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Colonic metabolites of berry polyphenols: the missing link to biological activity?

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The absorption of dietary phenols, polyphenols and tannins (PPT) is an essential step for biological activity and effects on health. Although a proportion of these dietary bioactive compounds are absorbed intact, depending on their chemical structure and the nature of any attached moiety (e.g. sugar, organic acid), substantial amounts of lower molecular weight catabolites are absorbed after biotransformation by the colon microflora. The main products in the colon are (a) benzoic acids (C_6–C_1), especially benzoic acid and protocatechuic acid; (b) phenylacetic acids (C_6–C_2), especially phenylacetic acid *per se*; (c) phenylpropionic acids (C_6–C_3), where the latter are almost entirely in the dihydro form, notably dihydrocaffeic acid, dihydroferulic acid, phenylpropionic acid and 3-(3′-hydroxyphenyl)-propionic acid. As a result of this biotransformation, some of these compounds can each reach mM concentrations in faecal water. Many of these catabolites are efficiently absorbed in the colon, appear in the blood and are ultimately excreted in the urine. In the case of certain polyphenols, such as anthocyanins, these catabolites are major products *in vivo*; protocatechuic acid is reported to represent a substantial amount of the ingested dose of cyanidin-3-*O*-glucoside. The major catabolites of berries, and especially blackcurrants, are predicted based on compositional data for polyphenols from berries and other sources. Since microbial catabolites may be present at many sites of the body in higher concentration than the parent compound, it is proposed that at least a part of the biological activities ascribed to berry polyphenols and other PPT are due to their colonic catabolites.

Anthocyanins: Berry: Blackcurrant: Chlorogenic acids: Flavonoids: Gut microflora: Phenylacetic acid: Phenylpropionic acid: Protocatechuic acid

Dietary polyphenols are found in fruits and vegetables, and in products derived from plants such as fruit beverages, tea, coffee and dark chocolate. Epidemiological evidence shows that consumption of polyphenol-rich foods reduces the risk of CVD and associated conditions[1], and human intervention studies have supported this association[2]. The absorption and metabolism of flavonoids is quite well described for some compounds, such as quercetin and (−)-epicatechin[3]. However, although the absorption of certain other phenols, polyphenols and tannins (PPT) such as procyanidins, chlorogenic acids and anthocyanins has been described in the literature, the levels of the parent compounds in blood after a high dose, or a large amount of PPT-rich food, are very low compared with other flavonoids[4,5]. In contrast, intervention studies show that procyanidin-rich, chlorogenic acid-rich or anthocyanin-rich foods affect certain biomarkers, but because the concentration of parent compounds to be expected in blood after consumption is too low to affect these biomarkers[6], other bioactive substances such as metabolites may be responsible and must be identified. It is proposed that these 'missing' components are the colonic catabolites, and that they are potentially important compounds mediating some of the biological activities and health benefits of polyphenol-rich foods.

In excess of 8000 phenolic compounds have been reported, and they are widely dispersed throughout the plant kingdom. The nature of those occurring in foods and beverages has been reviewed[7,8]. Briefly, they can be subdivided into flavonoids (anthocyanins, chalcones and dihydrochalcones, flavanols or catechins, flavanones, flavones, flavonols, isoflavones and proanthocyanidins or condensed tannins) and non-flavonoids (benzoic acids, cinnamic acids and cinnamic acid conjugates, such as the chlorogenic acids, and gallic acid esters or hydrolysable tannins). To these must be added the 'derived polyphenols', substances that are characteristic of many traditionally processed foods and beverages (coffee, black tea, matured red wine, etc.) but which do not occur in healthy intact plant tissue. Collectively, these three major groups will in this review be referred to as PPT. In addition, this review will discuss the aromatic and phenolic acids produced from these PPT by the gut microflora, many of which do not occur preformed in the diet. These acids can be classified by the number of carbons in the side chain (one to five), and whether the side chain is saturated or unsaturated, and whether it carries an aliphatic hydroxyl. Specimen structures are shown in Fig. 1.

Abbreviations: GIT, gastro-intestinal tract; PPT, phenols, polyphenols and tannins.

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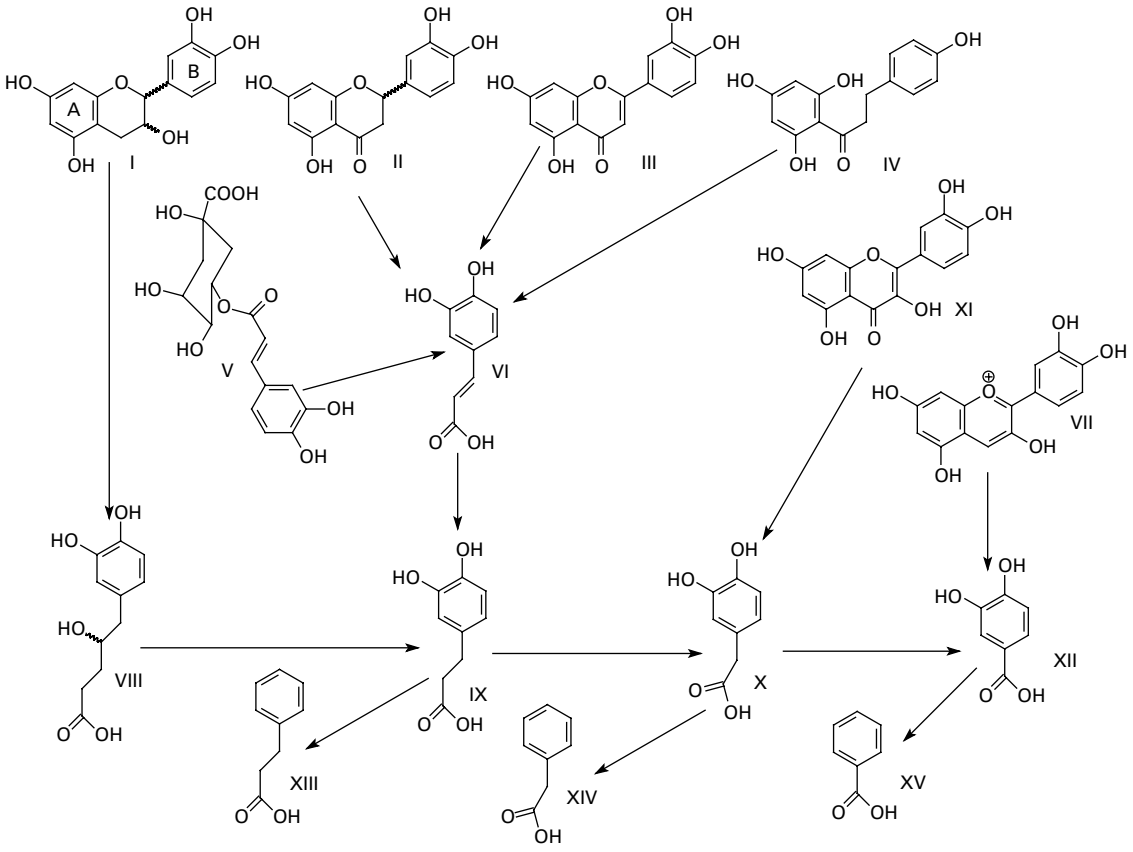

Fig. 1. Structures of selected phenols, polyphenols and tannins, and selected gut microflora catabolites. A and B indicate the A- and B-ring, respectively, of a typical flavonoid. Typical structures are shown for flavanols (catechins) (I); flavanones (II); flavones (III); dihydrochalcones (IV); chlorogenic acids (cinnamate conjugates) (V); cinnamic acids (C_6-C_3) (VI); anthocyanidins (VII); phenylvaleric acids (C_6-C_5-γ-OH) (VII); phenylpropionic acids (C_6-C_3) (IX. XIII); phenylacetic acids (C_6-C_2) (X, XIV); flavonols (XI); benzoic acids (C_6-C_1) (XII, XV). Note: it is not possible to show all possible substrates, intermediates and pathways but those shown are representative.

Polyphenol breakdown in the colon

Bacterial composition of the human gut

The first part of this review is concerned primarily with the capability of the microflora in the distal gastro-intestinal tract (GIT) to transform PPT provided by the diet. A detailed discussion of the complex nature and composition of this microflora is beyond the scope of this review, but some basic statistics are instructive. The GIT is home to between some 10 and 100 trillion microbes[9], approximately 10-times as many cells as found in the human body[10]. The corresponding microbiome is said to contain more than 100-times as many genes as the human genome[9]. Each portion of the GIT (mouth, oesophagus, stomach, small intestine and large intestine) has a distinctive flora, but the majority of the organisms are located in the distal GIT, where the concentration of some 500 bacterial species may reach $10^{11}-10^{12}$ colony forming units/g, and account for some 35–50 % of the contents[11]. The flora contains bacteria, archaea and eukarya, and the bacterial component has been studied the most extensively. Three genera, *Bacteroides* spp., *Clostridium* spp. and *Eubacterium* spp., each account for approximately 30 % of the organisms present[9], but the colon also has a significant content of *Fusobacterium* spp., *Peptostreptococcus* spp. and *Bifidobacterium* spp.[11,12] These bacteria are accompanied by a single methanogenic archaeon, *Methanobrevibacter smithii*[9,13]. The eukarya include protozoa, but those found in the GIT seem never to have been studied with reference to PPT catabolism.

It is generally accepted that colonisation of the GIT of neonates starts immediately after birth. The type of delivery (passage through the birth canal *v.* caesarean section), type of diet (breast *v.* formula feeding) and environment affect the colonisation pattern[14,15]. The composition of the gut flora of a 2-year-old child is essentially the same as that of an adult[16], but the variations in microflora composition associated with age, diet and health status are still not fully determined[17]. A study of faecal samples from 230 volunteers, aged 20–50 years, from four European countries has demonstrated marked country–age interactions. The proportions of bifidobacteria were 2- to 3-fold higher in the Italian study population than in any other study group, and this effect was independent of age. Higher proportions of enterobacteria were found in all elderly volunteers independent of the location[18]. There is good evidence that comparatively minor changes in the diet of rats can produce significant changes in the catabolites eventually produced from dietary PPT[19], but the extent to which the human microflora can be modulated by diet is unknown.

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Models used to study colonic catabolism

The distal GIT is conveniently viewed as an anaerobic fermentation vessel in which polysaccharides, proteins, lipids and xenobiotics such as PPT can be transformed. Several strategies have been used to investigate these transformations. *In vitro* studies frequently use either a defined bacterial strain[20,21], ileostomy fluid[22] or a flora from freshly voided human[23–27] or animal faeces[28–30] cultured in a suitable medium to which a defined substrate has been added, and monitor (i) disappearance of substrate, (ii) formation of catabolites or (iii) changes in the microflora[31,32]. Rarely all three parameters are studied at the same time. Typically controls would include (i) incubations containing test substrate but lacking the microbial culture to investigate purely chemical transformation, (ii) incubations containing test substrate and chloroform-inactivated culture to investigate substrate losses associated with cell binding and transformations associated with enzymes released after lysis of the micro-organisms and (iii) incubations containing basal medium and microbial culture in order to monitor catabolites arising from basal metabolism and to distinguish these from catabolites produced from the test substance. The culture may be static[27] or dynamic[33]. The various types of study used to examine microbial transformation of PPTs are shown in Table 1.

It is impossible to know how well these *in vitro* models reflect the human GIT *in vivo* but studies using gnotobiotic rats that have been associated with a specific GIT micro-organism do not produce the same catabolites as that of the organism incubated in an artificial medium[34] and one must assume that the models are imperfect. In particular, micro-organisms that bind to the GIT surface might be very different to those found in the lumen, and it is these 'unbound' organisms that might predominate in the voided stool from which the culture is prepared[17,35]. The micro-organisms that survive the culturing process will depend on how rigorously oxygen was excluded and the nature of the medium employed. Media for anaerobic micro-organisms are typically rich in protein or polypeptides and these can be incompatible with the recovery of PPT that bind strongly to protein thus preventing proper analysis of the transformations. Studies in the authors' laboratories suggest that the catabolism of PPT that are not strongly bound to protein is not significantly different from their catabolism in protein-free media, and protein-free media have been used to investigate the catabolism of procyanidins that would bind irreversibly to protein[36]. One advantage of these models is the requirement for comparatively small amounts of a scarce and costly pure substrate.

A second approach has been the use of laboratory animals, commonly rodents, but occasionally pigs. The rodents might be either (i) normal laboratory animals with their typical flora[37], (ii) animals treated with antibiotics (typically neomycin) to destroy the flora, (iii) gnotobiotic animals delivered under sterile conditions, and housed in sterile conditions and fed only on γ-irradiated diets[34,38] or (iv) gnotobiotic animals associated with a human-type flora[39]. The use of such animals has allowed the effects of the rodent flora and human-type flora to be compared, and the effects of the flora and mammalian metabolism to be distinguished. Radio-labelled test substances can be used and full pharmacokinetic studies can be performed with these animals, and the contents (microbial and chemical) can be compared at different sites in the GIT[37]. However, gnotobiotic animal facilities, are very expensive to maintain. An extensive review of the *in vitro* and animal models is available[40]. The early studies using these procedures, but focusing on flavonoids, have been reviewed[41–43].

A third approach is to use volunteers, either free-living or following prescribed diets, and to collect faeces[44], faecal water[45] and urine for analysis[4]. Normal volunteers can be compared with volunteers who have undergone an ileostomy but who are otherwise healthy enabling metabolism in the small intestine to be distinguished from that in the large intestine. These are discussed in more detail as follows.

Transformation of polyphenols by colonic microflora

The transformations of which the microflora are capable include *O*- and *C*-deglycosylation, the hydrolysis of esters

Table 1. Studies concerned with the transformation of phenols, polyphenols and tannins (PPT) by gut flora micro-organisms

		Animal studies		Volunteer studies	
PPT	Microbial *in vitro*	Normal flora	Germ free	Normal healthy	Healthy ileostomists
Benzoic acids	(203)				
Cinnamic acids	(92,204)	(34)	(34,38)	(205)	
Chlorogenic acids	(27,206–208)	(142,209)		(210)	(211)
Other Cinnamates	(27)				
Flavanols	(32,82,94,95,212,213)	(61,214–216)	(59,217)	(201)	(105)
Flavonols	(22,54,55,60,72,82,83,85,89,203,208,218–220)	(54,141,217,221,222)	(73)	(223)	(102,223)
Flavones	(28,46,56)	(56)			
Isoflavones	(20,21,29,74,78,224–231)		(39,232)	(233–238)	(239)
Flavanones	(82,89,240–242)	(243)	(217)	(244)	
Chalcones	(242)				
Dihydrochalcones		(245)	(217)		(106,108,211)
Anthocyanins	(23–25,56)	(30,37,56,141,246)		(4)	(107)
Gallotannins					
Ellagitannins	(47)	(97,99,246)			
Proanthocyanidins	(36,94,247–249)			(135,193,250)	
Lignans	(80,251–253)	(251,254,255)	(232)	(236,256,257)	
Derived polyphenols				(202,258)	
Amino acids	(57)				

and amides, and deglucuronidation of excreted mammalian metabolites. The aglycones are susceptible to aromatic dehydroxylation, demethoxylation and demethylation, and hydrogenation, α-oxidation and β-oxidation of the aliphatic elements generated following rupture of the aromatic ring[34,38,41–43,46–49]. Most investigations have focused on phenolic catabolites, i.e. those that retain at least one phenolic hydroxyl, but it is clear that complete dehydroxylation can occur producing aromatic catabolites[19,50]. Benzoic acid, however, can also be produced in human subjects by GIT microflora aromatisation of quinic acid[51]. It should also be noted that there is a significant, but often unquantified, yield of non-aromatic products, including oxaloacetate, CO_2, etc.[52]. A key step in the catabolism of flavonoids is the fission of the A-ring and loss of carbons C_5 to C_8 as oxaloacetate that is ultimately metabolised to CO_2[53]. Similarly, an oral-dosing volunteer study using [4-^{14}C]-quercetin demonstrated that approximately 50 % of the radioactivity was eliminated as CO_2[52], but the fate of the A-ring and B-ring was not investigated.

Dehydroxylation of *ortho*-dihydroxy and *ortho*-trihydroxy substrates can occur at either a *meta* or *para* position, but it is accepted that the hydroxyl at the *meta* position is removed less readily. Accordingly, *ortho*-dihydroxy and *ortho*-trihydroxy substrates yield predominantly *meta*-hydroxy and *meta*-dihydroxy catabolites, respectively[54,55]. Absence of a *para*-hydroxyl in the B-ring of flavonoids is considered to slow the degradation by the GIT microflora[28,56]. Studies using ^{2}H-labelled substrates and crude human gut flora have established that with 3-(4′-hydroxyphenyl)-propionic acid and 4-hydroxyphenyl-lactic acid, the aliphatic side chain may be moved about the aromatic ring, thus converting a 4-hydroxy substrate to the corresponding 3-hydroxy isomer[57,58]. The enzymes/micro-organisms responsible have not been characterised and their substrate specificity is unknown. Although it is possible that such an isomerisation might explain the apparent preference for removal of the *para*-hydroxyl from a 3,4-dihydroxy substrate, it would not explain the tendency for 3,4,5-trihydroxy substrates to be converted to 3,5-dihydroxy products.

Aromatic demethylation by rodent, pig and human GIT microflora has been observed *in vitro,* for example demethylation of 3′-*O*-methyl-(+)-catechin[59], 4′-*O*-methylgenistein (angolensin)[60], 6-methoxyapigenin[28] and phenolic acids formed from the B-ring of anthocyanins[24]. In contrast, the gut microflora of the rat, mouse and marmoset are unable to degrade 3-*O*-methyl-(+)-catechin[61], suggesting that the they cannot attack the C-ring aliphatic *O*-methyl group. Similarly, there is no evidence for demethylation of this compound when given orally to volunteers[62].

Comparatively few of the enzymes associated with these transformations have been characterised in microbes associated with the GIT, and the subtleties of substrate specificity, enzyme inhibition, etc., remain largely unknown. At least with regard to polysaccharide catabolism there appears to be few structures that cannot be degraded[9], and this situation might well apply also to PPT.

It has been demonstrated that some strains of the GIT micro-organism *Eubacterium oxidoreducens* can convert pyrogallol to dihydrophloroglucinol and 3-hydroxy-5-oxohexanoate. The phloroglucinol reductase and pyrogallol–phloroglucinol isomerase have been isolated and characterised[63–66]. This isomerase can be viewed as creating a new hydroxyl at C_2 in the phloroglucinol (1,3,5-trihydroxybenzene) equivalent to creating a hydroxyl at either C_6 or C_8 in the flavonoid A-ring. Such a hydroxylation of the flavonoid A-ring has been demonstrated with *Pseudomonas* spp.[67,68] but the general relevance of this observation to GIT transformation of PPT is uncertain because these facultative anaerobes are not typical of the GIT microflora.

Eubacterium ramulus has been reported at $4{\cdot}4 \times 10^7$ to $2{\cdot}0 \times 10^9$ colony forming units/g (*n* 20) of human faecal dry mass[69], and its growth is stimulated *in vivo* by dietary flavonoids[70]. *Eubacterium* spp. are well known for their ability to degrade flavonoids anaerobically, variously possessing deglycosylating activity, the ability to split the C-ring, chalcone isomerase and phloretin hydrolase activity[26,71–76]. These chalcone isomerase[76] and phloretin hydrolase[26,75] enzymes convert flavanones to the isomeric dihydrochalcones and the dihydrochalcones to a C_6–C_3 acid and phloroglucinol, respectively. The phloroglucinol will be rapidly converted to aliphatic products as described earlier. It is not known whether the *retro*-chalcone associated with anthocyanins in media with pH > 7[77] are substrates for the phloretin hydrolase.

Eubacterium limosum demethylates isoflavones such as biochanin A, formononetin and glycitein[78], and *Eubacterium* A-44 has a novel arylsulfotransferase but whether mammalian flavonoid sulphates can serve as donors is not known[79]. *Eubacterium* sp. SDG-2 and *Peptostreptococcus* sp. SDG-1 convert *seco*-isolariciresinol diglucoside to mammalian lignans[80], and this *Eubacterium* degrades various (3*R*)- and (3*S*)-flavanols and their 3-*O*-gallates to diaryl-propan-2-ols, 3,5-dihydroxyphenylvalerolactone and 3-hydroxyphenylvalerolactone. Interestingly, this organism can remove the *para*-hydroxyl from 3*R* flavanols such as (−)-epicatechin and (−)-catechin but not from the 3*S* flavanols such as (+)-catechin and (+)-epicatechin[81], and it has been demonstrated *in vitro* that the conversion of (+)-catechin to C_6–C_5 and C_6–C_3 catabolites required its initial conversion to (+)-epicatechin[32].

Some *Clostridium* spp. can cleave the C-ring of flavonoids, converting flavonols to a phenylacetic acid and presumably phloroglucinol. Activity with flavanones was much weaker and could not be detected with flavanols[82,83]. Some *Fusobacterium* spp. and *Bacteroides* spp. possess α-L-rhamnosidase[84,85], β-D-glucosidase[86,87], β-D-galactosidase[85] and β-D-glucuronidase[88] activities.

The PPT catabolites most frequently reported are aromatic/phenolic acids having 0, 1, 2 or 3 aromatic hydroxyls, 0, 1 or 2 methoxyls, and a side chain of between one and five carbons, which might be unsaturated and might bear an aliphatic hydroxyl. For most flavonoids, the acids produced retain the intact B-ring, but anthocyanins also yield 2,4,6-trihydroxybenzoic acid and the corresponding benzaldehyde (phloroglucinaldehyde) both derived from the A-ring[24,72,89,90]. There is evidence that these phloroglucinol derivatives might form from anthocyanins purely by chemical degradation at the prevailing pH value in the GIT[91]. Whether of chemical or microbial origin, these aromatic/phenolic acids are common to most PPT substrates so far investigated, although some variation of ring substitution and side chain length are substrate specific as summarised in Table 2.

Table 2. The nature of the aromatic and phenolic acids produced by the gut microflora from pure phenols, polyphenols and tannins (PPT) substrates

	Aromatic and phenolic acid catabolites containing 0, 1, 2 or 3 phenolic hydroxyls							
PPT	C_6-C_1	C_6-C_2	C_6-C_3	C_6-C_3-α-OH	C_6-C_3-β-OH	C_6-C_3-dihydro	C_6-C_5	C_6-C_5-γ-OH
Benzoic acids								
Cinnamic acids	(27)		(92,204,205)			(27,34,56,259)		
Chlorogenic acids	(27)		(207)			(27,142,206–208)		
Other Cinnamates	(27)		(260)			(27)		
Flavanols	(36)	(36)			(216)*	(36)	(36,213)	(36,81,213, 247,261)†
Flavonols		(28,54–56,60,72,73, 82,89,203,208,222)				(89,208)		
Flavones	(56)		(56)			(28,54,56)		
Isoflavones						(60)‡		
Flavanones	(56)	(89)	(56)		(262)*	(56,89,208,263)		
Chalcones								
Dihydrochalcones	(56)		(56)			(26,56,75)		
Anthocyanins	(24)§	(23)		(56)				
Gallotannins								
Ellagitannins								
Proanthocyanidins	(36)	(36,248,249)		(247)		(36,248,249)	(36,248)	(36,247,249)†
Lignans								
Derived polyphenols								
Amino acids		(57)		(57)		(57)		

* C_6-C_3 βOH (phenylhydracrylic acids) probably arise from mammalian metabolism of a microbial catabolite rather than directly by microbial catabolism.
† May occur also as lactones.
‡ In the case of isoflavones the aryl substituent is attached to C_2 of the C_6-C_3 dihydro side chain. For all other PPT, it is attached to C_3.
§ C_6-C_1 acids may form also by a purely chemical mechanism at the prevailing pH value.

Phenolic acids can be decarboxylated to the corresponding hydrocarbons[28,34,56,89,92,93], but the corresponding alcohols seem not to have been reported.

Certain larger mass intermediates have been characterised only during the catabolism of flavanols and proanthocyanidins (e.g. diaryl-propan-2-ols)[94,95], and catabolites such as *S*-equol, diarylbutanes and urolithins[96–99] are specific to the isoflavones, lignans and ellagitannins, respectively. Faecal water from free-living volunteers also contains some flavonoid aglycones indicating that aglycone degradation is not necessarily complete[45].

Models to study absorption in the colon compared with the small intestine

Although absorption is traditionally associated with the small intestine, the colon is also very capable of absorbing compounds; normally it is the supply of nutrients that limits absorption in the colon. There are several different models for studying absorption at these different sites. The most commonly used is to give a single bolus dose of a food or compound to volunteers and collect blood and urine over the next 24–48 h period. Compounds which are absorbed in the small intestine normally appear in the plasma within an hour and the maximum concentration of a compound at any time point in a pharmacokinetic study is usually less than 2·5 h, although this depends on how full the stomach is at the start, i.e. whether a meal was given or not. Compounds that appear after 3 h with T_{max} (a time point corresponding to maximum concentration of a compound at any time point in a pharmacokinetic study) >5 h are generally considered to have been absorbed mainly in the colon. The latter situation involves, almost without exception, a chemical or microbial transformation which converts the compound from non-absorbable to absorbable. The microflora can catalyse two basic steps at this stage: removal of a conjugated chemical moiety such as a sugar or organic acid, and breakdown of the PPT itself. These interactions are illustrated by the flavonol quercetin. Conjugation with a glucose moiety, such as quercetin-4′-*O*-glucoside found in onions, leads to absorption in the small intestine after deglycosylation by the brush border enzyme lactase phloridzin hydrolase[100], and the T_{max} is <1 h[101]. In contrast, if the quercetin is conjugated with a rhamnose moiety as in rutin (quercetin-3-*O*-rhamnoglucoside), a sugar which is not a substrate for the brush border enzyme lactase phloridzin hydrolase, then the compound is untouched in the small intestine and reaches the colon intact, where colonic microflora remove the terminal rhamnose and the glucose moieties[101]. The immediate product, quercetin aglycone, is then either absorbed intact and appears in the plasma as methylated, sulfated and/or glucuronidated conjugates, or is broken down by microflora as described previously. The inferior absorption of the quercetin component of rutin is thus due to degradation by microflora rather than less efficient colon absorption *per se*. This illustrates the dual role of the colon microflora for absorption and catabolism.

A second approach is to use healthy ileostomists, i.e. subjects who do not have a colon, because it has been removed by colectomy as a result of ulcerative colitis, Crohn's disease, familial polyposis or colon cancer complications. The end of the ileum is brought through the abdominal wall to form a stoma, usually on the lower right side of the abdomen. The GIT contents pass out of the body through the stoma and are collected in an individually fitted drainable pouch, which is worn at all times. The ileal fluids are easily accessible, their nature makes

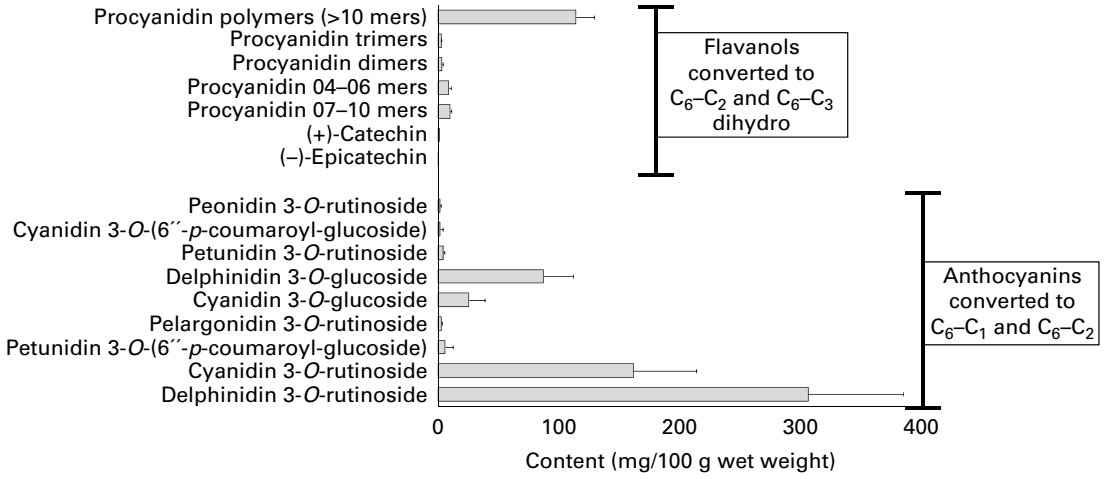

Fig. 2. Content of anthocyanidins and flavanols in blackcurrants (http://www.phenol-explorer.eu/, accessed October 2009).

them easier to analyse than some other biological fluids, and ileostomists are a good tool for comparing the catabolism of a given PPT presented in different foods or matrices. There are some limitations: the terminal ileum develops a (minimal) gut microflora, only disappearance is measured, and some of the subjects have increased gut permeability, electrolyte imbalance and a lower production of urine. Nevertheless, this model has been used successfully to examine the absorption of quercetin[102–104], flavanols and hydroxycinnamic acids[104,105], apple polyphenols[106], blueberry polyphenols[107], cider dihydrochalcones[108] and coffee hydroxycinnamates[267].

An alternative approach is to use sections of rat intestine; these are everted and then suspended in tissue culture, where they exhibit functionality for a short time (< 1 h). These rat everted sacs can then be tested for transport of various substances as required. The difference between absorption of phenolic acids in the small intestine and colon of the rat was examined using this model[109]. Ascending and descending colon transported dihydrocaffeic and dihydroferulic acids to the serosal (blood) side 1·5- and 3-fold faster than jejunal segments, implying a more efficient absorption in the colon for these catabolites, both major compounds derived from microbial catabolism of hydroxycinnamic acids and some flavonoids (Table 2). In addition, the two colon segments were much more efficient at effluxing glucuronides back to the luminal side[109].

The Caco-2 cell model is very commonly used as a model of small intestinal absorption. This cell line is very well characterised[110–112] but, unlike the small intestine and especially the colon, Caco-2 cells do not secrete mucus. The cell line was derived originally from colonic cells but monolayers of the cells differentiate to produce a small intestine-like morphology, with expression of sucrase and maltase, markers of the small intestine. An alternative colon model using combinations of Caco-2 (76 %) and mucus-secreting

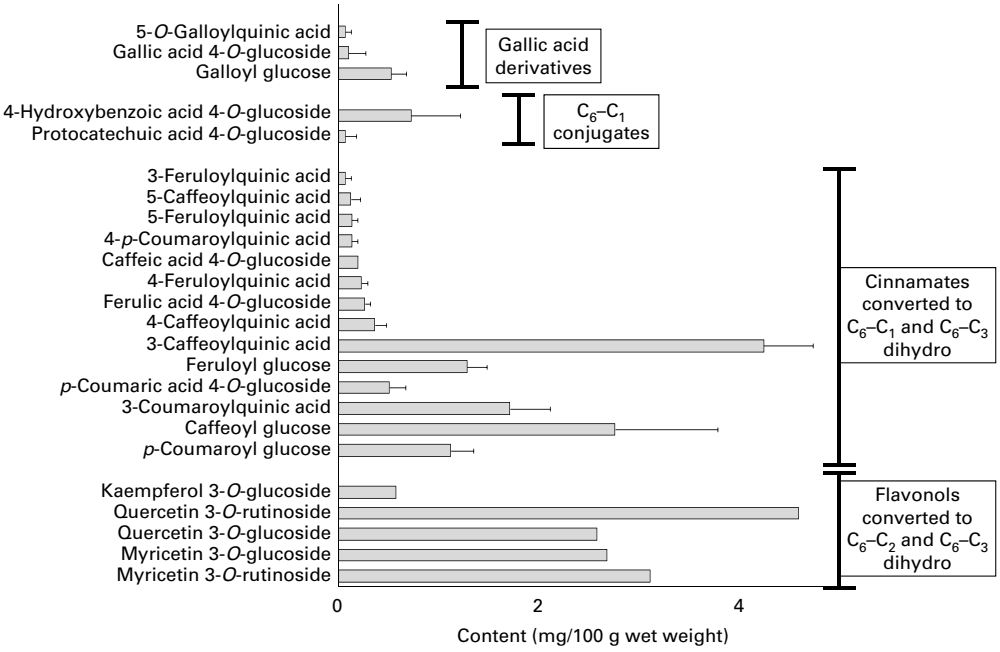

Fig. 3. Content of flavonols, cinnamates, benzoic and gallic acid derivatives in blackcurrants (http://www.phenol-explorer.eu/, accessed October 2009).

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HT-29 goblet cells (24 %) has been reported[109]. The presence of mucus in the co-culture system reduced ferulic acid transport by 23 %, and the HT29 cell component alone was responsible for glucuronidation of the supplied ferulic acid. The co-culture system also reduced the hydroxycinnamic acids to the respective dihydrocinnamic acids, and in this system, dihydroferulic acid was more efficiently transferred to the basolateral side than dihydrocaffeic acid, in contrast to the system using rat everted sacs[109].

Polyphenol content of blackcurrants and most abundant predicted catabolites from colonic microflora

The PPT content of the diet has been reviewed[7,113–115]. Berries are among the foods richest in polyphenols, many having an especially high content of anthocyanins, and some (e.g. blueberries) being rich also in chlorogenic acids. Blackcurrants contain various anthocyanins at a high-total concentration (approximately 600 mg/100 g with the 3-*O*-glucosides and 3-*O*-rutinosides of cyanidin and delphinidin usually dominant), a relatively high level of proanthocyanidins (approximately 140 mg/100 g, consisting of procyanidins and prodelphinidins), some flavonols (approximately 14 mg/100 g) and hydroxycinnamates (approximately 13 mg/100 g), and lower levels of hydroxybenzoic acids (approximately 1·5 mg/100 g) and catechins (approximately 1·2 mg/100 g)[116,117] (Figs. 2 and 3).

The next section of this review on the GIT transformation of dietary PPT is concerned with the potential for blackcurrants and blackcurrant-derived products to influence human health. Much of the information is derived from studies of similar PPT from other food sources. Table 2 shows that the classes of aromatic/phenolic acids that might be expected to form in the GIT tract is largely independent of the classes of PPT consumed. The dominance in blackcurrants of anthocyanins and proanthocyanidins will result primarily in the production of C_6-C_1 and C_6-C_3 dihydro acids, with some C_6-C_5-γ-OH products to be expected early in the degradation of the proanthocyanidins and an increasing yield of C_6-C_1 acids at later time points as the side chain is progressively shortened either by the microflora or by the mammalian enzyme systems. A percentage of the cinnamic acids might be absorbed as such but these also are reduced to the C_6-C_3 dihydro acids: the flavonols will yield C_6-C_2 acids, and these might also form from α-oxidation of C_6-C_3 dihydro acids and yield C_6-C_1 acids by the same mechanism.

The ring (C_6) hydroxylation pattern depends primarily on the B-ring hydroxylation and the precise biochemical competence of the gut microflora. As summarised in Table 3, the dominance of cyanidin and delphinidin glycosides suggests that initially protocatechuic and gallic acid are likely to be the dominant C_6-C_1 acids. However, in human subjects, gallic acid consumed as flavanol-3-*O*-gallates is rapidly converted to 3-*O*-methylgallic acid, 4-*O*-methylgallic acid and 3,4-*O*-dimethylgallic acid[118,119]. Although free gallic acid has been detected in human plasma after an oral dose (50 mg)[119], this mammalian metabolism is consistent with the failure to observe gallic acid in plasma after volunteers had consumed delphinidin[5].

Evidence for microbial biotransformations from animal and human intervention studies

Anthocyanins

The absorption and catabolism of anthocyanins from blackcurrants in rats and human subjects have been studied[120]. Four anthocyanins, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, were absorbed and excreted as the glycosylated forms in both rats and human subjects. In human subjects, only 0·05 % of the ingested anthocyanin dose was found in urine. Although, unexpectedly, the 3-*O*-rutinosyl anthocyanins were directly absorbed, the amount found in the urine was very low[120]. Co-administration of anthocyanins with phytic acid enhances plasma concentration significantly by an unknown mechanism[121]. In all studies on healthy subjects consuming anthocyanin-rich food, it is now well established that <2 % of the total anthocyanin dose is absorbed intact, as estimated from the amount of anthocyanin and conjugates either in urine[122] or in blood[123]. Using ileostomist subjects,

Table 3. The expected B-ring fragments for the common anthocyanidins and their known mammalian metabolites

		Known mammalian metabolites	
Anthocyanidin	Initial B-ring fragmentation product	Human	Animal
Pelargonidin	4-Hydroxybenzoic acid	Benzoic acid-4-*O*-sulphate[264] 4-Hydroxybenzoyl-Gly[264]	
Cyanidin	3,4-Dihydroxybenzoic (protocatechuic) acid	3-Methoxy-4-hydroxybenzoic (vanillic) acid[264] Protocatechuic acid conjugates in Caco-2 cells[91]	Methylated. glucuronidated or Gly conjugated metabolites including vanniloyl-Gly[147,149]
Delphinidin	3,4,5-Trihydroxybenzoic (gallic) acid	3-*O*-methylgallic acid[118] 4-*O*-methylgallic acid[118,119] 3,4-*O*-dimethylgallic acid[118]	Pyrogallol[265] Pyrogallol-1-*O*-β-D-glucuronide[265] 4-*O*-methylgallic acid-3-*O*-sulphate[265] 2-*O*-methylpyrogallol-1-*O*-β-D-glucuronide[265] 2-*O*-methylpyrogallol[265] 4-*O*-methylgallic acid[265]
Peonidin	3-Methoxy-4-hydroxybenzoic (vanillic) acid	Vanillic acid-4-*O*–sulphate or 4-*O*-β-D-glucuronide[266]	
Petunidin	3-Methoxy-4,5-dihydroxybenzoic acid		
Malvidin	3,5-Dimethoxy-4-hydroxybenzoic (syringic) acid		

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a high proportion of the anthocyanins from blueberry (up to 85 %, depending on the attached sugar moiety) traversed the small intestine unchanged and were found in the ileostomy bags; this amount would therefore reach the colon under physiological conditions and be subject to microbial degradation[107]. *In vitro* studies using human microflora in an anaerobic chamber have indicated that protocatechuic acid is one of the most likely major degradation products from anthocyanins having a 3,4-dihydroxy B-ring[23]. In a human intervention study on blood orange juice, the C_{max} in blood for cyanidin-3-*O*-glucoside was 1·9 nm, whereas the value for protocatechuic acid was approximately 250-fold higher (492 nM). It was calculated that the main product, protocatechuic acid, accounted for up to 70 % of the anthocyanin intake. Only 1·2 % of the anthocyanin dose ultimately appeared in urine, whereas urinary protocatechuic acid represented 28 % of the total anthocyanin dose. The protocatechuic acid appeared to be unconjugated[4]. Protocatechuic acid was also found in rat plasma after feeding cyanidin-3-*O*-glucoside[124] and after deglycosylation, cyanidin can breakdown spontaneously to give protocatechuic acid (very pronounced at physiological pH)[91]. After ingestion of black raspberries by pigs, the profile of compounds in the gastrointestinal tract was analysed. In the entire gut, protocatechuic acid was the major phenolic, followed by 4-hydroxycinnamic, caffeic, ferulic and 3-hydroxybenzoic acids. These accounted for approximately 6 % of the ingested anthocyanin[125] which is considerably less than the human study described previously.

After consumption of a high dose of strawberries by volunteers, the main phenolic acids in urine after 5 h were 4-hydroxybenzoic acid (10·4 mg/l), protocatechuic acid (0·7 mg/l), vanillic acid (1 mg/l) and genistic acid (1 mg/l)[126]. Strawberry anthocyanins are primarily pelargonidin glycosides with lesser amounts of cyanidin glycosides[122,127] that would be expected to yield 4-hydroxybenzoic acid and protocatechuic acid (Table 3), but these acids are normal preformed constituents of strawberries[128].

Consumption of oats added to a purée of bilberries (glycosides of delphinidin, accompanied by lesser amounts of malvidin, peonidin and petunidin glycosides)[129–131] and lingonberries (cyanidin glycosides)[132] by volunteers resulted in urinary excretion of 3-methoxy-4-hydroxyphenylacetic (homovanillic) and vanillic acid, low amounts of syringic acid (from malvidin glycosides) but no gallic acid (which would have been expected from delphinidin glycosides). Urinary excretion of these acids was maximal at 4–6 h and intact urinary anthocyanins comprised $<0{\cdot}01$ % of the dose[5].

Procyanidins

When rats were fed procyanidin dimer B3, the major urinary catabolites were 3-(3′-hydroxyphenyl)-propionic, 3-hydroxycinnamic, 4-hydroxybenzoic and vanillic acids (total 6·5 % of intake). Feeding of the procyanidin trimer C_3 or a mixture of procyanidin polymers gave in addition 3-hydroxyphenylvaleric acid (C_6–C_5)[133]. When rats were fed ^{14}C-labelled procyanidin B2[134], approximately 80 % of the ^{14}C-radiolabel was absorbed and appeared in the urine. The ^{14}C-radiolabel appeared rapidly in the blood at low levels, but did not reach a maximum until 6 h, indicative of a major contribution of catabolism by colonic microflora. These two studies indicate that the majority (>70 %) of the colonic catabolites of procyanidin dimers are unknown. In human subjects, after consumption of procyanidin and catechin-rich chocolate, the main urinary catabolites were 3-(3′-hydroxyphenyl)-propionic acid, ferulic acid, 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, vanillic acid and 3-hydroxybenzoic acid[135].

Hydroxycinnamic acids

Hydroxycinnamic acids are most commonly found linked to a quinic acid moiety in fruits and foods. These compounds are called chlorogenic acids and the most common are caffeoylquinic acids, found at high levels in coffee. Fibre such as wheat bran is another source, where the hydroxycinnamic acids, particularly ferulic acid, are covalently linked to an arabinose sugar unit. Much of the understanding of the metabolism of hydroxycinnamates is derived from work on coffee. Chlorogenic acids are poorly hydrolysed by conditions in the stomach or small intestine. When coffee is given, there is a relatively small absorption of caffeic and ferulic acids in the small intestine and a low absorption of intact chlorogenic acids. The major absorption occurs in the colon, where dihydroferulic and dihydrocaffeic acids, products of microbial biotransformation, are the major products[136,137]. The important involvement of the colon is also supported by studies on ileostomists, where approximately 67 % of the dose of chlorogenic acids reaches the colon[138]. Dihydrocaffeic acid is one of the major phenolic acids in human faecal water[45] and has been detected in the plasma of coffee drinkers[139], in urine as the free form and mainly conjugated in human plasma after ingestion of artichoke leaf extracts[140], in human urine after chocolate intake[135] and in rat urine after ingestion of polyphenol-rich wine extract[141]. In summary, the major products from ingestion of chlorogenic acids are dihydrocinnamic acids in the plasma and urine. Studies on rats showed that the major 5-caffeoylquinic acid-derived phenolic acids, 3-hydroxybenzoic, 3-hydroxybenzoylglycine (3-hydroxyhippuric) and 3-hydroxycinnamic acids were from the caffeic acid moiety but that a significant portion of the hippuric acid (benzoylglycine) was produced from the quinic acid moiety[142].

Some older work on the tissue distribution of radiolabelled cinnamic acids is worth noting. Injection of ^{14}C-cinnamic acid to rats gave distribution in organs as shown in Fig. 4, with the remaining radioactivity in urine (48 %), faeces (25 %) and exhaled CO_2 (0·3 %)[143]. There was a substantial amount in skin and gonads. A later study showed that 82–90 % of orally administered *trans*-(3-^{14}C)-cinnamic acid was absorbed in rats as indicated by the amount present in urine after 24 h[144].

Once formed by microbial biotransformation, compounds must pass the colonic epithelium and enter the bloodstream. Mammalian enzymes will further transform the microbial products, mainly by conjugation, but also by β-oxidation. Conjugation can involve methylation (catalysed by the enzyme catechol-*O*-methyl transferase), sulfation (sulfotransferases), β-glucuronidation (UDP-glucuronosyl transferases), glycinylation (via a CoA thioester) and glutaminylation

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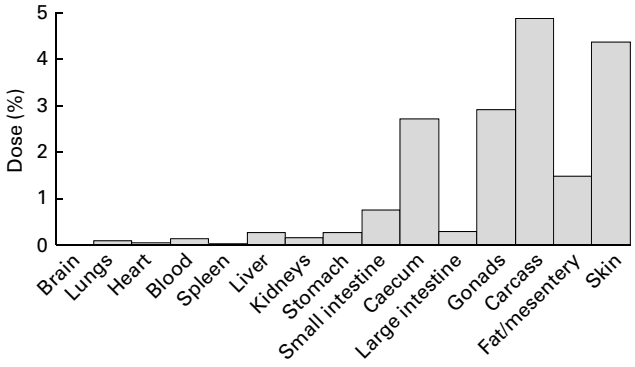

Fig. 4. Distribution of radiolabel in rat tissues after injection of ^{14}C-cinnamic acid to rats. The remaining radioactivity was in urine (48·%), faeces (25·%) and exhaled CO_2 (0·3·%)[143].

(by conjugation with glutamine). Many of the enzymes involved exist as multiple isoforms with different but overlapping specificities, typical of those acting on xenobiotics.

Properties of benzoate derivatives (C_6–C_1) (e.g. protocatechuic and 4-hydroxybenzoic acids)

Absorption and metabolism of C_6–C_1

Both protocatechuic acid and phloroglucinaldehyde are transported by Caco-2 cells, and then metabolised to sulphate and glucuronide conjugates by Caco-2 cells[91] (Fig. 5). Studies with Caco-2 cells have shown that benzoic acid and the three mono-hydroxybezoic acid isomers are substrates for the monocarboxylate transporter[145]. 3-Methoxy-4-hydroxyphenylacetic (homovanillic) acid is a substrate for the rat organic anion transporter[146]. Following absorption, catechol-*O*-methyl transferase methylates protocatechuic acid to vanillic acid[147]. Of the conjugating enzymes, UDP-glucuronosyl transferases 1A6 in human liver microsomes is active on protocatechuic aldehyde[148]. In addition, protocatechuic aldehyde is converted to approximately 70·% protocatechuic acid by guinea pig liver slices by the enzymes aldehyde oxidase, xanthine oxidase and aldehyde dehydrogenase[149], but approximately 20·% was unidentified polar conjugates of protocatechuic acid. The conversion of caffeic acid to protocatechuic acid did not occur in the absence of a gut microflora in germ-free rats[34].

Biological effects of C_6–C_1

In a rat model of induced carcinogenesis, protocatechuic acid prevented 4-nitroquinoline-1-oxide-induced oral carcinogenesis, *N*-methyl-*N*-nitrosourea-induced carcinogenesis in the glandular stomach, azoxymethane-induced carcinogenesis in the colon and diethylnitrosamine-induced carcinogenesis in the liver[150,151]. The doses of protocatechuic acid were expressed only as a percentage of the diet, but can be

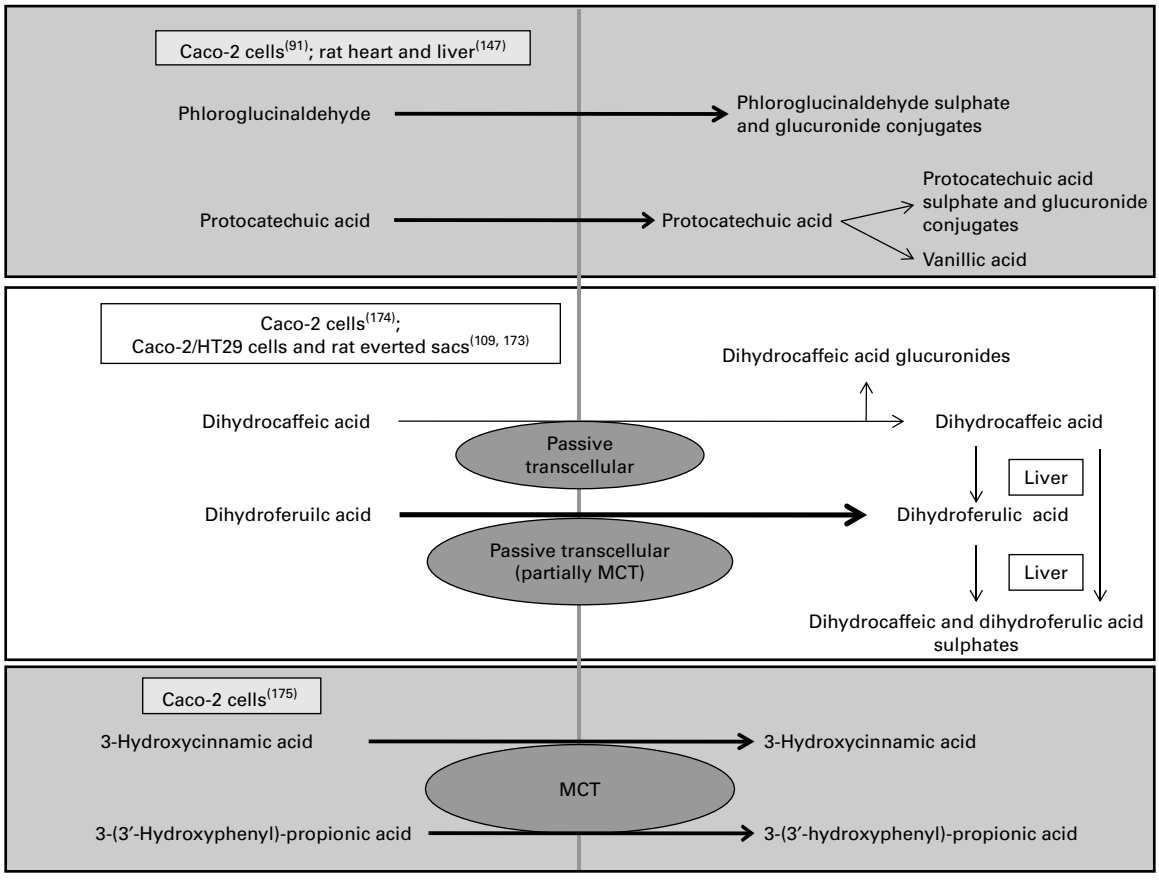

Fig. 5. Summary of absorption and metabolic pathways of C_6–C_1 and C_6–C_3 compounds in the gastrointestinal tract.

estimated as approximately 10–40 mg/kg body weight per d assuming that a 200 g rat typically consumes 20 g diet per d.

Several papers have reported *in vitro* activity of protocatechuic acid on cultured cells, and in some investigations, this microbial catabolite is more potent than cyanidin glycoside, an anthocyanin that gives rise to substantial levels of protocatechuic acid *in vivo*[4]. For example, in human neuronal SH-SY5Y cells, protocatechuic acid (100 μM) is more potent than cyanidin-3-*O*–glucoside (100 μM) at inhibiting hydrogen peroxide-induced apoptotic events, including mitochondrial function and DNA fragmentation. However, neither was effective below 25 μM[152]. Protocatechuic acid induces c-jun *N*-terminal kinase-dependent hepatocellular carcinoma cell death at concentrations > 50 μM[153].

Protocatechuic acid at concentrations above 500 μM promoted time-dependent and concentration-dependent migration of human adipose-tissue derived stromal cells *in vitro* possibly via effects on matrix metalloproteinase-2[154], and promoted cell proliferation of cultured rat neural stem cells, with reduced apoptosis possibly via repression of caspase-3 activation[155]. Significantly lower production of reactive oxygen species was seen following treatment with protocatechuic acid (6 μM for 7 d or 30 μM for 4 d) but suppression of caspase-3 required 30 μM for both durations. The relative effectiveness of lower doses longer term is encouraging and suggests that dietary exposure over a long period might perhaps promote better health over the subsequent years.

Several investigations have addressed lipid and cholesterol metabolism. Protocatechuic acid, the major component of the Chinese functional medicine, Danshen, has reported anti-angina efficacy[147]. Protocatechuic acid is methylated and then diffuses into mitochondria where it is conjugated with CoA. The result is that fatty acid oxidation is decreased, as shown by a lower acyl CoA/CoA ratio in heart, which in turn activates pyruvate dehydrogenase, a key and irreversible step in carbohydrate oxidation. This could switch heart energy substrate preference from fatty acid to glucose that would be beneficial for ischaemic heart conditions. There was no detectable accumulation of protocatechuic acid in tissues[147]. Protocatechuic acid at 0·5 g/kg diet given to rats (estimated as approximately 10 mg/kg body weight per d) produced changes in cholesterol and lipid metabolism. The serum total cholesterol, HDL-cholesterol and VLDL-cholesterol were all lower in the protocatechuic acid-treated group, suggested to be partly as a result of induction (estimated by mRNA) of hepatic LDL receptor, apoB, apoE, lecithin-cholesterol acyltransferase and hepatic TAG lipase[156].

Properties of phenylacetate derivatives (C_6–C_2)

Absorption and metabolism of C_6–C_2

Phenylacetate is an endogenous product of phenylalanine metabolism, is present at low levels in the mammalian circulation and is conjugated with glutatmine during metabolism. Its pharmacokinetic parameters have been well studied in a clinical setting in patients owing to its proposed effects on cancer. When phenylacetate was administered as an intravenous infusion as part of a phase I trial in children with refractory cancer, the half life was approximately 1 h, and phenylacetate was conjugated with glutatmine to form phenylacetylglutatmine[157]. After oral consumption of phenylacetate, the concentration of phenylacetate peaked at 2 h and 40 % was excreted in the urine after 40 h[158]. Intravenous radiolabelled ^{14}C-phenylacetate was rapidly taken up by rat brain and converted into ^{14}C-acetate[159]. Phenylacetate was well tolerated when infused into patients twice per d for weeks at a high dose (125 mg/kg body weight), and at these levels, phenylacetate induced its own clearance by 27 % during this period[160]. Both phenylketonuric and normal subjects eliminated an oral dose (80 mg) of [^{14}C]-phenylacetic acid in the urine almost entirely as phenacetylglutamine, showing that the glutamine conjugation mechanism is not defective in phenylketonuria and that it is able to cope with the large amounts of phenylacetic acid produced in this disorder[161]. The contents of the colon, examined as human faecal water, contained phenylacetic acid, 3-phenylpropionic acid, 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3-(4′-hydroxyphenyl)-propionic acid and 4-hydroxy-3-methoxycinnamic (ferulic) acid at high levels, up to 400 μM for phenylacetic acid and 3-phenylpropionic acid in one individual. The mean levels were 188, 197, 110, 64, 61 and 10 μM, respectively[162].

Biological effects of C_6–C_2

Phenylacetic acid (administered as sodium phenylacetate) is one component of a drug (together with sodium benzoate) which is given for the treatment of acute hyperammonemia[163]. As discussed previously, the pharmacokinetic behaviour is well characterised[163], and phenylacetylglutatmine and hippuric acids are products excreted in the urine after an intravenous dose. Phenylbutyrate, a prodrug that is metabolised into its active component, phenylacetate, *in vivo,* has been reported to extend lifespan in *Drosophila*[164]. Large doses of phenylbutyrate can have some toxic side effects[165], and it has been shown that phenylacetate and phenylbutyrate modulate medulloblastoma[166]. Some C_6–C_2 compounds may modulate cholesterol metabolism (see below). When given orally, a single high dose of phenylacetate did not affect plasma glucose concentration nor gluconeogenesis in type II diabetic patients[158]. Phenylacetate affects cell growth and proliferation[166–168], inhibits prenylation and has been tested in both phase I and II clinical trials. 3,4-Dihydroxyphenylacetic acid exhibited antiproliferative activity on prostate and colon cancer cells[33]. 3-Phenylpropionic acid, 3-hydroxyphenylacetic acid and 3-(4′-hydroxyphenyl)-propionic acid, found at high concentrations in faecal water, decreased the expression of cyclo-oxygenase-2 in HT29 cells at physiologically relevant concentrations[162].

Properties of phenylpropionate derivatives (C_6–C_3) (e.g. dihydrocaffeic and dihydroferulic acids)

Absorption and metabolism of C_6–C_3

As summarised in Table 2, C_6–C_3 acids can be formed from many PPT substrates including proanthocyanidins. However, information on the absorption and metabolism of C_6–C_3 acids has come largely from studies of chlorogenic acids, conjugates of cinnamic acids with quinic acid. Such conjugates are widespread in foods and beverages, but coffee, artichoke,

apples and blueberries are particularly rich[7,169,170] Approximately, 1 h after the consumption of coffee, ferulic, caffeic and isoferulic acids appear in the plasma at low levels indicating absorption in the small intestine[137]. This early appearance was characterised in more detail, and the circulating compounds included caffeic acid-3-*O*-sulphate and ferulic acid-4-*O*-sulphate, and sulphates of 3- and 4-caffeoylquinic acid lactones[136]. These lactones are formed from the chlorogenic acids during coffee roasting[169] and are rarely found in other foods or beverages. Several hours later (T_{max} >4 h), greater concentrations of dihydroferulic acid, dihydroferulic acid-4-*O*-sulphate and dihydrocaffeic acid-3-*O*-sulphate appear in blood, indicating colonic absorption after microbial transformation[137]. Dihydroferulic acid, dihydroferulic acid-4-*O*-sulphate, dihydrocaffeic acid-3-*O*-sulphate, ferulic acid-4-*O*-sulphate, dihydroferulic acid-4-*O*-glucuronide and feruloyl-glycine were major urinary components. These catabolites collectively represented approximately 29 % of the chlorogenic acid dose, indicating good absorption of the microbial catabolites of chlorogenic acids[136].

The colon pH value ranges from pH 5·7 to 6·7[171], and unless there are localised pockets of significantly lower pH value, these phenolic acids with pK values in the range pH 4–5[172] will be extensively ionised. The absorption of C_6–C_3 catabolites has been examined using Caco-2 cells and rat everted intestinal sacs (Fig. 5). Dihydrocaffeic acid is absorbed mainly by the passive transcellular route and enters the circulation mainly as the free form, although a small proportion is glucuronidated[109]. The dihydrocaffeic acid is either rapidly sulphated, or methylated then sulphated, by the liver[173], so that the major metabolites in the circulation are free dihydroferulic acid, dihydrocaffeic acid-3-*O*-sulphate or dihydroferulic acid-4-*O*-sulphate. Dihydroferulic acid in the gut is absorbed partially by the passive transcellular route, but with some involvement of a monocarboxylic transporter[109,174]. 3-Hydroxycinnamic acid and its dihydroform, 3-(3′-hydroxyphenyl)-propionic acid, are both absorbed at least partially by the monocarboxylic transporter in Caco-2 cells[175].

Once absorbed in the colon, sulphate conjugation occurs primarily in the liver. In human subjects, sulphotransferase 1A1 is the most active isoform for sulfation of caffeic, isoferulic and dihydrocaffeic acids, whereas sulphotransferase 1E1 is most active towards the other methylated forms, i.e. ferulic and dihydroferulic acids[176]. Glucuronidation occurs to a much lesser extent. The levels of dihydrocaffeic acid-3-*O*-sulphate were approximately 45-fold greater than the corresponding glucuronides, and the amount of dihydroferulic acid-4-*O*-sulphate in urine was much greater than the amount of dihydroferulic acid-4-*O*-glucuronide. The only cinnamic acid for which the glucuronides predominated was isoferulic acid[176].

Cinnamic acids, as distinct from the dihydrocinnamic acids, have rarely been reported as gut flora catabolites of flavonoids. Rats excrete 4-hydroxycinnamic acid in urine after the consumption of apigenin, phloridzin and naringenin[56].

Biological effects of C_6–C_3

Excess solar UV radiation produces damage and initiates immune response and inflammation in skin, sometimes leading to cancer. Dihydrocaffeic and caffeic acids, but not dihydroferulic and ferulic acids, reduced the cytotoxicity and pro-inflammatory cytokine production (IL-6 and -8) in HaCaT cells, a keratinocyte model, following UV radiation[177], and dihydrocaffeic and 4-hydroxycinnamic acids inhibited UV-B damage in human conjunctival cells as assessed using the DNA damage marker, 7,8-dihydro-8-oxo-2′-deoxyguanosine[178]. 4-Hydroxycinnamic acid, but not protocatechuic acid, decreases basal oxidative DNA damage in rat colonic mucosa[179]. In CCD-18 colon fibroblast cells stimulated with IL-1β, dihydrocaffeic, dihydroferulic and dihydroxyphenylacetic acids attenuated PG E_2 demonstrating an anti-inflammatory effect in this system. In addition, dihydrocaffeic acid diminished the expression of the cytokines IL-1β, IL-8 and TNF-α, reduced malonyldialdehyde levels and reduced oxidative DNA damage (measured as 7,8-dihydro-8-oxo-2′-deoxyguanosine) in distal colon mucosal tissue in the dextran sodium sulphate-induced colitis model in rats[180]. Animal and studies *in vitro* also suggest that some C_6–C_2[181] and especially C_6–C_3[182] catabolites interfere with various enzymes in the mevalonate pathway including 3-hydroxy-3-methylglutaryl-CoA reductase, the rate limiting enzyme in hepatic cholesterol biosynthesis, albeit at concentrations unlikely to occur in plasma. However, these observations are of interest since commodities rich in PPT that would yield such catabolites, and the catabolites when given *per os*, have been shown to inhibit platelet aggregation[183] or to have cholesterol-lowering activity in animal[182,184–188] and human studies[189], and such gut flora catabolites may have contributed to the *in vivo* effect. Interference in the mevalonate pathway, particularly 3-hydroxy-3-methylglutaryl-CoA reductase inhibition, may have broader human significance[190].

Properties of phenylvalerate derivatives (C_6–C_5)

Following the consumption of green tea flavanols or proanthocyanidin-rich extracts, several C_6–C_5 catabolites have been detected in human plasma and urine as methyl and/or glucuronide/sulphate conjugates[191–193].

Prebiotic effects of polyphenols on the microflora

Although the fact that biotransformation of dietary PPT by the gut microflora occurs has been known for many years, the possibility that either the untransformed PPT or the phenolic acid catabolites might modify the composition and biochemical competence of the gut microflora has attracted comparatively little attention. Changes over 20 d in the catabolites produced from punicalagin have been attributed to changes in the GIT microflora[97]. There is evidence from *in vitro* models[31,32] and *in vivo* studies using human subjects, pigs and sheep that PPT and/or their catabolites influence the composition of the gut microflora, for example lowering the colonic pH value, suppressing bacteroides and pathogenic *Clostridium perfringens* and *Clostridium difficile*, and increasing the proportion of bifidobacteria and eubacteria without inhibiting lactic acid bacteria[31,194–196], but the exact mechanisms are uncertain[197]. The concentrations of benzoic acid, phenylacetic acid, phenylpropionic acid and 3-(3′-hydroxyphenyl)-propionic acid in human faecal water can each

reach millimolar concentrations[44,45] which has the obvious potential to modulate bacterial growth.

Caffeic acid, a comparatively minor component, can reach concentrations (approximately 50 μM)[45] well in excess of the concentration shown *in vitro* to produce 50 % inhibition in the growth of the opportunistic pathogen *Listeria monocytogenes*[198]. Various flavonoid aglycones are also found in faecal water, but at low concentrations (< 3 μM). There is some evidence also that some pathogenic protozoa are inhibited by some flavonoids (IC_{50} or EC_{50} values in the range 15–50 μM)[199,200] but the effect of the aromatic/phenolic acid catabolites does not seem to have been investigated. Although the yield of phenolic/aromatic acids is variable (up to × 10) between individuals and is not necessarily normally distributed[44,45,201,202], the potential for suppressing some pathogens and for a prebiotic effect in some individuals clearly exists.

Summary, conclusions and recommendations for future research

The colonic microbiota transform a very complex range of PPT substrates that will vary between individuals and occasions. These transfomations can be extensive, and while some microbial catabolites are substrate-specific (equol, urolithins, mammalian lignans), certain catabolites are common to many of the major substrates, and this implies that the spectrum of catabolites produced is less complex and qualitatively less variable than the spectrum of substrates consumed. The catabolites most likely to dominate are the C_6–C_1, C_6–C_2 and C_6–C_3 dihydro acids derived from cinnamates, anthocyanins and most other flavonoids. These microbial catabolites are often better absorbed than the parent compounds, because of the mechanism of absorption, the large absorptive area available in the colon and the high concentrations (approaching millimolar) in the colonic lumen.

Therefore, we propose that these microbial catabolites could be responsible for a significant proportion of the biological activity derived from consumption of fruits, vegetables and other plant derived products such as fruit drinks, wine, coffee and tea.

The biological activity of these microbial catabolites would be in addition to the biological activity of any absorbed parent compound and its mammalian metabolites, and the potential for synergy between microbial catabolites and the parent compound and its mammalian metabolites could be important but has not been studied. Because of the potential importance of the microbial catabolites, we need to understand better the factors controlling (i) their production and whether this can be modulated advantageously, (ii) their absorption and mammalian metabolism and (iii) their biological activities both *in vitro* and *in vivo*.

The existing epidemiological data must be revisited and extended so as to take account of substances not present in the diet *per se*. For a given substrate consumption, there is often a large inter-individual variation (approximately × 10) in catabolite yields, subsequent plasma concentrations and also variation in the concentration–time profile. It is essential to define the factors responsible, whether manifest in the microflora, in the host or in both. Similarly it is important to determine the potential of PPT and their catabolites to modulate the gut microflora and hence potentially their own production. The answers to these are crucial if we are to understand and exploit the effect of PPT consumption on human health.

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