazzi I, Enslen M, and Offord EA. Plasma kinetics in man of epicatechin from black chocolate. *Eur J Clin Nutr* 53: 22–26, 1999.
49. Saito M, Hosoyama H, Ariga T, Kataoka S, and Yamaji N. Anti-ulcer activity of grape seed extract and procyanidins. *J Agric Food Chem* 46: 1460–1464, 1998.
50. Salah N. Polyphenolic flavanols as scavengers of aqueous phase radicals and chain-breaking antioxidants. *Arch Biochem Biophys* 322: 339–346, 1995.
51. Sanbongi C, Suzuki N, and Sakane T. Polyphenols in chocolate which have antioxidant activity, modulate immune function in humans in vitro. *Cell Immunol* 177: 129–136, 1997.
52. Sanbongi C, Osakabe N, Natsume M, Takizawa T, Gomi S and Osawa T. Antioxidative polyphenols isolated from Theobroma cacao. *J Agric Food Chem* 46: 454–457, 1998.
53. Santos-Buelga C and Scalbert A. Proanthocyanidins and tannin-like compounds: nature, occurrence, dietary intake and effects on nutrition and health. *J Food Sci Agric* 80: 1094–1117, 2000.
54. Scalbert A and Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr* 130: 2073S–2085S, 2000.
55. Scheline RR. Metabolism of oxygen heterocyclic com-

pounds. In: *CRC Handbook of Mammalian Metabolism of Plant Compounds.* Boca Raton, FL: CRC Press, 1991, pp. 279–284.
56. Spencer JPE, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, and Rice-Evans C. The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett* 458: 224–230, 1999.
57. Spencer JPE, Choudry F, Pannala AS, Srai SK, Debnam ES, and Rice-Evans C. Decomposition of cocoa procyanidins in the gastric milieu—implications for absorption. *Biochem Biophys Res Commun* 272: 236–241, 2000.
58. Spencer JPE, Schroeter H, Kuhnle G, Srai SKS, Tyrrell RM, Hahn U, and Rice-Evans C. Epicatechin and its in vivo metabolite, 3′-*O*-methyl epicatechin, protect human fibroblasts from oxidative stress-induced cell death involving caspase-3 activation. *Biochem J* 354: 493–500, 2001.
59. Sperker B, Backman T, and Kroemer HK. The role of β-glucuronidase in drug disposition and drug targeting in humans. *Clin Pharmacokinet* 33: 18–31, 1997.
60. Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, and Fujiki H. Wide distribution of [^{3}H] (−)epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* 19: 1771–1776, 1998.
61. Terao J. Dietary flavonoids as antioxidants in vivo: conjugated metabolites of (−)-epicatechin and quercetin participate in antioxidative defence in blood plasma. *J Med Invest* 46: 159–168, 1999.
62. Tsuchiya H, Sato M, Kato H, Okubo T, Juneja LR, and Kim M. Simultaneous determination of catechins in human saliva by high-performance liquid chromatography. *J Chromatogr B* 703: 253–258, 1997.
63. Unno T and Takeo T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats. *Biosci Biotechnol Biochem* 59: 1558–1559, 1995.
64. Van het Hof KH, Wiseman SA, Yang CS, and Tuburg LBM. Plasma and lipoprotein levels of tea catechins following repeated tea consumption. *Proc Soc Exp Biol Med* 220: 203–209, 1999.
65. Wahlgren RA, Karnaky KJ, Lindenmayer GE, and Walle T. Efflux of dietary quercetin-4′-glucoside across human intestinal caco-2 cell monolayers by apical multidrug resistance-associated protein-2. *J Pharmacol Exp Ther* 294: 830–836, 2000.
66. Walle UK, Galijatovic A, and Walle T. Transport of the flavonoid chysin and its conjugated metabolites by the human intestinal cell line Caco-2. *Biochem Pharmacol* 58: 431–438, 1999.
67. Waterhouse AL, Shirley JR, and Donovan JL. Antioxidants in chocolate. *Lancet* 348: 834, 1996.
68. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, and Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 7: 351–354, 1998.
69. Yang CS, Lee M, and Chen LS. Human salivary tea catechin levels and catechin esterase activities: implication in human cancer prevention studies. *Cancer Epidemiol Biomarkers Prev* 8: 83–89, 1999.
70. Yoshino K, Suzuki M, Sasaki K, Miyase T, and Sano M. Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. *J Nutr Biochem* 10: 223–229, 1999.
71. Zhu M, Chen Y, and Li RC. Oral absorption and bioavailability of tea catechins. *Planta Med* 66: 444–447, 2000.

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2. Jeong-Keun Kim, Keun-Tae Park, Hyun-Sun Lee, Mijin Kim, Young-Hee Lim. 2012. Evaluation of the inhibition of mushroom tyrosinase and cellular tyrosinase activities of oxyresveratrol: comparison with mulberroside A. *Journal of Enzyme Inhibition and Medicinal Chemistry* **27**:4, 495-503. [CrossRef]
3. David Vauzour, Katerina VafeiadouFlavonoids and Neuroinflammation **20121374**, . [CrossRef]
4. David Vauzour, Katerina Vafeiadou, Jeremy P. E. SpencerPolyphenols 110-145. [CrossRef]
5. Azadeh Ebrahimi, Hermann Schluesener. 2012. Natural polyphenols against neurodegenerative disorders: Potentials and pitfalls. *Ageing Research Reviews* . [CrossRef]
6. Sam-Long Hwang, Ping-Hsiao Shih, Gow-Chin Yen. 2012. Neuroprotective Effects of Citrus Flavonoids. *Journal of Agricultural and Food Chemistry* **60**:4, 877-885. [CrossRef]
7. Gordon van't Slot, Katrin Rose, Anna Engemann, Hans#Ulrich HumpfThe Pig Cecum Model – A Powerful Tool for Studying the Intestinal Metabolism of Flavonoids **1093**, 463-486. [CrossRef]
8. Jack W. Blount, Mario Ferruzzi, Dan Raftery, Giulio M. Pasinetti, Richard A. Dixon. 2012. Enzymatic synthesis of substituted epicatechins for bioactivity studies in neurological disorders. *Biochemical and Biophysical Research Communications* **417**:1, 457-461. [CrossRef]
9. H Jacobs, G H Koek, R Peters, M Moalin, J Tack, W J van der Vijgh, A Bast, G R Haenen. 2011. Differences in Pharmacological Activities of the Antioxidant Flavonoid MonoHER in Humans and Mice Are Caused by Variations in Its Metabolic Profile. *Clinical Pharmacology & Therapeutics* . [CrossRef]
10. Vito Verardo, Ylenia Riciputi, Maria Cristina Messia, Melania Vallicelli, Luisa Falasca, Emanuele Marconi, Maria Fiorenza Caboni. 2011. Dietary fiber and flavan-3-ols in shortbread biscuits enriched with barley flours co-products. *International Journal of Food Sciences and Nutrition* **62**:3, 262-269. [CrossRef]
11. Pradeep K. Sharma, Min He, Leo J. Romanczyk, Hagen Schroeter. 2010. Synthesis of [2-13C, 4-13C]-(2R,3S)-catechin and [2-13C, 4-13C]-(2R,3R)-epicatechin. *Journal of Labelled Compounds and Radiopharmaceuticals* **53**:10, 605-612. [CrossRef]
12. Orly Weinreb, Tamar Amit, Silvia Mandel, Moussa B. H. Youdim. 2009. Neuroprotective molecular mechanisms of (-)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties. *Genes & Nutrition* **4**:4, 283-296. [CrossRef]
13. E. Pappas, K. M. Schaich. 2009. Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability. *Critical Reviews in Food Science and Nutrition* **49**:9, 741-781. [CrossRef]
14. Katerina, Ana Rodriguez Mateos, David Vauzour, Jeremy Spencer, VafeiadouDietary Flavonoids as Neuroprotective Agents **20091218**, . [CrossRef]
15. Emma Ramiro-Puig, Margarida Castell. 2009. Cocoa: antioxidant and immunomodulator. *British Journal of Nutrition* **101**:07, 931. [CrossRef]
16. Teresa Pérez-Berezo, Emma Ramiro-Puig, Francisco J. Pérez-Cano, Cristina Castellote, Joan Permanyer, Àngels Franch, Margarida Castell. 2009. Influence of a cocoa-enriched diet on specific immune response in ovalbumin-sensitized rats. *Molecular Nutrition & Food Research* **53**:3, 389-397. [CrossRef]
17. Thomas P. Kenny , Shang-an Shu , Yuki Moritoki , Carl L. Keen , M. Eric Gershwin . 2009. Cocoa Flavanols and Procyanidins Can Modulate the Lipopolysaccharide Activation of Polymorphonuclear Cells In Vitro. *Journal of Medicinal Food* **12**:1, 1-7. [Abstract] [Full Text PDF] [Full Text PDF with Links]

18. David Vauzour, Katerina Vafeiadou, Ana Rodriguez-Mateos, Catarina Rendeiro, Jeremy P. E. Spencer. 2008. The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes & Nutrition* **3**:3-4, 115-126. [CrossRef]
19. Laura Bonfili, Valentina Cecarini, Manila Amici, Massimiliano Cuccioloni, Mauro Angeletti, Jeffrey N. Keller, Anna M. Eleuteri. 2008. Natural polyphenols as proteasome modulators and their role as anti-cancer compounds. *FEBS Journal* . [CrossRef]
20. Jeremy P. E. Spencer. 2008. Flavonoids: modulators of brain function?. *British Journal of Nutrition* **99**:E-S1. . [CrossRef]
21. Jeremy P. E. Spencer. 2008. Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society* **67**:02, 238-252. [CrossRef]
22. Xenofon Tzounis, Jelena Vulevic, Gunter G. C. Kuhnle, Trevor George, Jadwiga Leonczak, Glenn R. Gibson, Catherine Kwik-Uribe, Jeremy P. E. Spencer. 2008. Flavanol monomer-induced changes to the human faecal microflora. *British Journal of Nutrition* **99**:04. . [CrossRef]
23. Karen A. Cooper, Jennifer L. Donovan, Andrew L. Waterhouse, Gary Williamson. 2008. Cocoa and health: a decade of research. *British Journal of Nutrition* **99**:01. . [CrossRef]
24. Jeremy P. E. Spencer, Manal M. Abd El Mohsen, Ann-Marie Minihane, John C. Mathers. 2008. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *British Journal of Nutrition* **99**:01. . [CrossRef]
25. Jeremy P. E. Spencer. 2007. The interactions of flavonoids within neuronal signalling pathways. *Genes & Nutrition* **2**:3, 257-273. [CrossRef]
26. C LAURENT, P BESANCON, B CAPORICCIO. 2007. Flavonoids from a grape seed extract interact with digestive secretions and intestinal cells as assessed in an in vitro digestion/Caco-2 cell culture model. *Food Chemistry* **100**:4, 1704-1712. [CrossRef]
27. Susan E. Pollard, Gunter G.C. Kuhnle, David Vauzour, Katerina Vafeiadou, Xenofon Tzounis, Matthew Whiteman, Catherine Rice-Evans, Jeremy P.E. Spencer. 2006. The reaction of flavonoid metabolites with peroxynitrite. *Biochemical and Biophysical Research Communications* **350**:4, 960-968. [CrossRef]
28. Montse Pinent, Cinta Bladé, M. Salvadó, Mayte Blay, Gerard Pujadas, Juan Fernández-Larrea, Lluís Arola, Anna Ardévol. 2006. Procyanidin Effects on Adipocyte-Related Pathologies. *Critical Reviews in Food Science and Nutrition* **46**:7, 543-550. [CrossRef]
29. Christian Heiss, Petra Kleinbongard, Andrè Dejam, Sandra Perré, Hagen Schroeter, Helmut Sies, Malte Kelm. 2005. Acute Consumption of Flavanol-Rich Cocoa and the Reversal of Endothelial Dysfunction in Smokers. *Journal of the American College of Cardiology* **46**:7, 1276-1283. [CrossRef]
30. Jeremy SpencerClassification, Dietary Sources, Absorption, Bioavailability, and Metabolism of Flavonoids **20052271**, . [CrossRef]
31. DAVID J. LOREN, NAVINDRA P. SEERAM, RISA N. SCHULMAN, DAVID M. HOLTZMAN. 2005. Maternal Dietary Supplementation with Pomegranate Juice Is Neuroprotective in an Animal Model of Neonatal Hypoxic-Ischemic Brain Injury. *Pediatric Research* **57**:6, 858-864. [CrossRef]
32. Jeremy SpencerInteractions of Flavonoids and Their Metabolites with Cell Signaling Cascades **20055079**, 353-378. [CrossRef]
33. Robert J Williams, Jeremy P.E Spencer, Catherine Rice-Evans. 2004. Flavonoids: antioxidants or signalling molecules?. *Free Radical Biology and Medicine* **36**:7, 838-849. [CrossRef]
34. Jeremy P.E Spencer, Manal M Abd El Mohsen, Catherine Rice-Evans. 2004. Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity. *Archives of Biochemistry and Biophysics* **423**:1, 148-161. [CrossRef]
35. Sharmila Basu-Modak, Matthew J Gordon, Laura H Dobson, Jeremy P.E Spencer, Catherine Rice-Evans, Rex M Tyrrell. 2003. Epicatechin and its methylated metabolite attenuate UVA-induced

oxidative damage to human skin fibroblasts. *Free Radical Biology and Medicine* **35**:8, 910-921. [CrossRef]

36. Francene M Steinberg, Monica M Bearden, Carl L Keen. 2003. Cocoa and chocolate flavonoids: Implications for cardiovascular health. *Journal of the American Dietetic Association* **103**:2, 215-223. [CrossRef]
37. Catherine A. Rice-Evans , Debasis Bagchi . 2001. Nutritional Proanthocyanidins, Flavonoids, and Related Phenols. *Antioxidants & Redox Signaling* **3**:6, 939-940. [Citation] [Full Text PDF] [Full Text PDF with Links]