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# Absorption, Metabolism, and Bioavailability of Flavonoids

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## INTRODUCTION

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. They comprise 2-phenylbenzo-γ-pyrones, -dihydropyrones, -dihydropyrans, and -pyryliums. Variations in the oxygen-containing heterocyclic ring give rise to catechins (dihydropyrans), flavonols and flavones (γ-pyrones), flavanones (dihydropyrones), and anthocyanidins (pyryliums) (Fig. 1). Attachment of the second benzene ring to the 3 instead of the 2 position creates isoflavonoids (Fig. 1). In addition, the basic structure of flavonoids allows a multitude of substitution patterns in the two benzene rings within each class of flavonoids. Over 4000 different naturally occurring flavonoids have been described (1), and this list is still growing.

The major dietary sources of flavonoids are vegetables, fruits, and beverages such as tea and red wine (2–7). Kühnau (4) estimated the total flavonoid intake in the United States to be 1 g/day expressed as glycosides or 650 mg/day expressed as aglycones, but most likely this estimate is too high. New, more specific food analyses (2,3) suggested that the Dutch intake of flavonols and flavones is 23 mg/day (expressed as aglycones) (8), as opposed to Kühnau's estimate of 115 mg/day (expressed as aglycones) in the United States (4).

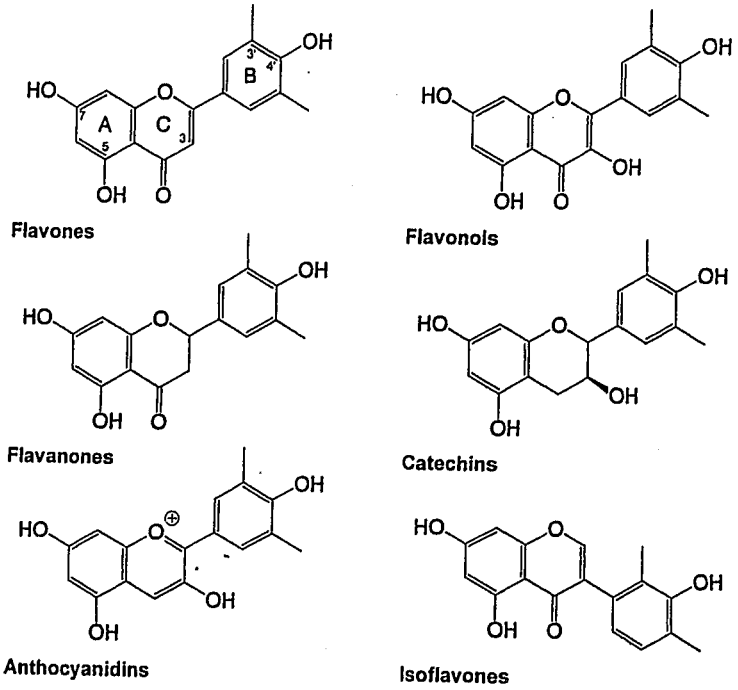


**Figure 1** Subclasses of flavonoids. Classification is based on variations in the heterocyclic c-ring.

## History

It was observed in 1936 that a mixture of two flavanones decreased capillary permeability and fragility in humans. This gave rise to a claim for vitamin action of flavonoids (vitamin P). Doubts about the evidence for these claims prompted the U.S. Council on Foods and Nutrition to issue a report on the absorption and excretion of flavonoids (9). It was concluded that flavonoids are probably largely destroyed in the mammalian gastrointestinal tract, thus strengthening the skepticism about "vitamin P." The possibility was suggested that one or more metabolites were responsible for the potential therapeutic effects.

In the 1950s and 1960s, research on absorption and metabolism of flavonoids was advanced by Booth, Das, and Griffiths, who published many articles exploring the metabolic routes of various flavonoids in animals. Several review articles describe these achievements (4,10,11). How-

ever, most of these studies used high doses, mainly because the analytical techniques used for identification of metabolites lacked sensitivity. The details of the metabolic pathway still needed to be unraveled.

### Recent Developments

A multitude of in vitro studies suggested that flavonoids inhibited, and sometimes induced, a large variety of mammalian enzyme systems (1). Some of these enzymes are involved in cell division and proliferation, platelet aggregation, detoxification, and inflammatory and immune responses. Thus, it is not surprising that effects of flavonoids on different stages in the cancer process, on the immune system, and on hemostasis were reported in cell systems and animals (1,12). Recently, much attention has been paid to the antioxidant properties of flavonoids, caused by their ability to scavenge oxygen free radicals (13–15). Oxygen free radicals and lipid peroxidation might be involved in atherosclerosis, and a role has also been suggested in cancer and chronic inflammation (16). There is indeed some epidemiological evidence for an inverse association of the intake of flavonols and flavones with subsequent coronary heart disease (17), although the association is still controversial (18). No association of flavonoid intake and cancer risk in humans has been established (17).

### Scope of This Review

The increasing awareness of a potential beneficial role of flavonoids in human health provided new perspectives for flavonoid research. Knowledge of the pharmacokinetics and bioavailability of flavonoids in humans is indispensable to fully evaluate this role. Metabolic transformations of flavonoids in the human system may be crucial for their biological effect. This chapter focuses on the fate of dietary flavonoids, except isoflavonoids, in mammals, and thus updates the reviews published (4,10,11). Pharmaceutical preparations are dealt with only where this helps to understand general principles of bioavailability.

## ABSORPTION

The major questions here are to what extent flavonoids are absorbed from the gastrointestinal tract and which factors affect absorption. Absorption of flavonoids from the diet was long considered to be negligible, as most of the flavonoids, except catechins, are present in plants bound to sugars as

glycosides, and these were considered nonabsorbable. Studies with germ-free rats indeed showed that large amounts of unchanged glycosides were excreted in feces, whereas only small amounts of glycosides were found in feces of rats with a normal microflora (19). Evidently, enzymes that can split these predominantly β-glycosidic bonds were not secreted into the gut or present in the intestinal wall. Bacteria in the colon were able to hydrolyze flavonoid glycosides (20–22), but at the same time degraded the liberated flavonoid aglycones. In addition, the absorption capacity of the colon is far less than that of the small intestine. The assumption that only free flavonoids (aglycones) are absorbed by the gut and that glycosides are not is a classic example of "conventional wisdom"—it was never seriously questioned, even though there was little evidence to support it.

## Absorption of Pure Compounds

Balance studies with radioactively labeled flavonoid aglycones were used in the 1970s and 1980s to quantify the absorption of (+)-catechin, quercetin, and flavanones in rodents, monkeys, and humans (Table 1), always without their attached sugars. In these studies total radioactivity was measured in urine, feces, expired air, and sometimes also in tissues (28,31). As a consequence, the excreted radioactivity included the intact administered compound, if any, and metabolites that contained radioactive atoms. Catechins and their microbial degradation products were well absorbed as judged by excretion of 47–58% of the total administered radioactivity into urine. The administered dose did not seem to be an important variable, and absorption in rodents, monkeys, and humans was similar. It was suggested (23) that the radioactivity not accounted for (~20%) had possibly been incorporated into tissues. Excretion of unchanged catechin aglycones in urine was only 0.1–2% of the dose.

Radioactive quercetin aglycone was less well absorbed than catechins, with only 4–13% recovered in urine. About 40% was excreted with feces. The high excretion of radioactivity associated with $CO_2$ could originate to some extent from absorbed quercetin metabolites through β-oxidation of phenylpropionic acids. In rats, 1% of the administered dose of quercetin was excreted as quercetin (conjugates) (28). However, after oral administration of quercetin aglycone to humans, neither the aglycone nor its conjugates could be detected in urine (29). These investigators concluded that less than 1% of the administered quercetin could have been absorbed (29). This conclusion was based on the limit of detection of their analytical method. Absorption of the flavanone aglycones and their metabolites was some-

**Table 1** Summary of Studies on Absorption of Flavonoids

| Compound | Species | Dose (mg/kg body weight) | Excretion (% of dose) | | | | Ref. |
|---|---|---|---|---|---|---|---|
| | | | Urine | $CO_2$ | Feces | Total | |
| (+)-[U-$^{14}$C]Catechin | Rat, guinea pig | 200 | 58 | 18 | 1 | 77 | 23 |
| | Monkey | 125 | 54 | – | 2 | 56 | 24 |
| | Human | 25 | 55 | – | – | – | 25 |
| 3-[$^{14}$C]Methoxy-(+)-catechin | Human | 30 | 47 | – | – | – | 26 |
| [random-$^{14}$C]Quercetin aglycone | Rat | 15 | 4 | 12 | 33 | 79 | 27 |
| [4-$^{14}$C]Quercetin aglycone | Rat | 630 | 13 | 41 | 47 | 100 | 28 |
| | Rat, bile duct cannulated | 630 | 21[a] | 34 | 13 | 68 | 28 |
| Quercetin aglycone | Human | 60 | <1[b] | – | 53 | – | 29 |
| Quercetin aglycone | Human | 1.4 | 0.1[b] | – | 76 | 100 | 30 |
| Rutin | Human | 1.4[c] | 0.1[b] | – | 83 | 100 | 30 |
| Quercetin glucosides from onions | Human | 1.2[c] | 0.3[b] | – | 48 | 100 | 30 |
| [3-$^{14}$C]Hesperetin aglycone | Rat | 1.5 | 33 | 39 | 15 | 89 | 31 |
| [2-$^{14}$C]Flavanone aglycone | Rat | 100 | 28 | 0 | 71 | 99 | 32 |

– = Not determined.
[a]Urine + bile.
[b]Total quercetin, including conjugates.
[c]Expressed as quercetin equivalents.

what higher than that of quercetin aglycone: about 30% was excreted with urine.

## Absorption of Flavonoids from Foods

Previous studies did not address absorption of flavonoids from foods, but only of pure aglycones. We ourselves were interested in the absorption of flavonoids from regular foods, and in humans rather than in animals. To circumvent the problem of microbial degradation, we employed ileostomy subjects (30). To our surprise, the quercetin glycosides from onions were absorbed far better than the pure aglycone (Table 1). Absorption from onions was 52% of the ingested amount, while only 24% of the aglycone and 17% of rutin (quercetin-3-*O*-rutinoside) were absorbed. A small percentage (<0.5%) of the absorbed quercetin was excreted into urine as the intact quercetin molecule, conjugated or otherwise. Thus, glycosides can be absorbed in humans as such without prior hydrolysis by microorganisms. Evidence for direct absorption of glycosides was also found in rats; oral administration of naringin (5,4′-dihydroxyflavanone-7-neohesperidoside) and hesperidin (5,3′-dihydroxy-4′-methoxyflavanone-7-rutinoside) showed that the parent glycosides were secreted with bile (33), which implied that glycosides were transported across intestinal membranes.

As far as the catechins are concerned, epigallocatechins present in green tea were shown to be absorbed in rats. The following compounds were identified in the portal vein after their oral administration: (−)-epigallocatechin-3-gallate (EGCg), (−)-epigallocatechin (EGC), (−)epicatechin-3-gallate (ECg), and (−)-epicatechin (EC) (34,35). It was reported that 2% of ingested green tea catechins were excreted into urine in humans (36).

Data on the mechanisms of flavonoid absorption across the intestinal membrane itself are scarce. The absorption of (+)-catechin, epicatechin-2-sulfonate, and 7,3′,4′-tri-*O*-(β-hydroxyethyl)quercetin-3-rutinoside was studied in the rat everted sac model, and the rate of passive transport was in the order epicatechin-2-sulfonate > (+)-catechin > trihydroxyrutinoside (37). However, of these three flavonoids, only (+)-catechin was absorbed in an in situ perfused small intestine segment of the rat (38). This demonstrated the limitations of the everted sac model.

In conclusion, the absorption of flavonoid aglycones in rats was estimated at 4–58% of ingested radioactive aglycones as judged by the amount of radioactivity excreted into urine. Contrary to the common belief that only aglycones can be absorbed, flavonol glycosides were well absorbed in humans without prior hydrolysis by microorganisms, and similar observa-

tions have been made in rats. Only a small fraction of the flavonols subsequently excreted with urine had an intact flavonoid structure.

## METABOLISM

### Introduction

The metabolism of flavonoids is relevant because a major portion of administered flavonoids is excreted in the urine after only more or less extensive modification in the body. Thus, a potential biological effect predicted from in vitro studies may be modulated in vivo due to metabolism after ingestion of the parent compounds. The major questions left to answer are which products are formed and to what extent and what their potential biological effect is. In the metabolism of flavonoids, two compartments are important. The first consists of tissues in the body, such as the liver, where biotransformation enzymes act upon absorbed flavonoids and their absorbed colonic metabolites. The second metabolically active compartment is the colon, where microorgansims degrade unabsorbed flavonoids and flavonoids are absorbed and then secreted with bile.

#### Metabolism by the Liver

Flavonoids absorbed as such as well as their degradation products absorbed from the colon after bacterial action are subsequently metabolized by enzymes located mainly in the liver. The kidney and the small intestine might also contain enzymes capable of biotransformation of flavonoids (39). The general phase I biotransformation reactions introduce or expose polar groups (39). These may be less relevant to naturally occurring flavonoids and their colonic degradation products because they already contain several polar hydroxyl groups. Indeed, phase I transformations have been reported almost exclusively for synthetic flavonoids lacking hydroxyl groups (11). Conjugation of these polar hydroxyl groups with glucuronic acid, sulfate, or glycine constitutes phase II biotransformation reactions (39), which have been reported both for flavonoids and for their colonic metabolites. The water-soluble conjugates thus formed can be excreted into urine. In addition, the molecular weight increases, which promotes secretion into bile (40). Finally, *O*-methylation by the enzyme catechol-*O*-methyltransferase plays an important role in the inactivation of the catechol moiety (41), i.e., the two adjacent (*ortho*) aromatic hydroxyl groups, of flavonoids and their colonic metabolites.

#### Metabolism by the Colonic Flora

Flavonoids can reach the colon in two different forms: as unabsorbed flavonoids passing through the small intestine and as absorbed flavonoids

secreted as conjugates into the duodenum via the gall bladder. In the colon both are stripped of their sugar moieties, glucuronic acids, and sulfates by glycosidases, glucuronidases, and sulfatases of colonic bacteria (42). Hydrolysis by bacterial enzymes enables absorption in the colon because the aglycones formed are less polar (39,43). As a result, secreted glucuronides and sulfates can be reabsorbed, thus entering an enterohepatic cycle. Another possibility exists in that the heterocyclic oxygen-containing ring is split. The subsequent degradation products can evidently be absorbed because they are found in urine. These include a variety of phenolic acids, which, depending on the hydroxylation pattern, are antioxidants themselves (44) and may thus contribute to the biological effects of dietary flavonoids. The type of ring fission depends on the type of flavonoid; as a result, primary ring fission products of catechins, flavonols, and flavones and flavanones are all different. Hydroxyl groups are necessary for ring cleavage, and the hydroxylation pattern of the flavonoids determines their susceptibility to microbial degradation in the colon (21). Free hydroxyl groups at positions 5 and 7 together with a free hydroxyl group at the 4′ position are necessary for ring fission of the heterocyclic C-ring (21). Whether or not the position of the free hydroxyl group in ring B is essential is not known. A flavonoid that has only one free hydroxyl group in ring A at position 7, e.g., 7,4′-dihydroxyflavone, withstands ring fission. Whether two hydroxyls in ring A or a single hydroxyl group at position 5 are sufficient for ring fission is not known. The presence of *O*-methyl substitution in these essential positions reduces susceptibility to cleavage. Methylation of the 3-hydroxyl group in the C-ring of (+)-catechin also increases resistance to ring scission. The most widespread dietary flavonoids have a 5,7,3′,4′-hydroxylation pattern, which will enhance ring cleavage by bacteria after hydrolysis of the glycosides in the colon. Thus, the formation of potentially active metabolites through bacterial degradation in the colon is highly dependent on the structural details of the dietary flavonoids involved.

## Catechins

After oral administration of labeled catechins to humans, some 50% of the radioactivity is recovered in urine, only 0.5–3% of which is in the form of the catechin aglycone. Thus, catechins are extensively metabolized.

### Enzymatic Transformations of Catechins in Body Tissues

Unfortunately phase I transformation reactions for catechins have not been described; information on metabolism in liver and other organs is limited to the attachment of various substituents to existing hydroxyl groups, and

most of this information is qualitative only. This subject evidently needs closer study.

Sulfates and glucuronides of (+)-catechin were identified after oral administration of (+)-catechin to rodents, monkeys, and humans (Table 2). Intravenous and intraperitoneal administration of this flavonoid to rodents and monkeys also produced these conjugates. Thus, *glucuronidation* and *sulfation* in body tissues was demonstrated. Rats and humans excreted sulfates and glucuronides of 3-methoxy-(+)-catechin into urine, plasma, and bile after intravenous injection of this compound (26,51). Because catechins are polyhydroxylated compounds, several sites offer themselves for binding of glucuronic acid or sulfate. In human urine, two glucuronides of 3,3′-dimethoxy-(+)-catechin and of 3-methoxy-(+)-catechin and a sulfate conjugate of 3-methoxy-(+)-catechin were found (26). The position of these groups could not be determined. Two different sulfates and a mixed sulfate-glucuronide of (+)-catechin were detected in perfusate and bile of a perfused isolated rat liver (52). After oral administration of (−)-epigallocatechin-3-gallate and (−)-epicatechin of green tea to human volunteers, the major conjugates found in plasma were sulfates, whereas the (−)-epigallocatechin circulated as the glucuronide (36). Some 20% of the (−)-epigallocatechin-3-gallate was also present as unconjugated compound. In addition to conjugates of (+)-catechin, glucuronides of the main colonic metabolites, the three valerolactones and 3-hydroxyphenylpropionic acid (Fig. 2), were identified in urine of humans (47). Sulfates of δ-(3-hydroxyphenyl)-γ-valerolactone and 3-hydroxyphenylpropionic acid were also present.

In vitro incubation of (+)-catechin in liver homogenates produced 3′-methoxy-(+)-catechin, thus showing that *O-methylation* had occurred (49). Purified catechol-*O*-methyl transferase (E.C.2.1.1.3) was also able to form the 3′-methoxy compound (49). Only the 3′-hydroxyl group of (+)-catechin or 3-methoxy-(+)-catechin was methylated, suggesting that catechol-*O*-methyl transferase was also involved in vivo. Additional evidence for the role of catechol-*O*-methyl transferase in *O*-methylation came from experiments with oral administration of valerolactones, the primary bacterial ring fission products of (+)-catechin, to guinea pigs (46). Only δ-(3-4-dihydroxyphenyl)-γ-valerolactone was *O*-methylated, whereas δ-(3-hydroxyphenyl)-γ-valerolactone, which lacks a catechol group, was not *O*-methylated.

In conclusion, catechins were metabolized by liver enzymes to give sulfates, glucuronides, and mixed sulfates-glucuronides, which were excreted into bile, urine, and plasma. In addition, *O*-methylated catechin conjugates were produced by catechol-*O*-methyl transferase in the liver. Because of the specificity of this enzyme, only *ortho*-hydroxy-methoxy me-

**Table 2** Metabolites of (+)-Catechin Found After Oral (p.o.) or Intravenous (i.v.) or Intraperitoneal (i.p.) Administration to Various Species

| Metabolite | Species | Dose (mg/kg body weight) | Body fluid | Ref. |
|---|---|---|---|---|
| (+)-Catechin aglycone | Rat | i.v. 100 | Urine | 45 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. and i.p. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 47 |
| | Human | p.o. 25 | Urine, plasma | 25 |
| (+)-Catechin glucuronide(s) | Rat | i.v. 100 | Urine, bile | 45 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. and i.p. 125 | Urine, bile | 24 |
| | Human | p.o. 80 | Urine | 47 |
| | Human | p.o. 25 | Urine | 25 |
| (+)-Catechin sulfate(s) | Guinea pig | p.o. 150 | Urine | 46 |
| | Human | p.o. 80 | Urine | 47 |
| | Human | p.o. 25 | Urine | 25 |
| (+)-Catechin or its conjugates | Human | p.o. 45 | Serum | 48 |
| 3′-*O*-Methyl-(+)-catechin | Rat | p.o. 10 | Bile | 49 |
| | Human | p.o. 25 | Urine, plasma | 25 |
| δ-(3-Hydroxyphenyl)-γ-valerolactone | Rat, guinea pig | p.o. 200 | Urine, feces | 23 |
| | Rat | i.v. 100 | Urine | 45 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. and i.p. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 47 |
| δ(3,4-Dihydroxyphenyl)-γ-valerolactone | Rat, guinea pig | p.o. 200 | Urine, feces | 23 |
| | Rat | i.v. 100 | Urine | 45 |
| | Guinea pig | p.o. 150 | Urine | 46 |

| | | | | |
|---|---|---|---|---|
| | Monkey | p.o. and i.p. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 47 |
| δ-(4-Hydroxy-3-methoxyphenyl)-γ-valerolactone | Rat, guinea pig | p.o. 200 | Urine | 23 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 47 |
| 3-Hydroxyphenylpropionic acid | Rat | p.o. 200 | Urine | 23 |
| | Rat | p.o. 200 | Urine | 50 |
| | Rat | i.v. 100 | Urine | 45 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 47 |
| | Human | p.o. 25 | Urine | 25 |
| 4-Hydroxyphenylpropionic acid | Rat | i.v. 100 | Urine | 45 |
| 3-Hydroxyphenylhydracrylic acid | Monkey | p.o. and i.p. 125 | Urine | 24 |
| | Human | p.o. 80 | Urine | 24,47 |
| 4-Hydroxyphenyllactic acid | Rat | i.v. 100 | Urine | 45 |
| 4-Hydroxyphenylacetic acid | Rat | i.v. 100 | Urine | 45 |
| 3-Hydroxyhippuric acid | Rat | p.o. 200 | Urine | 23 |
| | Rat | p.o. 200 | Urine | 50 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. 125 | Urine | 24 |
| | Human | p.o. 25 | Urine | 25 |
| 3-Hydroxybenzoic acid | Guinea pig | p.o. 200 | Urine | 23 |
| | Guinea pig | p.o. 150 | Urine | 46 |
| | Monkey | p.o. 125 | Urine | 24 |
| | Human | p.o. 25 | Urine | 25 |

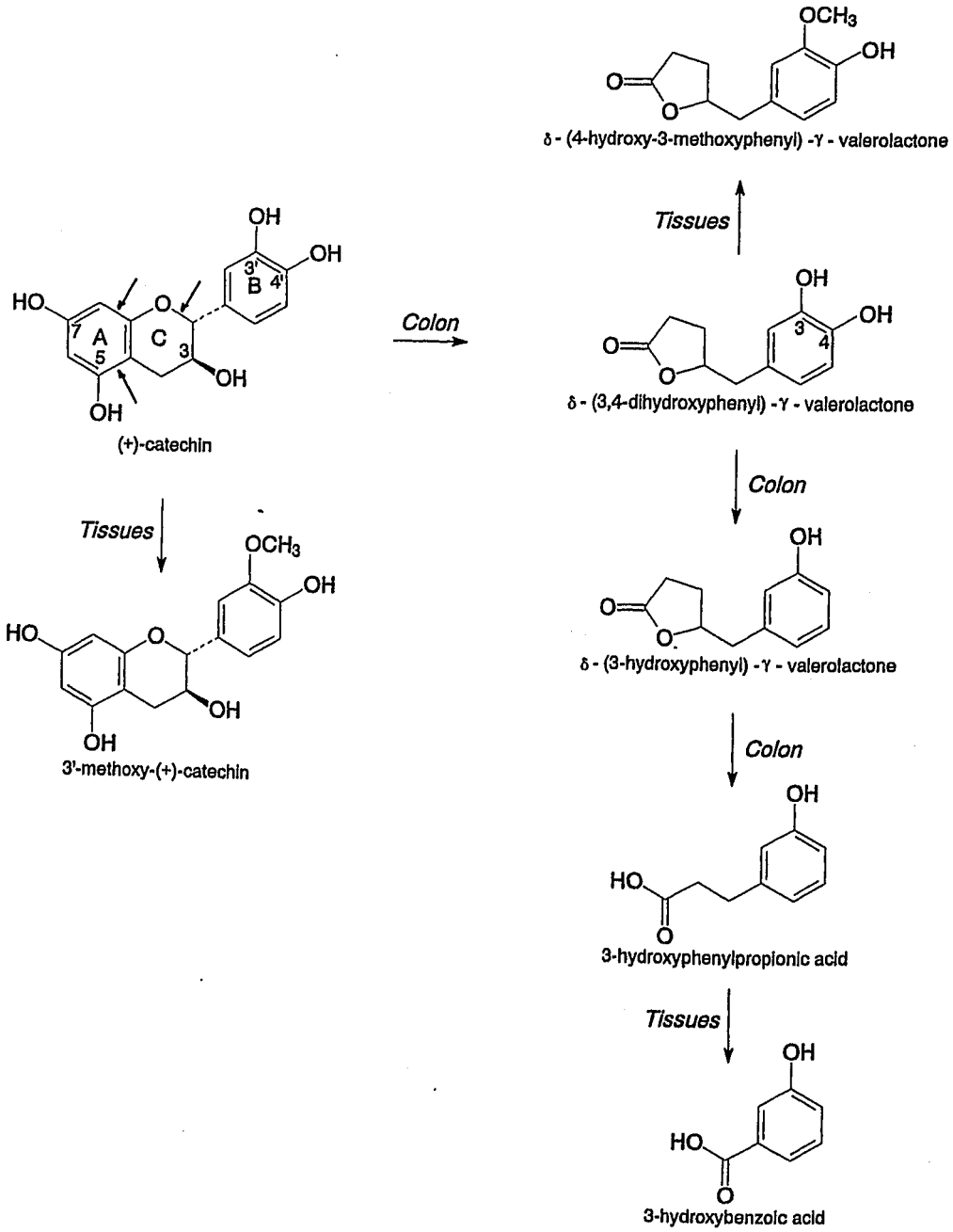


**Figure 2** Metabolic reactions of catechins in body tissues and colon. (+)-Catechin is shown as an example. Conjugation reactions are not shown.

tabolites were formed. These phase II reactions occurred in rodents as well in humans. Types of glucuronides depended on species, and preference for sulfation was found in humans (26).

## Bacterial Ring Cleavage of Catechins in the Colon

According to Das et al. (23), the catechin ring is cleaved by microorganisms at the positions indicated by the arrows in Figure 2. This type of fission is decisive for the basic structures of the successive metabolites: valerolactones (phenyl-$C_5$: a benzene ring with a side chain of 5 C-atoms), phenylpropionic acids (phenyl-$C_3$), and benzoic acids (phenyl-$C_1$). Variations in substituent patterns of these basic structures occurred and were to some extent species dependent (Table 2). Identification of the valerolactones was pioneered by Watanabe (53,54) and was the first step in the elucidation of the bacterial metabolism of (+)-catechin in the colon. Catechin labeled with $^{14}C$ in the A-ring only ([ring A-$^{14}C$]catechin) and uniformly $^{14}C$-labeled catechin ([U-$^{14}C$] catechin) were used to further substantiate this general scheme (23). Oral administration of valerolactones to rats and guinea pigs (23,46) gave rise to the propionic and benzoic acids depicted in Figure 2. The free hydroxyl group at the 3 position of catechin was essential for ring fission in the colon by bacteria, as 3-methoxy-(+)-catechin was resistant to ring fission in rat, mouse, marmoset (55), and humans (26).

Animal experiments showed that heterocyclic ring fission of (+)-catechin was wholly mediated by microorganisms in the colon. In the presence of antibiotics that kill the microorganisms, the ring fission products were not produced (46,50). These metabolites also were formed upon in vitro incubation of catechin with intestinal contents, and again their formation could be suppressed by addition of antibiotics. Ligation of the bile duct prevents bile that contains conjugated (+)-catechin after intravenous injection to flow into the small intestine. After intravenous injection of (+)-catechin to bile duct–ligated rats, no ring fission products were detectable in urine (45), again showing the crucial role of the gut.

In rats, biliary circulation was an important phenomenon in catechin metabolism. Studies with bile duct-cannulated rats showed that about 40% of orally administered absorbed (+)-catechin was secreted with the bile into the small intestine (11). Only glucuronide or sulfate conjugates of catechins and 3′-methoxy-(+)-catechin (Fig. 2), the major hepatic metabolite (49), were secreted with the bile. Catechins secreted with bile were prone to microbial degradation. Subsequently, after hydrolysis of the conjugates, catechin and its phenolic acid and lactone metabolites were reabsorbed (45,49). About 60% of the metabolites of 3-methoxy-(+)-catechin that

were secreted with the bile were reabsorbed in the first enterohepatic circulation (55).

In conclusion, bacteria of the colon cleaved the heterocyclic ring of (+)-catechin to form phenyl-$C_5$ and phenyl-$C_3$ metabolites, which were absorbed and were excreted with urine both in rodents and in humans. In rats secretion of catechin conjugates into bile exposed them anew to bacterial degradation. Presence of a methoxy group at position 3 in (+)-catechin made the molecule resistant to ring fission in rodents as well as in humans. Ring fission of other types of catechins was not studied.

## Extent of Catechin Metabolism and Species Differences

Unconjugated (+)-catechin, valerolactones, and phenolic acids excreted in urine represented only 3% of the orally administered [U-$^{14}$C]-(+)-catechin in rats (23). As 58% of the dose was excreted in urine (Table 1), only 5% of this radioactivity was identified. On oral administration of [U-$^{14}$C]-(+)-catechin to monkeys, a considerably higher percentage, 20% of the dose, was excreted in urine as unconjugated catechin and its phenyl-$C_5$, phenyl-$C_3$, and phenyl-$C_1$ metabolites (24). Catechin accounted for 3%, and the main metabolite δ-(3-hydroxyphenyl)-γ-valerolactone for 8% of the dose; conjugates of some of these metabolites and of catechin were present, but were not quantified. Ingestion of [U-$^{14}$C]-(+)-catechin by human volunteers showed that ring fission was only a minor metabolic route (25); 90% of the urinary radioactivity (50% of the dose) was composed of conjugates of (+)-catechin and 3′-methoxycatechin, and their aglycones accounted for 3% of the dose. Oral administration of 3-[$^{14}$C]-methoxy-(+)-catechin to humans showed that less than 0.5% of the dose was excreted unchanged in urine; major metabolites were conjugates of the parent compound and of 3,3′-dimethoxycatechin (26). *O*-methylation of 3-methoxy-(+)-catechin was less important in humans than in rodents, where *O*-methylation was almost 100% (26). Thus in humans a major part of (+)-catechin is absorbed and subsequently excreted with urine as conjugates.

Only monkeys and humans excreted 3-hydroxyphenylhydracrylic acid in urine after an oral (+)-catechin dose (Table 2) (24,47). The traces of 4-hydroxyphenolic acids in urine reported in one study with rats (45) are puzzling because only 3-hydroxyphenolic acids are expected based on the scheme depicted in Figure 2. Possibly some of these metabolites originate from dietary tyrosine (50).

Thus, only a few percent of orally administered (+)-catechin and 3-methoxy-(+)-catechin escaped metabolism. The major metabolic reactions were conjugation and *O*-methylation performed by liver cells. In humans, ring fission by bacteria in the colon was only of minor importance

for these two catechins. Very limited data were found on the metabolism of an important group of dietary catechins, the epicatechins of tea.

## Flavonols

### Enzymatic Transformations of Flavonols in Body Tissues

As for catechins, phase I transformation reactions for flavonols have not been described, and this part of their metabolism awaits study. The role of the liver in rats in *glucuronidation* and *O-methylation* was demonstrated by intraperitoneal injection of rutin (quercetin-3-rutinoside) and quercetin (56). 3′-Methoxyquercetin-3-rutinoside and its glucuronide and conjugates of quercetin, isorhamnetin (3′-methoxyquercetin), and rutin were found in bile. Both 3′-methoxyquercetin and 4′-methoxyquercetin were reported in the urine and bile of rats (28). The presence of the 4′-methoxy isomer was confirmed by NMR and a specific chemical reaction. Again, only *o*-hydroxy-methoxy metabolites were found (Table 3), suggesting that catechol-*O*-methyl transferase was involved (41). *Sulfation* of quercetin was studied by using perfusion of isolated rat liver (52). Two double sulfate-glucuronide conjugates constituted 85% of the biliary secreted sulfate-containing conjugates. Sulfation in male rats was twice that in female rats. In vitro incubations with unfractionated sulfotransferases of rat liver confirmed these results.

Human data are limited. We found circumstantial evidence for the presence of quercetin conjugates in urine and plasma after oral administration of dietary quercetin to human subjects (30,60,61); acid hydrolysis of urine and plasma increased the concentration measured. We also found 3′-methoxyquercetin in plasma and urine of these subjects (P.C.H. Hollman et al., unpublished).

Thus, in rats major enzymatic metabolic reactions of flavonols were located in the liver, and they were similar to those of catechins: glucuronidation and sulfation of hydroxyl groups, and *O*-methylation of catechol groups. Conjugation and *O*-methylation of quercetin also occurred in humans.

### Bacterial Ring Cleavage of Flavonols in the Colon

The proposed flavonol-specific sites of ring fission are depicted by the arrows in Figure 3. The proposed scheme accounts for the phenylacetic acids (phenyl-$C_2$) and the phenylpropionic acids (phenyl-$C_3$) found in various species (Table 3). However, direct experimental proof for these types of ring fission in flavonols is not available. The phloroglucinol (1,3,5-trihydroxybenzene) and phloroglucinolcarboxylic acid (2,4,6-trihydroxybenzoic acid) found in urine of rats after oral administration of quercetin

**Table 3** Metabolites of Quercetin Found After Oral (p.o.), Intravenous (i.v.), or Intraperitoneal (i.p.) Administration to Various Species

| Metabolite | Species | Dose (mg/kg body weight) | Body fluid | Ref. |
|---|---|---|---|---|
| Quercetin glucuronide(s) or sulfate(s) | Rat | i.p. and p.o. 30 | Bile | 56 |
| Quercetin glucuronide(s) | Rat | p.o. 630 and i.p. 315 | Urine and bile | 28 |
| Quercetin sulfate(s) | Rat | p.o. 630 and i.p. 315 | Urine | 28 |
| 3′-Methoxyquercetin conjugate | Rat | i.p. and p.o. 30 | Bile | 56 |
| 3′-Methoxyquercetin and its glucuronide | Rat | p.o. 630 and i.p. 315 | Urine and bile | 28 |
| 4′-Methoxyquercetin | Rat | p.o. 630 and i.p. 315 | Urine and bile | 28 |
| 3-Hydroxycinnamic acid | Rat | p.o. | Urine | 57 |
| 3-Hydroxyphenylpropionic acid | Rat | p.o. | Urine | 57 |
| 3,4-Dihydroxyphenylacetic acid | Rat, rabbit, guinea pig, human | p.o. | Urine | 58 |
| | Rat | p.o. 320 | Urine | 59 |
| | Rat | p.o. | Urine | 57 |
| 4-Hydroxy-3-methoxyphenylacetic acid | Rat, rabbit, guinea pig, human | p.o. and i.p. | Urine | 58 |
| | Rat | p.o. 320 | Urine | 59 |
| | Rat | p.o. 25 | Urine | 27 |
| | Rat | p.o. | Urine | 57 |
| 3-Hydroxyphenylacetic acid | Rat, rabbit, guinea pig, human | p.o. | Urine | 58 |
| | Rat | p.o. 320 | Urine | 59 |
| | Rat | p.o. 25 | Urine | 27 |
| | Rat | p.o. | Urine | 57 |
| 3-Hydroxybenzoic acid | Rat | p.o. | Urine | 57 |

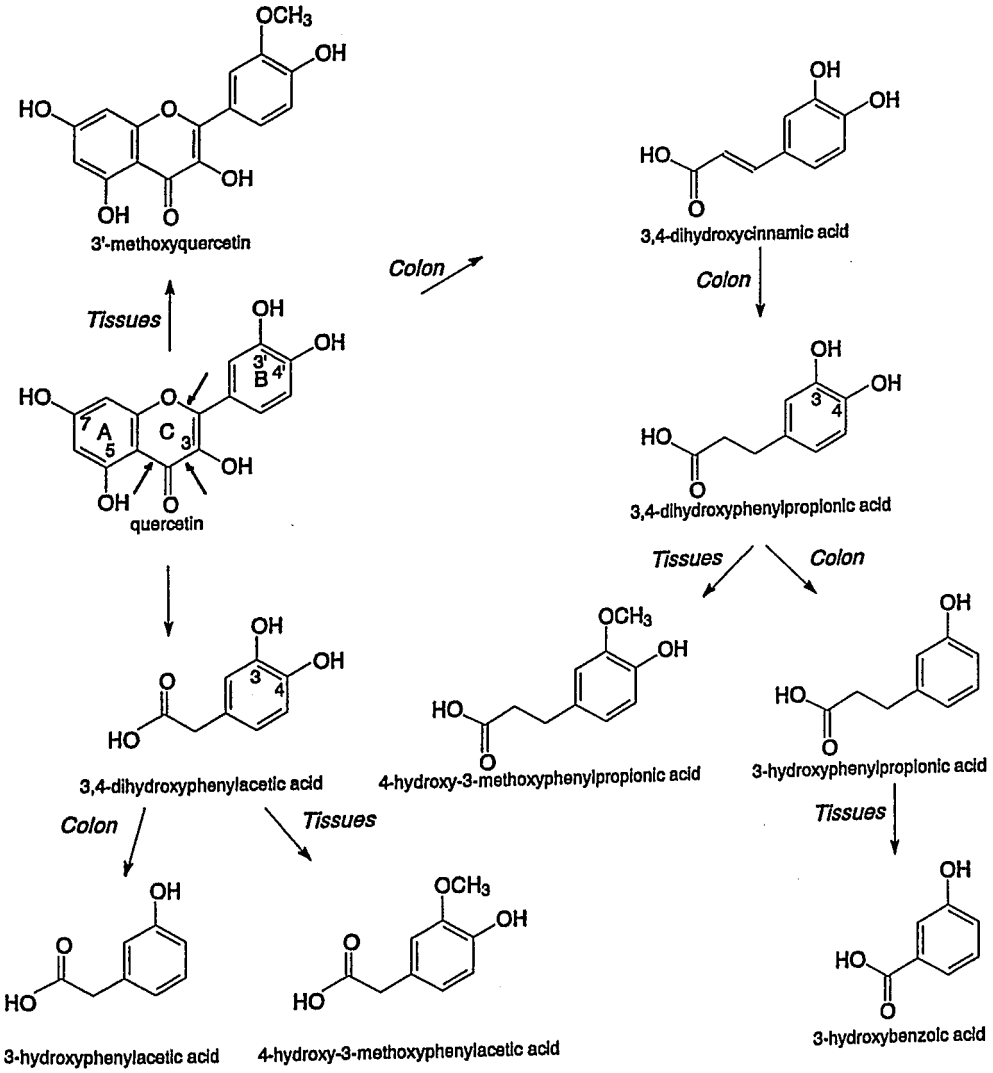


**Figure 3** Metabolic reactions of flavonols in body tissues and colon. Quercetin is shown as an example. Conjugation reactions are not shown.

pointed to metabolites with an intact A-ring of quercetin (62), but they turned out to be analytical artefacts (27,59). Oral administration of 3,4,5-trihydroxyphenylacetic acid to rats produced 3,4-dihydroxy- and 3-hydroxyphenylacetic acid, which are metabolites of myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone) (22). Similar to (+)-catechin, these results indicated

that the phenolic acids formed only had an intact B-ring (Fig. 3). Microorganisms mediated these dehydroxylation reactions.

The phenylacetic acids, typical for the proposed ring fission of quercetin, rutin (quercetin-3-*O*-rutinoside), and myricetin were not found in rats treated with an antibiotic to suppress microorganisms (22,57,63). In vitro anaerobic incubation of myricetin, myricitrin (myricetin-3-*O*-rhamnoside) (22), and rutin (63) with rat cecal microorganisms also produced the metabolites observed in urine after oral administration. Mucosal membranes of the small intestine as well as contents of duodenum, jejunum, and ileum were unable to metabolize quercetin in rats (28), as opposed to the contents of cecum and colon. Thus, microorganisms in the colon cause ring fission of flavonols. The absence of these ring fission products after intraperitoneal injection of rutin in bile duct-cannulated rats is an additional indication for the exclusive role of microorganisms in ring fission of flavonols (63). Biliary circulation of quercetin was indicated by the occurrence of glucuronides and sulfates of quercetin (28,56) in bile. However, no data are available about the extent of biliary secretion and reabsorption.

In plant foods flavonols mainly occur as glycosides. As expected, the metabolites of quercetin and of rutin, the 3-rutinoside of quercetin, were similar (Tables 3 and 4); microorganisms of the colon probably first hydrolyzed rutin to produce quercetin. The rutinose moiety was also removed from quercetin-7-*O*-(β-hydroxyethyl)rutinoside by microorganisms in the colon of dogs (65), but this aglycone was stable against ring fission. It is likely that the β-hydroxyethyl group is resistant to microbial hydrolysis. This means that substituents bound to hydroxyls that are resistant to hydrolysis by the gut microorganisms can have a profound effect on the stability of the ring system. However, the microflora of rats was capable of ring fission of quercetin-7-*O*-(β-hydroxyethyl)rutinoside (66), but failed in the case of quercetin-7,3′,4′-tri-*O*- and quercetin-5,7,3′,4′-tetra-*O*-(β-hydroxyethyl)rutinoside. Another interesting observation was made by Griffiths (22), who found that robinetin (3,7,3′,4′,5′-pentahydroxyflavone) was not degraded to phenolic acids in rats and also was stable upon incubation with microorganisms. Robinetin lacks the hydroxyl group at position 5 as compared to myricetin, which is degraded as expected (Table 4).

In summary, bacteria of the colon cleaved the heterocyclic ring of flavonols to form phenyl-$C_3$ and phenyl-$C_2$ metabolites, which were absorbed and excreted into urine. These metabolites were found in rodents as well as in humans, although most of these studies were performed with rodents. Glycosylation could not stabilize the ring structure as opposed to substituents that formed nonhydrolyzable bonds with hydroxyl groups. Secretion into bile of flavonol conjugates may contribute to additional bacterial degradation.

**Table 4** Metabolites of Rutin, Kaempferol, and Myricetin Found After Oral (p.o.) or Intraperitoneal (i.p.) Administration to Various Species

| Flavonol | Metabolite | Species | Dose (mg/kg body weight) | Body fluid | Ref. |
|---|---|---|---|---|---|
| Rutin (quercetin-3-*O*-rutinoside) | Rutin glucuronide | Rat | i.p. 30 | Bile | 56 |
| | 3,4-Dihydroxyphenylacetic acid | Rat, rabbit, guinea pig, human | p.o. | Urine | 58 |
| | 3-Hydroxyphenylacetic acid | | | | 58 |
| | 4-Hydroxy-3-methoxyphenylacetic acid | | | | 58 |
| | Quercetin-3-*O*-rutinoside glucuronide | Rat | i.p. 30 | Bile | 56 |
| | 3′-Methoxy-3-*O*-rutinoside and its glucuronide | | | | 56 |
| [2′,5′,6′-$^2$H]Rutin | 3,4-Dihydroxyphenylacetic acid | Human, rat | p.o. 10 rat: | Urine | 64 |
| | | | p.o. 100 | | 63 |
| | 4-Hydroxy-3-methoxyphenylacetic acid | Human, rat | | | |
| | 3-Hydroxyphenylacetic acid | Human, rat | | | |
| | 3,4-Dihydroxytoluene | Human, rat | | | |
| | $\beta$-3-Hydroxyphenylhydracrylic acid | Human | | | 64 |
| | 3-Hydroxyphenylpropionic acid | Rat | | | 63 |
| Kaempferol (5,7,3,4′-tetrahydroxyflavone) and kaempferol-7-rhamnosido-3-galactorhamnoside | Kaempferol | Rat | p.o. 300 | Urine | 21 |
| | 4-Hydroxyphenylacetic acid | Rat | p.o. 300 | Urine | 21 |
| Myricetin and myricetin-3-*O*-rhamnoside | Myricetin | Rat | p.o. 300 | Urine | 22 |
| | 3,5-Dihydroxyphenylacetic acid | | | | |
| | 3-Hydroxyphenylacetic acid | | | | |

### Extent of Flavonol Metabolism

Quantitative studies are limited and are available only for quercetin. Rats did not excrete aglycone in urine after oral administration of quercetin aglycone (28); 1.7% of the dose was excreted as glucuronide and sulfate conjugates of quercetin, while monomethoxylated quercetin conjugates accounted for 3.6%. This accounts for about half of the urinary metabolites in these rats, as 13% of the administered radioactivity was excreted in urine (Table 1).

In humans, less than 1% of the orally administered aglycone was estimated to reach the circulation unchanged in humans (29). We found that humans who were fed quercetin or quercetin glycosides excreted only 0.1–0.3% of the dose as unchanged quercetin or its conjugates in urine (Table 1), whereas absorption amounted to 20–50% (30). In these human subjects less than 0.5% of the dose was excreted as 3′-methoxyquercetin (P.C.H. Hollman et al., unpublished).

These data indicate that quercetin is extensively metabolized in rats and humans. Only a small part of these metabolites has been quantified. In contrast with (+)-catechin in humans, quercetin is metabolized only to a limited extent via conjugation with sulfate, glucuronic acid, or *O*-methylation.

## Flavones and Flavanones

### Enzymatic Transformations of Flavones and Flavanones in Body Tissues

Evidence for oxidative phase I reactions of flavones was found in guinea pigs. After intraperitoneal and oral administration of a synthetic flavone lacking hydroxyl groups, both 4′- and 3′,4′-flavone were excreted in urine (67). Oral administration of flavanone lacking hydroxyl groups to rats introduced hydroxyl groups at the 3 or 6 position (32,68), and these metabolites were excreted in urine. However, proof for the involvement of the liver was not given. Several metabolites formed by reduction of the carbonyl group, for instance, flavan-4-α-ol, were identified. However, the corresponding reduction of flavone was never found (67). Phase II reactions were demonstrated by many workers. *Conjugation* of baicalein (5,6,7-trihydroxyflavone) and baicalein-6-glucuronide was shown in rats: five conjugates were identified in bile (69). Baicalein conjugated with two glucuronic acid molecules and the mixed conjugate containing one glucuronic acid and one sulfate predominated (69). This is in accordance with observations that high molecular weight and high polarity of compounds facilitate their secretion with bile (40). A study with isolated perfused rat liver (70)

showed that diosmin (5,3′-dihydroxy-4′-methoxyflavone-7-rutinoside) was secreted with bile as such and as its glucuronide conjugate; diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone) was only secreted as sulfate and glucuronide conjugates. Oral administration of naringin (5,4′-dihydroxyflavanone-7-rhamnoglucoside) and hesperidin (5,3′-dihydroxy-4′-methoxyflavanone-7-rutinoside) to rats showed that, besides the glucuronides, the parent glycosides were also secreted into bile (33). On oral administration of diosmetin to rats, its glucuronide appeared within minutes in portal venous blood, and no aglycone could be detected (71). This suggests that the glucuronide was produced on absorption at the level of the intestinal mucosa. It is documented (72) that intestinal mucosa were important for extrahepatic glucuronidation. In vivo as well as in vitro (rat liver microsomes), 5-hydroxyflavone was glucuronidated. This is remarkable, because the 5-hydroxyl group is strongly stabilized by the 4-keto group or is involved in chelation. Glucuronidation in rats of 7,5-dihydroxyflavone occurred mainly at the 7-position and of 5,7,3′-trihydroxyflavone at the 7- and 3′-position (71).

Tangeretin (5,6,7,8,4′-pentamethoxyflavone) was *O-demethylated* by rat and human liver microsomes (73). The metabolites formed were not identified separately.

Anaerobic incubation of [3-$^{14}$C]hesperetin with rat cecal microorganisms only produced phenylpropionic acids and no $^{14}CO_2$ or phenylbenzoic acids. This suggested that *β-oxidation* of the propyl chain of the phenylpropionic acids was not mediated by bacterial enzymes, but by mammalian enzymes (31).

In conclusion, flavones and flavanones were metabolized by liver enzymes to give sulfates, glucuronides, and mixed sulfates-glucuronides, which were excreted into bile, urine, and plasma. Only glucuronides and sulfates were secreted with bile, except for rhamnoglucosides, which were secreted as such. Glucuronidation in the intestinal mucosa was observed. These studies were only performed with rats. In addition, *O*-methylation of catechol groups and *O*-demethylation occurred in humans and rats. β-Oxidation of phenylpropionic acids was found in rats.

## Bacterial Ring Cleavage of Flavanones and Flavones in the Colon

The specific sites of ring fission for flavones and flavanones are shown by the arrows in Figure 4. The proposed scheme accounts for the phenylpropionic acids (phenyl-$C_3$) reported in the body fluids of various species after various flavones and flavanones (Table 5,6). A study with $^{14}$C-labeled hesperetin ([3-$^{14}$C]5,7,3′-trihydroxy-4′methoxyflavanone) in rats identified

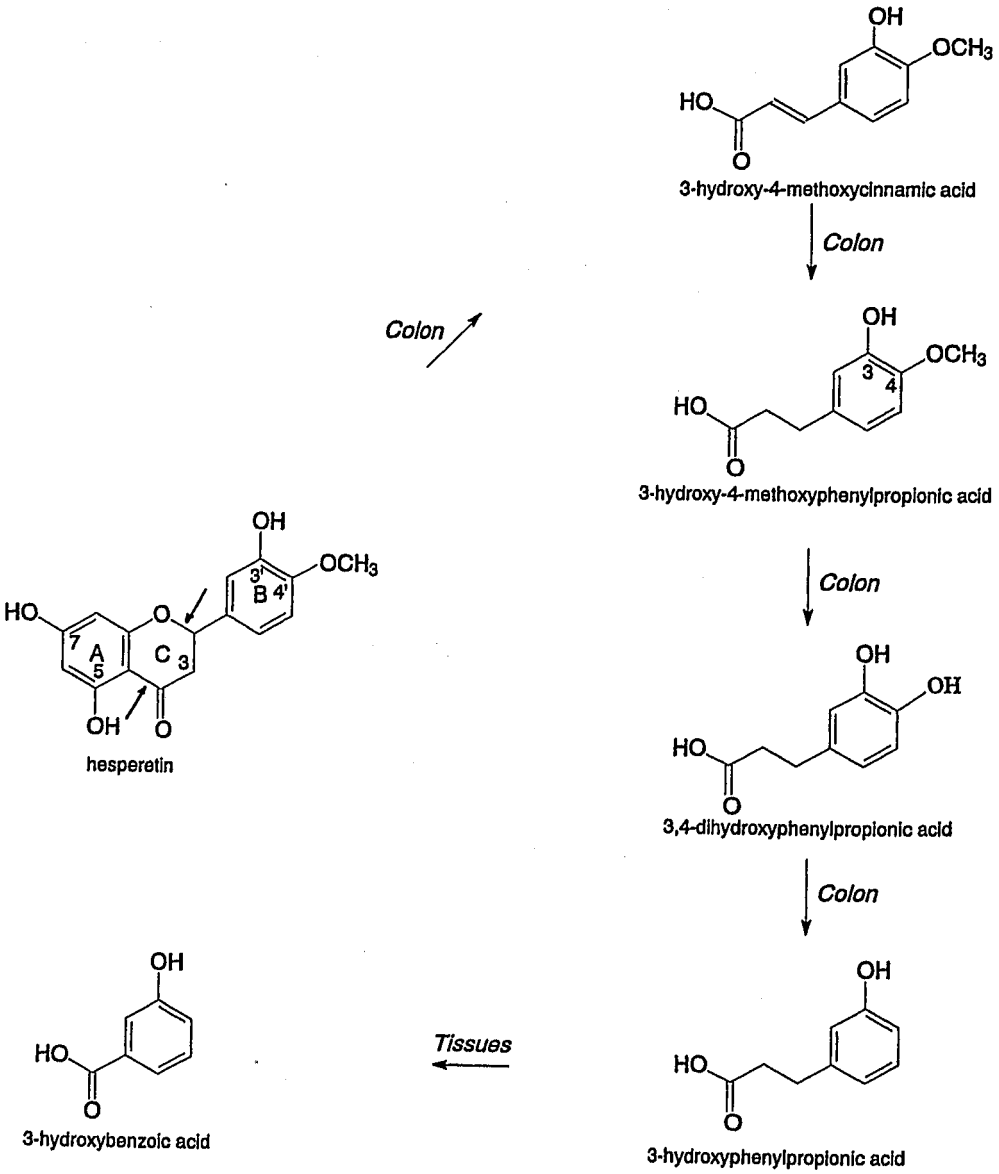


**Figure 4** Metabolic reactions of flavanones in body tissues and colon. Hesperetin is shown as an example. Flavones show similar reactions. Conjugation reactions are not shown.

**Table 5** *Metabolites of Flavones Found after Oral (p.o.) or Intraperitoneal (i.p.) Administration to Rats*

| Flavone | Metabolite | Species | Dose (mg/kg body weight) | Body fluid | Ref. |
|---|---|---|---|---|---|
| Flavone | Flavone aglycone | Rat | p.o. 150, i.p. | Urine | 74 |
| | Flav-3-ene | Rat | p.o. 150, i.p. | Urine | 74 |
| | 4′-Hydroxyflavone | Rat | p.o. and i.p. | Urine | 67 |
| | 3′,4′-Dihydroxyflavone | Rat | p.o. and i.p. | Urine | 67 |
| Tricetin (5,7,3′,4′,5′-pentahydroxyflavone) | 3,5-Dihydroxyphenylpropionic acid | Rat | p.o. | Urine | 22 |
| Tricin (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone | 3,5-Dihyroxyphenylpropionic acid | Rat | p.o. | Urine | 22 |
| 5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone | 3,5-Dihyroxyphenylpropionic acid | Rat | p.o. | Urine | 22 |
| 5,6,7-Trihydroxyflavone and its 7-*O*-β-glucuronide | 5,6,7-Trihydroxyflavone glucuronides, sulfates, and mixed conjugates | Rat | p.o. | Bile | 69 |
| | 6-Methoxy,5,7-dihydroxyflavone | Rat | p.o. | Bile | 69 |
| Diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone) | Diosmetin-7,3′-diglucuronide | Rat | p.o. 100 | Urine, whole blood | 71 |
| | Diosmetin-3′-glucuronide | Rat | p.o. 100 | Urine, whole blood | 71 |
| | Diosmetin glucuronide | Rat | p.o. 600 | Urine | 75 |
| | Diosmetin aglycone | Rat | p.o. 600 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Rat | p.o. 600 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rat | p.o. 600 | Urine | 75 |
| Diosmin (5,3′-dihydroxy-4′-methoxyflavone-7-rutinoside) | Diosmetin aglycone | Rat | p.o. 1200 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Rat | p.o. 1200 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rat | p.o. 1200 | Urine | 75 |
| Luteolin (5,7,3′,4′-tetrahydroxyflavone) | Luteolin aglycone | Rat | i.p. 40 | Urine | 76 |
| | Monomethoxylated luteolin (2 isomers) | Rat | i.p. 40 | Urine, bile | 76 |

**Table 6** Metabolites of Flavanones After Oral (p.o.) or Intravenous (i.v.) Administration to Various Species

| Flavanone | Metabolite | Species | Dose (mg/kg body weight) | Body fluid | Ref. |
|---|---|---|---|---|---|
| Liquiritigenin (7,4′-dihydroxyflavanone) | Liquiritigenin aglycone | Rat | i.v. 5 | Plasma | 77 |
| | Liquiritigenin-4′-glucuronide, -7-glucuronide, -4′,7-disulfate, -4′-glucuronide-7-sulfate, -7glucuronide-4′-sulfate | Rat | i.v. 5 | Plasma and/or bile | 77 |
| Eriodictyol (5,7,3′,4′-tetrahydroxyflavanone | Eriodictyol glucuronide | Rat | p.o. 900 | Urine | 75 |
| | 5,7,4′-Trihydroxy-3′-methoxyflavanone | Rat | p.o. 900 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Rat | p.o. 900 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rat | p.o. 900 | Urine | 75 |
| Homoeriodictyol (5,7,4′-trihydroxy-3′methoxyflavanone) | Homoeriodictyol aglycone | Rat | p.o. 450 | Urine | 75 |
| | Homoeriodictyol glucuronide | Rat | p.o. 450 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Rat | p.o. 450 | Urine | 75 |
| | 4-Hydroxy-3-methoxyphenylpropionic acid | Rat | p.o. 450 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rat | p.o. 450 | Urine | 75 |
| Hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone) | Hesperetin aglycone | Rat | p.o. 450 | Urine | 75 |
| | Hesperetin glucuronides | Rat | p.o. 150 | Bile | 33 |
| | | Rat | p.o. 450 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Human | p.o. 30 | Urine | 75 |
| | | Rat | p.o. 450 | Urine | 75 |
| | 4-Hydroxyphenylpropionic acid | Rat | p.o. 1.5 | Urine | 31 |
| | | Rat | p.o. 30-150 | Urine | 33 |
| | 3,4-Dihydroxyphenylpropionic acid | Rat | p.o. 1.5 | Urine | 31 |
| | 3-Hydroxy-4-methoxyphenylpropionic acid | Rat | p.o. 1.5 | Urine | 31 |
| | 3-Hydroxy-4-methoxyphenylhydracrylic acid | Human | p.o. 30 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rat | p.o. 450 | Urine | 75 |

| | | | | | |
|---|---|---|---|---|---|
| Hesperidin (5,3′-dihydroxy-4′-methoxyflavanone-7-rutinoside) | Hesperidin | Rat | p.o. 150 | Bile | 33 |
| | Hesperetin aglycone | Rabbit | p.o. 330 | Urine | 75 |
| | | Rat | p.o. 450 | Urine | 75 |
| | Hesperetin glucuronides | Rat | p.o. 150 | Bile | 33 |
| | | Rabbit | p.o. 330 | Urine | 75 |
| | | Rat | p.o. 450 | Urine | 75 |
| | 3,4-Dihydroxyphenylpropionic acid | Rabbit | p.o. 330 | Urine | 75 |
| | 4-Hydroxy-3-methoxyphenylpropionic acid | Rabbit | p.o. 330 | Urine | 75 |
| | 3-Hydroxyphenylpropionic acid | Rabbit | p.o. 330 | Urine | 75 |
| | | Rat | p.o. 450 | Urine | 75 |
| | 4-Hydroxyphenylpropionic acid | Rat | p.o. 30-150 | Urine | 33 |
| | 3-Hydroxy-4-methoxyphenylhydracrylic acid | Human | p.o. 30 | Urine | 75 |
| | 3-Hydroxycinnamic acid | Rabbit | p.o. 330 | Urine | 75 |
| | | Rat | p.o. 450 | Urine | 75 |
| | 3-Hydroxyhippuric acid | Rabbit | p.o. 330 | Urine | 75 |
| | 3-Hydroxybenzoic acid | Rabbit | p.o. 330 | Urine | 75 |
| | 4-Hydroxy-3-methoxybenzoic acid | Rabbit | p.o. 330 | Urine | 75 |
| Naringenin (5,7,4′-trihydroxyflavanone) | Naringenin glucuronides | Rat | p.o. 150 | Bile | 33 |
| | | Rat | p.o. 300 | Urine | 78 |
| | Naringenin aglycone | Rat | p.o. 300 | Urine | 78 |
| | 4-Hydroxyphenylpropionic acid | Rat | p.o. 30–150 | Urine | 33 |
| | | Rat | p.o. 300 | Urine | 78 |
| | 4-Hydroxycinnamic acid | Rat | p.o. 300 | Urine | 78 |
| | 4-Hydroxybenzoic acid sulfate | Rat | p.o. 300 | Urine | 78 |
| Naringin (5,4′-dihydroxyflavanone-7-neohesperidoside) | Naringenin aglycone | Rat | p.o. 150 | Bile | 33 |
| | | Rat | p.o. 600 | Urine | 78 |
| | Naringenin glucuronide | Rat | p.o. 150 | Bile | 33 |
| | | Rat | p.o. 600 | Urine | 78 |
| | 4-Hydroxyphenylpropionic acid | Rat | p.o. 30–150 | Urine | 33 |
| | 4-Hydroxycinnamic acid | Rat | p.o. 600 | Urine | 78 |
| | 4-Hydroxybenzoic acid sulfate | Rat | p.o. 600 | Urine | 78 |

the predicted $^{14}C$-labeled phenylpropionic acids (31). Anaerobic incubation of [3-$^{14}C$]hesperetin with cecal microorganisms did not produce $^{14}CO_2$, which indicated that $\beta$-oxidation of the propyl chain of the phenylpropionic acid was not caused by bacterial enzymes but by mammalian enzymes.

The dose clearly affected the metabolites formed. After a very low oral dose, only ring-cleavage products were found in urine (31), whereas a more than 100-fold increase of the oral dose, the common dose in these experiments, also produced metabolites with an intact ring structure (33,75).

The synthetic flavone (67) and flavanone (74), both lacking hydroxyl groups, were not cleaved by microorganisms, as no phenolic acids were excreted, and metabolites did not change after administration of antibiotics (67). Experiments with various flavones with 5,7-hydroxylation showed that at least one free hydroxyl group in ring B was required for ring fission (22).

To summarize, microorganisms in the colon of rats cleaved the heterocyclic ring only of hydroxylated flavones and flavanones to form phenyl-$C_3$ metabolites, which were absorbed and excreted into urine. Metabolism of flavanones by bacteria in rodents and humans led to similar metabolites. Human data on the metabolism of flavones are not available.

## Anthocyanidins

The limited data available on the metabolism of anthocyanidins indicate that these flavylium flavonoids are metabolized to a much more limited extent than other flavonoids. Cyanidin (3,5,7,3′,4′-pentahydroxyflavylium) was not converted to phenolic metabolites when incubated with rat cecal bacteria (20). Delphinidin 3,5,7,3′,4′,5′-hexahydroxyflavilium) and malvin (7,4′-dihydroxy-3′,5′-dimethoxy flavylium-3,5-diglucoside) fed to rats or incubated with microorganisms were not metabolized to identifiable compounds, but phenolic compounds could be excluded (22). After intravenous administration of an extract of *Vaccinium myrtillus* to rats, 20% of the administered dose (based on direct colorimetric measurement) was excreted as such into urine (79).

## Phenolic Acids

Ring fission of flavonoids generates phenolic acids, which are absorbed and excreted into urine (Tables 1–6). The primary ring fission products (Figs. 2–4) are susceptible to supplemental metabolism by bacteria in the colon and, after absorption, also by enzymes in body tissues. The major questions are:

What are the metabolic reactions acting upon these primary phenolic acids, and What affects these reactions?

## Degradation of Primary Ring Fission Products of Flavonoids by Bacteria in the Colon

In vitro incubations of cinnamic (phenyl-$C_3$), phenylpropionic (phenyl-$C_3$), and phenylacetic (phenyl-$C_2$) acids with rat cecal bacteria (80) demonstrated that these microorganisms performed the following metabolic reactions:

*Dehydroxylation* of 3,4-dihydroxyphenylpropionic and 3,4-dihydroxyphenylacetic acids to produce 3-hydroxyphenolic acids (22).
*Demethylation* of *o*-hydroxymethoxyphenolic acids.
*Reduction* of the double bond of cinnamic acids to produce phenylpropionic acids.
*Decarboxylation* of cinnamic and phenylacetic acids, but only when a hydroxyl group at position 4 was present. Decarboxylation of the phenylpropionic acids did not occur.

Also, after oral administration of 3,4,5-trihydroxyphenylacetic acid, a proposed primary fission product of myricetin, to rats, dehydroxylation was confirmed and produced 3,4-dihydroxyphenylacetic and 3-hydroxyphenylacetic acid, which were excreted in urine (22). Dehydroxylation of δ-(3,4-dihydroxyphenol)-γ-valerolactone, the primary ring fission product of (+)-catechin, was demonstrated in guinea pigs (46).

Species differences in these metabolic reactions of bacteria were observed. In rabbits no decarboxylation of 3,4-dihydroxyphenylacetic acid occurred (81). *β-Hydroxylation* of phenylpropionic acids, which produced phenylhydracrylic acids, was only observed in humans and monkeys (24,63,64,75), and it was demonstrated that bacteria in the colon carried out this reaction.

## Enzymatic Transformations of Phenolic Acids in Body Tissues

*Conjugation* of 3-hydroxybenzoic acid with *glycine* to form 3-hydroxyhippuric acid occurred in rodents and humans (Table 2), probably in the renal tissues (82). Conjugation with *glucuronic acid* or *sulfate* of valerolactones (47), of phenylpropionic acids (24,31,46,47) and phenylacetic acids (59) was generally found. *o*-Hydroxymethoxy phenolic acids excreted in urine (Tables 2–6) could have originated from ring fission of *O*-methylated flavonoids secreted with bile. However, absorbed ring fission products were also *O-methylated* in guinea pigs (46), rats (83–85), and humans (85). Phenylpropionic acids were converted to benzoic acids by *β-oxidation* of the

propyl chain in all species (23–25,31,46,57,75). It was shown that only tissue enzymes could have been involved.

In conclusion, administration of flavonoids in vivo yielded a range of substituted phenolic acids in urine (Tables 2–6). The presence of these compounds could be explained by the bacterial and tissue enzymatic reactions that act upon phenolic acids. Species differences in these metabolic reactions occurred to some extent.

## PHARMACOKINETICS

A quantity of major interest in assessing the biological effects of flavonoids, or indeed of any food component or drug, is the bioavailability. Bioavailability quantitates the exposure of the body (excluding gut and liver) to the substance in question. Bioavailability is often mistakenly equated with absorption. However, bioavailability also includes first-pass metabolism. Bioavailability is defined as the percentage of the ingested flavonoid amount that enters the blood circulation intact after passage through the liver (40). It is determined experimentally by giving a flavonoid orally and intravenously and then measuring the ratio of the areas under the plasma flavonoid concentration versus time curves (AUCs). The fate of flavonoids in the body after ingestion is determined by their absorption, distribution, and elimination, each of which has its own rates and extents. Pharmacokinetic parameters are needed to describe and to predict these processes. Subsequently, predictions of dosage dependency of plasma levels, achievable plasma levels, and accumulation can be made.

### Catechins

The maximum plasma concentration of (+)-catechin and its metabolites was reached after 1–3 h and elimination half-life of the aglycone was about 1 h (Table 7). By the time that the maximum concentration in plasma was reached, only about 10% of the catechin was present unchanged (25). Differences between $C_{max}$s (48,86) are probably explained by the inclusion (42) or exclusion (86) of conjugates in the data. $^{14}C$ activity was present in plasma up to 120 h, indicating a long persistence of metabolites (25). A linear relation between the administered dose and the area under the plasma concentration versus time curve (AUC) was found in humans (86). This indicates that there was no saturable gastrointestinal absorption and/or no dose-dependent first-pass effect of the liver up to an oral dose of 30 mg/kg. The rate of absorption of 3-methoxy-(+)-catechin (26) was similar to that of (+)-catechin (Table 7). However, the elimination of half-life of total

**Table 7** Pharmacokinetic Parameters of Flavonoids

| Flavonoid | Species | Dose (mg/kg body weight) | $C_{max}$ (ng/ml) | $t_{max}$ (h) | $t_{1/2}$ (h) | Method | Ref. |
|---|---|---|---|---|---|---|---|
| (+)-Catechin | Human ($n$ = 3) | p.o. 45 | 15000 | 1–2 | 1.3 | Serum; photometric | 48 |
| (+)-Catechin | Human ($n$ = 6) | p.o. 8 | 590 | 1.6 | 1.3 | Serum; aglycone HPLC | 86 |
| (+)-Catechin | Rabbit ($n$ = 8) | i.v. 15 | 11000 | — | 0.75 | Plasma; aglycone HPLC | 87 |
| [U-$^{14}$C](+)-Catechin | Human ($n$ = 3) | p.o. 30 | 12000[a]<br>1500[b] | 3[a]<br>3[b] | —<br>— | Plasma | 25 |
| 3-[$^{14}$C]Methoxy-(+)-catechin | Rat ($n$ = 3) | i.v. 30 | 100000[a]<br>9500[b] | 0.1[a]<br>0.1[b] | 6.5[a]<br>$<1$[b] | Plasma | 51 |
| 3-Methoxy-(+)-[U-$^{14}$C]catechin | Human ($n$ = 3) | p.o. 30 | 50000[a]<br>11000[b] | 2[a]<br>2[b] | 10[a]<br>$\ll 10$[b] | Plasma | 26 |
| Green tea | Human | p.o. | | | | Plasma; total HPLC | 36 |
| (−)-epigallocatechin (EGC), EGC-3-gallate (EGCg), (−)-epicatechin (EC), EC-3-gallate (ECg) | ($n$ = 4) | 1.3 EGCg<br>1.2 EGC<br>0.5 ECg<br>0.5 EC | 144<br>140<br>$<1$<br>60 | 4[d]<br>1[d]<br>—[d]<br>1[d] | —<br>—<br>—<br>— | | |
| Quercetin | Human | i.v. 1.5 | 3700 | 0.1 | 2.4 | Plasma; aglycone fluorimetric | 29 |
| | ($n$ = 6) | p.o. 65 | $<100$ | — | — | | |
| Flavonol glycosides from *Gingko biloba* extract | Human ($n$ = 2) | p.o. ? | 28–140 | 2–2.5 | — | Plasma; total flavonols HPLC | 88 |
| Quercetin glucosides from onions | Human | p.o. 0.9[c] | 200 | 2.9 | 17 | Plasma; total quercetin HPLC | 61 |
| | ($n$ = 9) | p.o. 0.9[c] | 225 | 0.7 | 28 | | 60 |

**Table 7** Continued

| Flavonoid | Species | Dose (mg/kg body weight) | $C_{max}$ (ng/ml) | $t_{max}$ (h) | $t_{1/2}$ (h) | Method | Ref. |
|---|---|---|---|---|---|---|---|
| Quercetin glycosides from apples | Human ($n$ = 9) | p.o. 1.4[c] | 90 | 2.5 | 23 | Plasma; total quercetin HPLC | 60 |
| Quercetin-3-rutinoside | Human ($n$ = 9) | p.o. 1.4[c] | 90 | 9.3 | – | Plasma; total quercetin HPLC | 60 |
| [$^{14}$C]7-*O*-(β-Hydroxyethyl)-quercetin-3-rutinoside | Dog ($n$ = 2) | p.o. 22 | 8750 | 3–6 | – | Plasma; radioactivity | 89 |
| [$^{3}$H]Diosmin | Rat ($n$ = 5) | p.o. | – | 2 | – | Serum; radioactivity | 90 |
| Diosmin | Human ($n$ = 2) | p.o. 10 | 420 | 1 | 31.5 | Plasma; diosmetin aglycone HPLC | 91 |
| 5-Methoxyflavone | Rat ($n$ = 3) | i.v. 5 | 3200 | – | – | Plasma; aglycone GC-MS/MS | 92 |
| | | p.o. 10 | 1500 | 0.3 | – | | |
| | Dog ($n$ = 2) | i.v. 10 | 6500 | – | 0.3 | | |
| | | p.o. 10 | 2150 | 1 | – | Plasma; aglycone GC-MS/MS | 92 |

(p.o.: per os; i.v.: intravenous; $C_{max}$: maximum concentration measured; $t_{max}$: time to reach $C_{max}$; $t_{1/2}$: elimination half-life).

[a]Total radioactivity.

[b]Aglycone HPLC.

[c]Quercetin equivalents.

[d]Plasma was measured after 1 and 4 h.

$^{14}C$ activity after 3-methoxy-(+)-catechin was considerably higher, whereas the elimination of the parent 3-methoxy-(+)-catechin appeared to be very rapid. This could point to storage of 3-methoxy-(+)-catechin or a metabolite in tissues and subsequent slow release of metabolites. The rate of absorption of various epicatechins of green tea seemed to be dependent on the type of catechin.

Thus, pharmacokinetic data of catechins are scarce, and bioavailability has not been determined. Absorption was moderately rapid, and elimination of the (+)-catechin aglycone was rapid.

## Flavonols

After a high oral dose of quercetin aglycone, no quercetin aglycone was detected in plasma in humans (29) (Table 1). Possibly the major fraction of plasma quercetin is conjugated to glucuronic acid or sulfate. This could explain why Gugler et al. (29) detected no quercetin in plasma: they determined only the aglycone, using a method with a high limit of detection (100 ng/ml). Flavonol glycosides showed moderate to rapid absorption in humans (60,61,88). We (60,61) compared the absorption of quercetin from onions, apples, and rutin and found distinct differences in the rates of absorption. Onions contain mainly quercetin-β-glucosides, whereas apples contain a mixture of quercetin-β-D-galactosides, and β-D-xylosides, whereas quercetin is bound to a disaccharide in rutin. We hypothesized that the rapid and better absorption of the quercetin glucoside in onions was caused by the glucose transporter in the small intestine (30,60). Indeed, model studies (93) showed that naphthol glucosides were transported by the active $Na^+$-glucose transporter across the intestinal wall of rats. The elimination of quercetin from plasma was slow in our studies, which implied that quercetin may accumulate in plasma throughout the day with repeated dietary intake. The bioavailability of quercetin in apples and of rutin were both 30% of that in onions. The important role of the sugar moiety in the absorption of quercetin was also found in a study with ileostomy subjects who lack a colon with the bacterial flora (30). The quercetin glucoside in onions was very well absorbed, whereas absorption of the pure quercetin aglycone and quercetin rutinoside was modest. The rate of urinary excretion of total quercetin in these subjects was highest after ingestion of the glucosides. Thus, the different types of glycosides in these foods could affect absorption and metabolism.

In conclusion, absolute bioavailability of flavonols has not been determined. The relative bioavailability and rate of absorption of quercetin varied between food sources.

## Flavones

Diosmin, the rutinoside of diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone), was not detectable in plasma after oral administration to human volunteers (91). Hydrolysis of diosmin to diosmetin had occurred, and the elimination of diosmetin from plasma was rather slow (Table 7). Serum elimination of tritium was very slow after administration of [$^3$H]diosmin to rats (90). Absolute bioavailability of 5-methoxyflavone was studied in rats and dogs and was high: 25% for rats, and 53% for dogs.

## Tissue Distribution of Flavonoids

The extended elimination times observed for quercetin and diosmin in humans (Table 7) could point to temporary storage of flavonoids or their metabolites in tissues. Studies on tissue distribution were carried out with various labeled flavonoids. After oral administration of [$^3$H]-diosmin to rats, the highest concentration of $^3$H was found in liver (90). However, $^3$H started to accumulate in tissue of veins and arteries after 4 hours and still was increasing at the last time (48 h) measured. It was suggested that a metabolite was accumulating in these tissues. The radioactivity associated with 3-[$^{14}$C]methoxy-(+)-catechin was only recovered with the contents of the alimentary tract; this was caused by enterohepatic circulation of the major metabolite (55). The distribution of radioactivity in tissues of the rat after oral administration of [4-$^{14}$C]quercetin (28) showed no evidence for accumulation in any tissue. Six hours after administration the highest radioactivity (0.3% of the administered dose per gram of wet organ) was found in the kidney, with liver and blood having somewhat lower concentrations. Because of the position of the label in the quercetin molecule, only quercetin and metabolites with an intact ring structure and phenylpropionic acids would have been detected. Recovery of radioactivity in organs of rats (liver, kidneys, spleen, stomach, and gut) was 1.4% of the activity of orally administered [3-$^{14}$C]hesperetin (31).

More than 98% of the quercetin in human plasma was bound to proteins (29). Binding of quercetin to human albumin was 70–80% (94). These observations were confirmed by ultrafiltration (95); after ultrafiltration to exclude proteins larger than 30 kDa, quercetin was absent in the filtrate of plasma of rats fed quercetin and rutin. Binding of flavonoids to proteins is well documented (96). Quercetin and rutin but not (+)-catechin or 3-methoxy-(+)-catechin were selectively bound to platelets of rabbits in vitro (97).

Thus, the limited data available do not point to storage of intact

flavonoids in tissues. However, evidence for accumulation of metabolites was found for diosmin.

## SUMMARY AND CONCLUSIONS

Most studies on absorption and metabolism of flavonoids have focused on measuring their urinary metabolites in rodents. Absorption was estimated by measuring urinary excretion using pure aglycones administered at unphysiologically high doses. Absorption as measured this way depended on the type of flavonoid and was between 4 and 58%. The extent of absorption from dietary sources is largely unknown; for instance, data on the catechins of tea, a major dietary source, are virtually absent. In the diet, most flavonoids except catechins are present not as aglycones but as glycosides. It used to be thought that intact glycosides are not absorbed. However, recently it was found that quercetin glucoside was in fact absorbed much better than the aglycone; this topic thus needs rethinking. Proteins in the diet may theoretically affect flavonoid absorption because they bind polyphenols (96). Circumstantial evidence for reduced absorption of tea polyphenols by complexation with milk proteins was found in humans (98); ingestion of tea caused a significant increase of the plasma antioxidant capacity, but not when tea was consumed with milk. However, these authors did not determine polyphenols in plasma. Research on this interaction needs to be done.

The two major sites of flavonoid metabolism are the liver and the colonic flora. Only the liver has been investigated as a metabolic organ. Other tissues such as intestine wall and kidneys may play a role. Phase I biotransformation reactions of liver enzymes have been described only for synthetic flavonoids lacking hydroxyl groups, but evidence for phase II biotransformation is abundant. Absorbed flavonoids and their absorbed colonic metabolites are glucuronidated and sulfated by the liver in humans as well as in rodents, but the types of glucuronides and the preference for sulfation may vary between species. *O*-methylation of catechol groups is found in humans and rodents. Unabsorbed flavonoids and flavonoid conjugates secreted with bile into the gut are degraded by bacteria in the colon. Hydrolysis of conjugates and glycosides and ring fission of the aglycones to phenolic acids are the major bacterial reactions. In rats the metabolites are then absorbed and excreted in urine. However, very few quantitative data on metabolism are available. In the metabolism of (+)-catechin, ring fission is of minor importance in humans. With other flavonoids, the rather high excretion of $CO_2$, about one third of the dose, points to notable ring fission in rats.

In humans, conjugation and *O*-methylation of quercetin occurs only to a limited extent, whereas these reactions are of major importance for (+)-catechin metabolism. One would expect biliary secretion also to occur in humans, as glucuronides of flavonoids have molecular weights in excess of 500 (39). However, the significance of biliary secretion and reabsorption in humans is unknown.

Pharmacokinetic data on flavonoids are scarce, probably because selective and sensitive analytical methods to determine flavonoids and their metabolites in plasma, urine, and tissues were lacking. Absolute bioavailability of flavonoids has not been determined, but it is becoming evident that relative bioavailability of flavonols differs markedly between foods.

In order to evaluate the impact of dietary flavonoids on human health, we need more information on how the nature of the glycoside moiety and of the food matrix affect absorption and metabolism. Such studies should be performed at the low levels of intake that occur naturally, as the high doses used in earlier studies may produce artefacts. Identification of metabolites in body fluids and tissues is also an important goal for further research.

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