Flavonoids comprise the most common group of plant polyphenols in fruits and vegetables. More than 5000 different flavonoids have been described. The major subclasses of flavonoids (Fig. 1) include the flavones (e.g., apigenin, luteolin), flavonols (e.g., quercetin, myricetin), flavanones (e.g., naringenin, hesperidin), catechins or flavanols (e.g., epicatechin, gallobratechin), anthocyanidins (e.g., cyanidin, pelargonidin), and isoflavones (e.g., genistein, daidzein). The proanthocyanidins or condensed tannins are oligomeric and polymeric flavan-3-ols. Most of the flavonoids, except flavanoids having a pyrogallol structure are found as aglycones. The aglycone can be absorbed and/or further metabolized to simple phenolic acids or conjugated forms with glucuronic acid or sulfate. Flavan-3-ols (catechins) are unusual in that they are not glycosylated in the natural form. Anthocyanins present a major deviation from this generalization in that the intact anthocyanin is absorbed and the aglycone is generally not present in plasma or urine.

The potential role of microbial metabolism in the gastrointestinal tract is often overlooked. A large number of flavonoids and other phenolic compounds have recently been identified in colonic water of the human. Some of the phenolic or aromatic compounds were probably generated after ring fission from flavonoids by human gut microflora. Compared to polyphenolic flavonoids, the monophenolics may be present in considerable excess both in terms of concentration and number within the colon. Within the gastrointestinal tract the biological effect of polyphenolics is generally not present in plasma or urine.6

Although there are some similar pathways of metabolism for some of the flavonoids, there are striking contrasts which present interesting challenges. Similarities and contrasts are illustrated in anthocyanins, isoflavones, flavonols (quercetin), and proanthocyanidins. Hydrolysis of the flavonoid to the aglycone prior to absorption and subsequent conjugation to the glucuronide or sulfate form is thought to occur via enteric bacteria or enteric mucosal metabolism. The action of the enzyme lactase-phlorizin hydrolase, localized to the apical membrane of small intestinal epithelial cells, and/or cytosolic β-glucosidase, is important in the formation of the aglycone prior to absorption. Dietary flavonoid glucosides may also be hydrolyzed in the oral cavity by both bacteria and sloughed intestinal epithelial cells to deliver the biologically active aglycones at the surface of the epithelial cells. The aglycone can be absorbed and/or further metabolized to simple phenolic acids or conjugated forms with glucuronic acid or sulfate. Flavan-3-ols (catechins) are unusual in that they are not glycosylated in the natural form. Anthocyanins present a major deviation from this generalization in that the intact anthocyanin is absorbed and the aglycone is generally not present in plasma or urine.

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the amount of quercetin consumed and identified in tissues.\textsuperscript{10} Likewise, anthocyanins (cyanidin-3-O-glycoside) have been shown to be metabolized to the 3′-O-methyl derivatives (peonidin-3-O-glycosides) and 4′-O-methyl derivatives (isopeonidin-3-O-glycosides).\textsuperscript{5,6,11} Delphinidin-3′-O-glucoside has been reported to be methylated at the 4′ position in the B-ring of delphinidin,\textsuperscript{12} which is expected since delphinidin has the pyrogallol structure in the B-ring.

Conjugation of flavonoids with glucuronic acid or sulfate is also common. Glucuronidation of flavonols, flavanones, flavones and their glycosides have been observed. The extent of glucuronidation seems to be dependent on the available hydroxyl groups. Flavonoids that contained a 3′, 4′-ortho-dihydroxy (or catechol) B-ring were transferred predominantly as glucuronides, whereas the monohydroxylated B-ring phenolics were less predisposed to glucuronidation. Flavonoids with monohydroxymethylated B-rings seem to be less susceptible to glucuronidation in the small intestine but may be glucuronidated in the liver. Anthocyanins have been shown to be conjugated with glucuronic acid.\textsuperscript{5,6,11} Glucuronide/sulfate and methylated conjugates of quercetin appeared in the lymph of rats after quercetin administration.\textsuperscript{13} Catechin and epicatechin are extensively O-methylated during transfer across the jejunum, consistent with the reported activity of catechol-O-methyltransferases in
the jejunum. Up to 30% of flavan-3-ol compounds from catechin and epicatechin detected in the jejunal serosal fluid were O-methylated and an additional 20% were O-methylated and glucuronidated.14 Catechin and epicatechin, which may enter the portal vein at relatively high concentrations from ileal transfer, may be further metabolized to O-methylated and glucuronidated forms by phase II metabolism in the liver.

Proanthocyanidins represent another class of polyphenolics that are oligomers or polymers of flavan-3-ols. The common occurrence of proanthocyanidins in foods makes them an important part of the human diet.15 Procyanidins present a special situation when it comes to metabolism in that a significant proportion may exist as larger molecular weight polymers.15 In addition to polymers, procyanidins are present as monomers (catechin and epicatechin) and as oligomers. Epicatechin (and catechin) is absorbed from the intestinal tract and conjugated to glucuronide and/or sulfate form.16 Natsume et al.17 identified (-)-epicatechin-3′-O-glucuronide as the main metabolite in plasma and urine from humans and also the metabolites of 4′-O-methyl(-)-epicatechin-3′-O-glucuronide and 4′-O-methyl(-)-epicatechin-5′ or 7-O-glucuronoide. In rats, 3′-O-methyl(-)-epicatechin, (-)-epicatechin-7-O-glucuronide and 3′-O-methyl(-)-epicatechin-7′-O-glucuronide were identified. In humans, (-)-epicatechin as well as quercetin can be conjugated with glucuronic acid within the intestine via the UDP-glucuronosyl transferase enzyme resulting in 3′-O-glucuronide metabolites.

Trace quantities of procyanidin B1, B2, B3 and B4 dimers and the C2 trimer have been detected in urine.18 Oligomeric procyanidins do not appear to be depolymerized into monomeric flavan-3-ols to any extent during passage through the stomach and gastrointestinal tract.18-20 No evidence exists that suggests that any of the higher oligomers or polymers of procyanidins are absorbed. However, specific brain proteins have been shown to be modulated in rats by a dietary supplement of procyanidins from grape seed extract.21 However, we may have to begin to think ‘outside the box’ and consider possible ‘local’ effects of procyanidins in the gastrointestinal tract that may trigger some other signal that may reach the brain.

A large number of the studies completed to date on the metabolism and conjugation of flavonoids have been done using the rat as a model. However, it is becoming apparent that for some of the flavonoids the rat may not be the best model for extrapolation to human metabolism.22 In rats and monkeys significant amounts of diadzein are metabolized to equol; however, in the pig and at least 60-70% of humans no equol is produced. Rats produce protocatechuic acid from cyanidin-3-O-β-glucoside (C3G), with concentrations in plasma being eightfold higher than C3G.23 However, only trace amounts24 or no protocatechuic acid5,6,25,26 have been identified in pig or human plasma following consumption of anthocyanins. As indicated above, the glucuronide conjugates and/or methylated forms of (-)-epicatechin were different between the human and rats.17

An area of investigation that has received little attention is the possibility of interaction or synergism of different flavonoids and/or phenolic acid metabolites. Rechner27 demonstrated that a mixture of anthocyanins and colonic metabolites were active in lowering (10-40%) the activation of platelets (P-selectin expression) at ten times lower concentrations compared to the individual components. No activity could be identified when the components were tested individually at the equivalent concentration, indicating that synergism of the different components was causing the effect. Platelet reactivity to strong agonists such as collagen and ADP was not influenced.27

**IN VITRO VERSUS IN VIVO STUDIES**

In terms of individual flavonoids, the aglycone quercetin has been commercially available for some time. Thus, this compound has been used for numerous *in vitro* studies, with many of the published biological effects based mainly on these *in vitro* studies. However, quercetin is rapidly metabolized during the *in vivo* digestion/absorption processes. Until recently, we have had little knowledge of the presence of quercetin or its metabolites in tissues. Recently, Muroto and Terao13 demonstrated that quercetin, after being methylated and/or conjugated in the gastrointestinal mucosa of rats with glucuronide or sulfate, was transported in the lymph but not as quercetin aglycone. De Boer et al.10 published one of the most comprehensive studies of quercetin and quercetin metabolites in the tissues of rats and pigs. After 11 weeks of feeding quercetin, quercetin metabolites were identified in many rat tissues, with the highest concentrations found in lungs and the lowest in brain, white fat, and spleen. In a shorter-term pig study, liver and kidney contained high concentrations of quercetin metabolites, whereas brain, heart, and spleen had low concentrations.10 Quercetin was present in tissues primarily as methylated, sulfate, and glucuronidated conjugates. However, the presence of β-glucoronidase activity in rat lung, liver, and kidney may cause *in vivo* conversion of conjugated quercetin to the free aglycone.10

Several flavonoids have been identified in brain tissue of rats, including naringenin, puerarin, tangeretin, genistein, epicatechin, and anthocyanins.28,29 Anthocyanins (cyanidin-3-O-β-galactoside, C3G, cyanidin-3-O-β-arabinoside, malvidin-3-O-β-galactoside, malvidin-3-O-β-glucoside, malvidin-3-O-β-arabinoside, peonidin-3-O-β-arabinoside, and delphinidin-3-O-β-galactoside) were found in the cerebellum, cortex, hippocampus, and striatum of 19-month-old F344 rats fed blueberries for 10 weeks, but not in the controls.
This suggests that these polyphenolic compounds are able to cross the blood–brain barrier and localize in various brain regions important for learning and memory.\textsuperscript{28} Talavera \textit{et al.}\textsuperscript{29} measured anthocyanin distribution in the stomach, jejunum, liver, and kidney, as well as the brain in rats fed a blackberry anthocyanin-enriched diet for 15 days. The stomach tissue contained only native blackberry anthocyanins (cyanidin 3-O-glucoside and cyanidin 3-O-pentoside), while in jejunum, liver, and kidney native and methylated anthocyanins as well as conjugated anthocyanidins (cyanidin and peonidin monoglucuronides) were identified. Proportions of anthocyanin derivatives differed according to the organ considered, with the liver having the highest proportion of methylated forms. This study demonstrated the presence of the aglycone form in jejunum and plasma. In the brain, total anthocyanin content (blackberry anthocyanins and peonidin 3-O-glucoside) reached 0.25 mmol g\(^{-1}\) of tissue.\textsuperscript{29} Equol, an intestinal metabolite of diadzein, was found to be the predominant isoflavone phytoestrogen in breast tissue, with concentrations exceeding those in the serum. Concentrations of isoflavones were at least 100-fold higher in the urine than in serum and breast tissue.\textsuperscript{30} Additional studies of the concentration of flavonoids and their metabolites are needed to identify target tissues of for the various flavonoids, which may provide help in understanding their mechanisms of action in \textit{vivo}.

\textbf{IN VITRO STUDIES}

Considerable research has been done using cell culture systems to study effects of various flavonoids. Compounds that have been studied have depended largely upon commercially available aglycones. Based on the recent literature, the flavonoids that have been studied \textit{in vitro} may not represent what might be available to the tissues and cells \textit{in vivo}. Cells other than those lining the gastrointestinal tract will not likely be exposed to the anthocyanin aglycone. For other flavonoids, the methylated or conjugated forms may be the form primarily present in the cell. However, most of these conjugated forms are not commercially available or are difficult to prepare in sufficient quantities to study.

A further difficulty with studies performed in cell culture has to do with the concentrations of the polyphenolic compound used. In most \textit{in vitro} studies, the concentrations used have been in the millimolar concentration range. However, based on data on plasma concentrations (in the absence of much, if any, tissue concentrations) the highest concentrations are likely in the micromolar or nanomolar range. Plasma concentrations of anthocyanins have been reported to be in the 1–120 nM range in pig and human studies.\textsuperscript{5,6,11,26} Plasma concentration of quercetin and its metabolites were 23 and 108 \(\mu\)M in rats fed 1 g kg\(^{-1}\) and 10 g kg\(^{-1}\) quercetin, respectively, and 1.3 \(\mu\)M in pigs fed 500 mg kg\(^{-1}\) body weight per day. Serum concentrations of genistein and diadzein have been observed to be in the range 0.1–1.1 \(\mu\)M in rats, monkeys, pigs, and humans.\textsuperscript{22}

Recent studies have focused on the gene expression profile of various cells in culture treated with various flavonoids.\textsuperscript{31,32} The form of the flavonoid applied to the cells can be quite important. Differences have been observed between the glycoside form and the aglycone form of cyanidin-3-glucoside in terms of the genes that are affected and the direction of their regulation.\textsuperscript{31} Furthermore, investigators have not routinely determined the stability of the compounds in the culture system. Some anthocyanins are not stable longer than 2 h in some cell culture buffer systems (Wu and Prior, unpublished data), thus making interpretation of the results difficult in terms of assigning a response to a particular compound.

**SUMMARY**

It is becoming clear that study of the health effects of flavonoids is going to require a multidisciplinary approach in order to fully understand the often complex chemistry of the flavonoids as well as the biology and health effects. Future research needs increased focus on metabolism of flavonoids in the gut, the compounds that are being absorbed, and the concentrations and forms of the flavonoids and their metabolites that are present in various tissues. Careful selection of the animal species and/or cell culture system that has most relevance to human biology is also critical.

**REFERENCES**


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