The splanchnic metabolism of flavonoids highly differed according to the nature of the compound

Vanessa Crespy,1 Christine Morand,1 Catherine Besson,1 Nicole Cotelle,2 Hervé Vézin,2 Christian Demigné,1 and Christian Rémy1
1Laboratoire des Maladies Métaboliques et des Micronutriments, Institut National de la Recherche Agronomique Centre de Recherche de Clermont-Ferrand/Theix, 63122 Saint Genève-Champannelle; and
2Laboratoire de Chimie Organique et Macromoléculaire, Unité Propre de Recherche et d’Enseignement Associée 8009, Equipe Polyphénols, Université des Sciences et Technologies de Lille, 59655 Villeneuves d’Ascq, France

Submitted 11 June 2002; accepted in final form 21 January 2003

Crespy, Vanessa, Christine Morand, Catherine Besson, Nicole Cotelle, Hervé Vézin, Christian Demigné, and Christian Rémy. The splanchnic metabolism of flavonoids highly differed according to the nature of the compound. Am J Physiol Gastrointest Liver Physiol 284: G980–G988, 2003; 10.1152/ajpgi.00223.2002.—The absorption and splanchnic metabolism of different flavonoids (namely quercetin, kaempferol, luteolin, eriodictyol, genistein, and catechin) were investigated in rats after an in situ perfusion of jejunum plus ileum (14 mmol/min). Net transfer across the brush border ranged widely according to the perfused compound (from 78% for kaempferol to 35% for catechin). This variation seems linked to the lipophilicity of a given flavonoid rather than to its three-dimensional structure. Except for catechin, conjugated forms of perfused flavonoids were also detected in the intestinal lumen, but the extent of this secretion depended on the nature of the perfused compounds (52% for quercetin to 11% for genistein). For some of the perfused aglycones, biliary secretion was an important excretion route: 30% of the perfused dose for genistein but only 1% for catechin. Thus the splanchnic metabolism of flavonoid is controlled by several factors: 1) the efficiency of their transfer through the brush border, 2) the intensity of the intestinal secretion of conjugates toward the mucosal and serosal sides, respectively, and 3) the biliary secretion of conjugates. These data suggested that the splanchnic metabolism of perfused flavonoids depends on the nature of the compound considered, which in turn influences their availability for peripheral tissues.

absorption; biliary excretion; rats

FLAVONOIDS are phenolic secondary plant metabolites responsible for much of the color and flavor of plant foods. They are ubiquitous in fruits, vegetables, and beverages, with more than 4,000 chemically different flavonoids identified to date. They are assigned to different groups according on their structure: anthocyanins, flavonols, flavones, catechins, isoflavones, and flavonones (1, 8, 38, 43). Owing to their ubiquity in plants, humans are constantly exposed to a wide variety of flavonoids. A wealth of beneficial properties has been reported: antibacterial, antioxidant, anti-inflammatory, and anticarcinogenic effects (2, 21, 29) and estrogenic effects for isoflavones (33). Understanding the absorption and metabolism of dietary flavonoids is fundamental to determining their potential biological activity.

The small intestine is a crucial site for the absorption of dietary flavonoids. In this tissue, aglycones are metabolized by intestinal conjugation enzymes, and the resulting metabolites are then secreted toward the mucosal and/or serosal sides (5, 6, 10, 13, 37). Circulating forms of absorbed flavonoids have been identified as methylated and/or glucuronidated and/or sulfated metabolites (9, 12, 19, 23, 39), resulting from the conjugative activities of both liver and intestine (7). Flavonoid metabolites are then eliminated in urine and by the biliary route (18, 22, 35, 40). When excreted into the bile, flavonoid metabolites flow in the small intestine and reach the hindgut where they can be reabsorbed after their hydrolysis by the microflora (30).

Owing to the difficulties of an in vivo approach, many in vitro methods have been proposed to estimate the absorption and intestinal metabolism of flavonoids. Until now, literature data have not allowed a strict comparison of the fate of the different classes of flavonoids, essentially because of the diversity of the experimental models used (Caco-2 cells, everted intestine, intestinal perfusion). Accordingly, the present study was performed to compare the intestinal absorption and splanchnic metabolism of different flavonoids, using an in situ intestinal perfusion model. A representative compound of each major class of flavonoids was tested, using the same experimental pattern. All of the chosen compounds were aglycones possessing a catechol group, quercetin (flavonol), luteolin (flavone), eriodictyol (flavanone), and catechin (flavanol), except for kaempferol (another flavonol) and genistein (isoflavone) (Fig. 1).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
SPLANCHNIC METABOLISM OF FLAVONOIDS IN RATS

G981

Computational Chemistry

All the calculations were performed on an NT workstation (PIII-650 MHz processor) using Spartan Pro V 1.0.1 software. A conformational analysis was performed for all molecules on all rotatable bonds using the Monte Carlo method implemented in Spartan, and the structures found were then minimized using the molecular mechanics Merck Molecular Force Field MMF94. Then the structures were fully optimized at ab initio level using 3–21G* basis set with one polarization function (*) with the closed-shell Restricted Hartree Fock formalism (RHF/PM3 formalization). The log P values rendering the hydrophobic indication of the molecule were calculated using the method described in Ref. 14 implemented in the ab initio Spartan function.

Animals and Diets

Wistar rats weighing ~150 g were housed two per cage in temperature-controlled rooms (22°C) with a dark period from 2000 to 0800 and access to food from 1600 to 0800. They were fed a standard semipurified diet (73% wheat starch, 15% casein, 6% mineral mixture, 1% vitamin mixture, 5% corn oil) for 2 wk.

Animals were maintained and handled according to the recommendations of the Institut National de la Recherche Agronomique Ethics Committee, as legally required.

Sampling Procedure

Twenty-four-hour fasted rats were anesthetized with pentobarbital sodium (40 mg/kg body wt) and maintained alive throughout the perfusion period. After cannulation of the biliary duct, a perfusion of the jejunal plus ileal segment (from 5 cm distal from the flexura duodenojejunalis to the valvula ileocecalis) was prepared by introducing cannula at each extremity. This segment was continuously perfused in situ for 30 min with a physiological thermostated buffer (37°C) supplemented with 14 μM flavonoid aglycone (5 μl of a 140 mM solution in DMSO/50 ml buffer). The composition of buffer was as follows (in mM): 5 KH2PO4, 2.5 K2HPO4, 5 NaHCO3, 50 NaCl, 40 KCl, 10 tripotassium citrate, 2 CaCl2, 2 MgCl2, 8 glucose, and 1 taurocholic acid at pH 6.7. The flow rate was set at 1 ml/min. For all the perfused compounds we have checked beforehand 1) that they were stable in the buffer throughout the perfusion period and 2) that the steady state for their effluent flux was reached after 20 min. The fraction of the effluent collected at the exit of the ileum during the last 5 min was stored until analysis. The bile was collected throughout the perfusion, and the fraction corresponding to the 20–30 min period was stored and used for HPLC analysis.

At the end of the experiment, blood samples were withdrawn from the abdominal aorta and sampled in heparinized tubes. Plasma, bile, and effluent samples were acidified with 10 mM acetic acid (10 μl of 1 M acetic acid per ml of sample) and stored at −20°C until analysis.

HPLC Analysis

Sample treatment. Bile, plasma, and perfusate samples were spiked with 3.5 nmol/l of an appropriate internal standard: apigenin for quercetin, kaempferol, and eriodictyol experiments; tamarixetin for luteolin experiments; hesperetin for genistein experiments; and taxifolin for catechin experiments. All the standards used for the identification of the compounds were commercially available, except for 3′-O-methyl catechin, which was obtained by chemical O-methylation of catechin as previously described (11). The samples were then acidified (to pH 4.9) with 0.1 vol of 0.58 M acetic acid and incubated for 30 min at 37°C in the absence (unconjugated forms) or in the presence (total forms) of 5 × 106 U/l β-glucuronidase and 2.5 × 105 U/l sulfatase. Samples were then acidified (to pH 4.9) with 0.1 vol of 0.58 M acetic acid and incubated for 30 min at 37°C in the absence (unconjugated forms) or in the presence (total forms) of 5 × 106 U/l β-glucuronidase and 2.5 × 105 U/l sulfatase. Samples were extracted by adding 2.85 vol of methanol/200 mM HCl and centrifuged for 4 min at 14,000 g. The resulting supernatants were analyzed as described below. The concentrations of conjugated metabolites were calculated by the difference between the concentrations of aglycone measured before and after enzymatic hydrolysis. The absorption flux was calculated by the difference, initial perfused flux − effluent flux, with initial perfused flux (nmol/min) = initial flow rate (1 ml/min) × concentration (nmol/ml), and with effluent flux (nmol/min) = volume recovered (ml)/time of recovery (min) × concentration (nmol/ml).

Chromatographic conditions. To analyze the conjugated metabolites of the flavonoids, other than those of catechin, the HPLC system consisted of an autosampler (Kontron 360), a Diode Array Detector (set at 370 nm for flavonols, 350 nm for flavone, 290 nm for flavanone, and at 280 nm for...
genistein) and Software system for data recording and processing. The system was fitted with a 5-μm C18 Hypersil BDS analytic column (150 × 4.6 mm; Life Sciences International, Cergy, France). The mobile phases were 15% acetonitrile (solvent A) and 37% acetonitrile (solvent B), each containing 0.5% of H3PO4. The flow rate was set at 1 ml/min, and the chromatographic conditions were 0–2 min, 100% A; 2–22 min, linear gradient from 100% A to 100% B; 22–24 min, 100% B; 24–27 min, return to initial mobile phase conditions, and then equilibration for 8 min.

Catechin and catechin metabolites were analyzed by HPLC coupled to a multielectrode detection using an eight-electrode CoulArray model 5600 system (Eurosep, Cergy, France) with potentials set at 25, 100, 320, 400, 500, 700, 800, and 900 mV. Mobile phase A was 3% acetonitrile in 30 mM NaH2PO4 at pH 3.0, and mobile phase B was 20% acetonitrile in 30 mM NaH2PO4 at pH 3.0. The separation was performed at 35°C. The flow rate was set at 1 ml/min with a linear gradient from 0 to 50% B in 15 min and held at 50% B for 20 min. From 20 to 25 min, the gradient was increased to 100% B and then brought back to 100% A for 40 min. The specifications of the column were similar to those indicated above.

Statistics

Values are means ± SE, and the differences between values were determined by one-way ANOVA coupled with the Tukey-Kramer multiple comparisons test. Values of P < 0.05 were considered significant.

RESULTS

Fate of Flavonoids in the Intestine

Whatever the flavonoid tested, a fraction of the perfused dose was recovered intact in the effluent at the end of the perfusion, indicating its intestinal absorption was not complete (Table 1). The magnitude of this unabsorbed fraction was about 20–40% of the total perfused dose for all the compounds, except for catechin for which it reached 65%. These results suggest that the net transfer into the intestinal wall (Table 1), reflecting the transport through the apical membrane, depended on the nature of the compounds.

The determination of the net transfer into the intestinal wall shows a specificity of flavonoid uptake (5.25 ± 0.20 nmol/min for catechin and 11.45 ± 0.60 nmol/min for kaempferol). On the basis of these findings, we investigated the structure-activity relationships of the flavonoid pattern. Comparison of the basic chemical structures of the flavonoids tested and the net transfer values indicated that neither the position of the B ring at C2 (flavone/flavonol/flavanone) or C3 (isoflavone) nor the nature of the substitution at 3 (H or OH) affected their transfer through the brush border (Fig. 1, Table 1). However, the absence of a 4-oxo group on ring B ( flavanol) was associated with a lower net transfer.

To study the influence of the lipophilicity of the flavonoids on the efficiency of transfer across the intestinal membrane, log P was calculated for each of the tested substances (Table 2). The values obtained agree with those of the literature (32). We found that the most lipophilic molecules (kaempferol, quercetin, luteolin, eriodictyol, and genistein) displayed high transfer efficiencies, whereas catechin, which is much less lipophilic, was less readily transferred.

Net transfer efficiency was also compared with the three-dimensional structure of the molecules tested (Table 2). The dihedral angle indicates the position of the B ring relative to the C ring, i.e., the planarity of the whole molecule. Moreover, visualizing the molecule in its most stable conformation shows whether it is elongated or folded (Table 2). We see that a molecule with an elongated structure like luteolin is as efficiently transferred across the intestinal wall as eriodictyol, which nevertheless presents two structures, elongated and folded (Fig. 2), and which from Boltzman population analysis represents 62 and 38% of the species in solution, respectively. In these conditions, it seems that the three-dimensional structure of flavonoids may not be determinant in their interactions with the brush border.

All these data favor a relationship between net transfer and lipophilicity of the molecule, rather than its spatial arrangement.

For all the flavonoids tested, except for catechin, conjugated derivatives were recovered in the effluent (Table 1). These metabolites had an intestinal origin, because the biliary duct was cannulated before starting the perfusion. The rate of the intestinal secretion of conjugates was highly dependent on the nature of the

### Table 1. Fate of flavonoids absorbed by the gut

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Nonabsorbed, nmol/min</th>
<th>Net Transfer into Intestinal Wall, nmol/min</th>
<th>Secretion of Conjugated Forms into the Lumen, nmol/min</th>
<th>Net Absorption, nmol/min</th>
<th>Fraction of Net Transfer Secreted into Serosal side, %</th>
<th>Fraction of Net Transfer Secreted into Mucosal side, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>4.90 ± 0.40</td>
<td>9.80 ± 0.20</td>
<td>7.70 ± 0.40</td>
<td>2.10 ± 0.40</td>
<td>21 ± 3</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>3.20 ± 0.50</td>
<td>11.45 ± 0.60</td>
<td>2.94 ± 0.30</td>
<td>8.50 ± 0.40</td>
<td>74.5 ± 3</td>
<td>25.5 ± 2</td>
</tr>
<tr>
<td>Luteolin</td>
<td>4.70 ± 0.25</td>
<td>7.76 ± 0.10</td>
<td>2.66 ± 0.30</td>
<td>5.10 ± 0.30</td>
<td>66 ± 4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>5.40 ± 0.40</td>
<td>7.80 ± 0.50</td>
<td>2.14 ± 0.30</td>
<td>5.66 ± 0.60</td>
<td>73 ± 5</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Genistein</td>
<td>3.20 ± 0.25</td>
<td>9.80 ± 0.20</td>
<td>1.38 ± 0.30</td>
<td>8.40 ± 0.40</td>
<td>86 ± 3</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Catechin</td>
<td>9.70 ± 0.30</td>
<td>5.25 ± 0.20</td>
<td>0</td>
<td>5.25 ± 0.20</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. For each compound, the perfused fluxes were as follows (nmol/min): 14.70 ± 0.60 for quercetin, 14.70 ± 0.80 for kaempferol, 12.50 ± 0.70 for luteolin, 13.30 ± 0.40 for eriodictyol, 13.30 ± 0.40 for genistein, and 14.90 ± 0.60 for catechin. Nonabsorbed aglycone = flux of intact aglycone in the effluent at the end of the perfusion. Net transfer into the intestinal wall = (flux of perfused aglycone) − (flux of nonabsorbed aglycone). Secretion of conjugated forms into the lumen = (flux of the aglycone in hydrolyzed effluent) − (flux of the aglycone in nonhydrolyzed effluent). Net absorption = (Net transfer into the intestinal wall) − (Secretion of conjugated forms into the lumen).
perfused compound (Table 1). For quercetin, it reached 52% of the perfused dose but was markedly lower for the other tested compounds (11–20%). The intestinal metabolism of catechin differed unequivocally from that of the other compounds, since no trace of conjugated forms was found in the lumen at the end of the perfusion (Table 1).

For a given compound, the difference between the net transfer in the intestinal wall and the secretion of conjugates into the lumen has been made to estimate the net absorption (Table 1). Although the net transfer of quercetin in the intestinal wall reached 9.80 ± 0.20 nmol/min, the high rate of secretion of conjugated forms into the lumen (7.70 ± 0.40 nmol/min) resulted in a quite moderate rate of absorption (only 2.1 ± 0.40 nmol/min). This result was also reflected by the fraction of the net transfer in the intestinal wall directed toward the serosal side (21%) (Table 1). By contrast, kaempferol, luteolin, eriodictyol, and genistein presented a moderate intestinal secretion of their conjugates associated with a relatively high level of net absorption: 66 to 86% of the net transfer was directed toward the serosal side (Table 1). It was noteworthy that although kaempferol and quercetin belong to the same class of flavonoid, they presented marked differences in their intestinal metabolism. The net absorption of kaempferol (8.50 ± 0.40 nmol/min) was higher than that of quercetin (2.1 ± 0.40 nmol/min) owing to the less abundant secretion of kaempferol conjugates into the lumen.

For catechin, because no conjugates were secreted into the intestinal lumen, net transfer into the gut corresponded to its net absorption (5.25 ± 0.30 nmol/min) (Table 1).

All these data suggest that in the intestine, the efficiency of flavonoid absorption is modulated by the permeability of the apical membrane toward these compounds and by the extent of secretion of conjugates into the lumen.

**Biliary Secretion**

The cannulation of the biliary duct at the beginning of the perfusion and the collection of the biliary secre-

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Log P (±)</th>
<th>Net Transfer into the Intestinal Wall, nmol/min</th>
<th>Dihedral Angle @ O1C2C1C2</th>
<th>Spatial Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>2.15 (2.239 ± 1.000†)</td>
<td>9.80 ± 0.20</td>
<td>2.51*</td>
<td>elongated structure</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>2.94 (2.441 ± 1.000†)</td>
<td>11.45 ± 0.60</td>
<td>3.66*</td>
<td>elongated structure</td>
</tr>
<tr>
<td>Luteolin</td>
<td>2.53 (1.990 ± 0.388†)</td>
<td>7.76 ± 0.10</td>
<td>17.53* (17*)</td>
<td>elongated structure</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>2.02 (3.225 ± 0.836†)</td>
<td>7.80 ± 0.50</td>
<td>41.71* (−34.49*)</td>
<td>elongated structure, fold structure</td>
</tr>
<tr>
<td>Catechin</td>
<td>1.1 (0.452 ± 0.781†)</td>
<td>5.25 ± 0.20</td>
<td>42.81°(R,S)</td>
<td>elongated structure</td>
</tr>
<tr>
<td>Genistein</td>
<td>2.24–2.40 (3.424 ± 0.781†)</td>
<td>9.80 ± 0.20</td>
<td>179.32°</td>
<td>elongated structure</td>
</tr>
</tbody>
</table>

The value of the dihedral angle (O1C2C1C2) calculated from the crystallographic structure of quercetin, kaempferol and luteolin optimized at the ab initio RHF/3-21G* is in good agreement with the results published in Ref. 41. † Calculated using advanced Chemistry Development Software Solaris V4.67© 1994–2002 ACD. ‡ Data obtained in Ref. 32. § Data obtained in Ref. 41.

---

Fig. 2. Structures of the 2 most stable conformations of the eriodictyol molecule calculated by the Monte Carlo method before full optimization at RHF/PM3/3–21G* level. Heat of formation values are, respectively, −203.74 and −203.50 kcal/mol (left to right).
The biliary secretion of flavonoids, resulting from all the data obtained in the study and from the data presented in Tables 1, 3, and 4, is given in Fig. 3. The difference between the net absorption and the biliary secretion gave an estimate of the proportion of conjugated forms finally available for peripheral tissues (see Fig. 3). Quercetin was distinguishable from the other tested flavonoids by its high rate of intestinal secretion (52%). Thus only a small fraction of absorbed quercetin was finally available for peripheral tissues (9%) (Fig. 3). By contrast, this fraction was quite high for kaempferol.

**Table 3. Biliary secretion of perfused flavonoids**

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Biliary Secretion of Conjugates, nmol/min</th>
<th>3'-Methylated Form, µM</th>
<th>4'-Methylated Form, µM</th>
<th>Total Concentration of biliary conjugates, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>0.16 ± 0.05</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.07</td>
<td>57.55 ± 4.60</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>4.29 ± 0.40</td>
<td>30 ± 5</td>
<td>40 ± 10</td>
<td>159.55 ± 38.60</td>
</tr>
<tr>
<td>Luteolin</td>
<td>2.42 ± 0.20</td>
<td>38 ± 4</td>
<td>10 ± 6</td>
<td>168.71 ± 12.60</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>0.19 ± 0.10</td>
<td>40 ± 4</td>
<td>10 ± 6</td>
<td>321.50 ± 35.50</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.54 ± 0.60</td>
<td>76 ± 6</td>
<td>23 ± 6</td>
<td>402.60 ± 47.45</td>
</tr>
<tr>
<td>Catechin</td>
<td>14 ± 0.9</td>
<td>0.56 ± 0.20</td>
<td>13.40 ± 1.50</td>
<td>14.00 ± 1.50</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. Biliary secretion of conjugates (nmol/min) = (biliary flow rate × concentration of total forms present in bile).

Plasma Metabolites After Intestinal Perfusion

The analysis of plasma sampled from the aorta at the end of the perfusion indicated that whatever the perfused compound, the circulating metabolites were made up of glucuro- and/or sulfoconjugates, methylated or nonmethylated. This result shows that only conjugated forms of flavonoids absorbed by the gut were delivered to peripheral tissues.

The plasma levels measured differed markedly according to the perfused compound: 0.25 ± 0.05 µM for catechin, 0.48 ± 0.04 µM for eriodictyol, 1.04 ± 0.06 µM for luteolin, and 1.34 ± 0.20 µM for genistein. The two flavonoids tested, namely quercetin and kaempferol, resulted in quite similar plasma concentrations (0.71 ± 0.06 and 0.95 ± 0.12 µM, respectively).

**Table 4. Respective contributions of biliary and intestinal secretions in total secretion of conjugates found in lumen at end of perfusion**

<table>
<thead>
<tr>
<th>Total Secretion of Conjugated Forms, nmol/min</th>
<th>Biliary Secretion, %</th>
<th>Intestinal Secretion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>8.57 ± 0.50</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>4.29 ± 0.40</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Luteolin</td>
<td>4.25 ± 0.20</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>5.19 ± 0.30</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>Genistein</td>
<td>5.54 ± 0.60</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>Catechin</td>
<td>0.16 ± 0.05</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8.
(49%), in agreement with its relatively low rate of intestinal and biliary secretion (Fig. 3). These results suggest that the splanchnic metabolism of compounds belonging to the same class of flavonoid can markedly differ. Luteolin, eriodictyol, and genistein, which presented quite similar levels of total excretion into the lumen (20–30% of the perfused doses), exhibited relatively high availabilities for peripheral tissues (Fig. 3).

Among the tested flavonoids, catechin was a special case, first because no intestinal secretion of catechin conjugates occurred, and second because its biliary secretion was quite low (1%). In these conditions, almost all the catechin entering the intestinal cells, corresponding to 34% of the perfused dose, was available for peripheral tissues.

**DISCUSSION**

Flavonoid bioavailability is a complex field, and earlier studies on this topic have not simultaneously taken into account parameters such as intestinal absorption and splanchnic metabolism.

It is well established that factors such as the nature of glycosylation and the food matrix modulate the bioavailability of dietary flavonoids (15, 16). However, little is known about how they are transferred as aglycone through the brush border (rate and transport system) or about their intestinal metabolism and secretion. Theoretically, depending on its structure, a compound may cross the epithelial cell layer by several mechanisms: passive diffusion across the membrane, carrier-mediated transport, endocytosis, and paracellular diffusion through tight junctions. The mechanisms involved in flavonoid transport at the intestinal level are the subject of research but have not yet been elucidated. However, because of their structure, flavonoids probably do not cross the phospholipidic membrane by a passive diffusion system, but rather via a mechanism of facilitated diffusion or active transport.
With our in situ perfusion model, the efficiency of the flavonoid transfer through the brush border is inversely related to the level of intact flavonoid recovered in the effluent at the end of the perfusion. Because we failed to found detectable amounts of perfused compounds in intestinal mucosa extract (data not shown), we considered that the difference between the concentration measured in the initial perfusate and that found in the remaining perfusate reflected the magnitude of the transfer of the compounds through the brush border. Quercetin, kaempferol, luteolin, eriodictyol, and genistein were extensively absorbed in the gut (59–78% of the perfused dose), suggesting that their transfer through the brush border is not limiting for bioavailability. Recently, it was shown that genistein entered Caco-2 cells by a transepithelial transporter that was saturable when the concentration of genistein reached 50 μM (25). This result is corroborated by our data, since when perfused at 14 nmol/min (corresponding to a 14 μM dose), genistein was readily transferred in the intestinal wall (73% of the perfused dose).

Catechin is characterized by a relatively low transfer through the gut (only 35%), indicating that the transfer across the intestinal cells may be limiting for catechin bioavailability. In a previous study, we have found that the catechin absorption was directly proportional to the amount perfused (10). Although the mechanism of catechin absorption in the small intestine is not established, it appears probable that catechin enters the enterocytes by a passive diffusion system or by a transporter that was not saturated at concentrations up to 100 μM (10). However, in in vitro experiments, it has been reported that the absorption of genistein is slightly inhibited by quercetin (–50%) and catechin (–20%) (25). The authors suggested that the uptake of genistein might occur, at least partly, via a transport system common to other flavonoids (27). Even if this hypothesis of the existence of a transport system common to all flavonoids is correct, the affinity for the transporter and the rate of the transfer may still depend on the nature of the flavonoids. In our results, the position of the B ring at C2 or C3 (ring C) and the nature of the substitution at the 3 position (H or OH) did not influence the net transfer across the brush border. Thus if an active transport is involved, then the efficiency of the absorption may depend on the two hydroxyls at positions 5 and 7 on the A ring. Such a hypothesis could be tested using selectively synthesized 5- or 7-alkoxy or benzoxylflavonoids in our intestinal perfusion model. In addition, whereas the nature of the three-dimensional structure of the flavonoids does not seem to be determinant for the efficiency of transfer, the lipophilicity of the molecule seems important.

Many studies have emphasized the role of the intestinal conjugative enzymes in the metabolism of flavonoids (3, 5, 6, 42). The conjugates produced in the intestine (essentially glucuronides and sulfocjugates) are then secreted toward the serosal and mucosal sides (5, 6, 42). The recovery of only conjugated metabolites in the mesenteric vein (10, 13) indicated, first, that the intestinal conjugation occurred before the transfer of flavonoid into the serosal side and, second, that the system responsible for the transport of the aglycone is not present on the basolateral membrane.

The present study clearly shows that some of the conjugates synthesized in the enterocytes are secreted back in the intestinal lumen. This process could occur via MRP2, which has been reported to mediate the efflux of conjugated forms of chrysin into the luminal side of Caco-2 cells (42). However, the proportion of the conjugated forms secreted toward the serosal or toward the mucosal side depends markedly on the nature of the flavonoid. According to our data, quercetin conjugates produced in the intestine are copiously secreted into the mucosal side, whereas this secretion appears less abundant for the other perfused compounds. In any case, a high level of secretion of conjugates into the lumen is associated with a reduction of the net absorption of the perfused flavonoids. Thus a high intestinal secretion of flavonoid conjugates could correspond to a mechanism whereby the body reduces the bioavailability of quite reactive compounds such as quercetin (31).

All these data enabled us to define two control steps in the bioavailability of flavonoids: 1) transepithelial transport and 2) secretion of conjugates into the intestinal lumen and into the serosal side.

When flavonoid metabolites resulting from intestinal conjugation are secreted in the mesenteric vein, a proportion of them are taken up and metabolized by the liver (3, 4, 26) and then partially secreted in the bile (20, 34, 44, 45). In our experiments, the presence of flavonoids in the bile for all the tested compounds indicated that whatever their origin, flavonoids undergo enterohepatic cycling. The detection of methylated metabolites of quercetin, luteolin, eriodictyol, and catechin in the bile showed that the liver is an active site for the methylation of flavonoids, when they bear a catechol group. In the present study, we did not determine the precise nature of the metabolites resulting from the respective intestinal and hepatic conjugation, but, as shown by the forms recovered in the bile, they may be quite different. As reported in our previous study, catechin was present in the mesenteric vein as glucuronides (methylated or nonmethylated), whereas it was recovered as glucurono/sulfoconjugates (methylated or nonmethylated) in the bile (10). Taken together, these data strongly suggest that hepatocytes are a site of deconjugation for the circulating forms coming from the mesenteric vein and that the conjugative activity of the liver produced metabolites available for the bile and peripheral tissues.

The nature of the conjugation influences the elimination pathway of the circulating metabolites: the glucuronides are excreted in the bile to a much higher extent than the sulfates, which are preferentially eliminated in urine (24). The nature and the position of the conjugation also probably affect the availability of the circulating metabolites for peripheral tissues, but until now data on this purpose are lacking. The decrease in
flavonoid lipophilicity induced by their conjugation does not favor a passive diffusion of the conjugates through the membrane. However, it is quite probable that their uptake by the target cells involved specific transport systems, as it has already been shown for estrogen conjugates (28).

The activity of the biliary secretion strongly depends on the nature of the compounds. Some of them, such as catechin, are sparingly secreted in the bile (1%), whereas others, like genistein and eriodictyol, are extensively recycled (32 and 23% of the perfused dose, respectively). The importance of the biliary secretion in genistein bioavailability was previously suggested in a study reporting that 70–75% of genistein perfused in the small intestine was recovered in the bile over 4 h of perfusion (34). By contrast, the biliary secretion of quercetin and catechin appears quite limited, indicating that the biliary pathway probably plays a minor role in their elimination.

As the rat does not possess a gall bladder and because the liver did not constitute a site for flavonoid accumulation (19), it can be considered that the biliary flux is directly linked to the intestinal absorption flux. Moreover, the plasma concentrations measured after the intestinal perfusion of flavonoids are quite low and, by consequence, cannot constitute a significant pool. In these conditions, the compilation of the data obtained at the intestinal and biliary levels provides information about the fraction of perfused flavonoid ultimately available for peripheral tissues (see Fig. 3).

Because of its high secretion of conjugates in the intestinal lumen (58%), quercetin was poorly available for peripheral tissues (only 9%). By contrast, a relatively high level of perfused catechin (34%) reached peripheral tissues. These results are corroborated by experiments on rats fed a meal containing similar doses of quercetin or catechin showing that 4 h after the beginning of the meal, the plasma concentration of catechin was higher than that of quercetin (19).

It can be noted that quercetin and kaempferol, which belong to the same flavonoid class, presented quite different splanchnic metabolisms. The intestinal metabolism of kaempferol was not limiting for its bioavailability, unlike what was observed for quercetin. In these conditions, the fraction of kaempferol available for peripheral tissues was five times that of quercetin. Kaempferol differs from quercetin in having no catechol group, and this structural difference may be linked to changes in their splanchnic metabolism. However, despite the marked difference in the estimated systemic availability between quercetin and kaempferol, their respective plasma concentrations were quite similar. This could be due to a better elimination of plasma kaempferol conjugates by the kidney, leading to its rapid disappearance from plasma.

The availability of luteolin and eriodictyol for peripheral tissues appeared to depend on the intestinal and biliary secretions, whereas that of genistein was strongly controlled by the biliary secretion alone. It is difficult to compare the present in situ data with the published in vivo studies on luteolin, eriodictyol, and genistein bioavailability, because these are sparse and were conducted in quite different conditions (dose, administration mode) (17, 22, 27, 36). As a direct comparison of the literature data with our findings appears impossible, additional in vivo experiments will have to be performed in conditions that permit a true comparison of the bioavailability of these flavonoids.

In conclusion, the data presented here indicate that flavonoid bioavailability depends on three main factors: 1) efficiency of transfer through the brush border, 2) intensity of intestinal secretion of conjugates toward the mucosal and serosal sides, and 3) activity of biliary secretion. Also, the importance of each of these factors in the control of the splanchnic metabolism varies according to the molecule, making it impossible to state a general rule valid for the splanchnic metabolism of all flavonoids.

Further investigations must now be conducted to determine the mechanisms by which flavonoids enter the cells (intestinal, hepatic, and target cells) and the nature of the molecules responsible for the biological effects inside the cells. Thorough knowledge of these phenomena is crucial for a complete understanding of flavonoid bioavailability.

REFERENCES

11. Donovan JL, Luthria DL, Stremple P, and Waterhouse AL. Analysis of (+)-catechin, (−)-epicatechin and their 3’- and 4’-O-