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Gastrointestinal Absorption and Urinary Excretion of *trans*-Cinnamic and *p*-Coumaric Acids in Rats

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trans-Cinnamic acid (CIN) and *p*-coumaric acid (COU) are ingested by humans in their diet. While the metabolism and health benefits of CIN have been widely documented, little is known about its absorption sites, and there have been few studies dedicated to COU. The gastrointestinal sac technique demonstrated that CIN and COU are absorbed by all digestive organs in rats and partially transported via MCT-mediated carrier. Absorption was lowest in the stomach. Regardless of the organs that were studied, CIN was more efficiently absorbed than COU. After their individual oral administration to rats, CIN and COU were excreted in 0−24 h urine (0.3% and 23% of ingested CIN and COU, respectively). This suggests that COU was less metabolized than CIN. CIN and COU are absorbed across the digestive epithelium and subsequently interact with target tissues. Despite its lower gastrointestinal absorption, COU may have greater health benefits because it seems to be less metabolized than CIN.

KEYWORDS: ***trans*-Cinnamic acid; *p*-coumaric acid; gastrointestinal absorption; urinary excretion; rats**

INTRODUCTION

Phenolic acids and their derivatives are widely distributed in plants (*1*), mostly in grains (*1*−*3*), fruits, and vegetables (*4*). One group of plant phenolics is composed of monomeric cinnamic acid derivatives such as cinnamic, ferulic, *p*-coumaric, sinapic, and caffeic acids (*5*). In plants, cinnamic acid is converted into *p*-hydroxycinnamic (*p*-coumaric) acid, one of the more important precursors of lignin (*5*). *trans*-Cinnamic acid (CIN) is found abundantly in potatoes, strawberries, and raspberries, while *p*-coumaric acid (COU) is present in apple juice, tomatoes, carrots, red wine, coffee, crisp-bread, red raspberries, and strawberries (*6*, *7*). CIN and COU are thus ubiquitous in the plant kingdom and are ingested by animals and humans in their regular diet.

CIN and its derivatives have a broad therapeutic spectrum, including antimicrobial (*8*, *9*) and antifungal (*10*) activity. Olasupo et al. (*9*) demonstrated in vitro the antimicrobial activity of CIN against two Gram-negative bacteria, *Salmonella typhimurium*, and *Escherichia coli*. The minimum inhibitory concentrations of CIN were 7.5 and 5.0 mmol/L for *S. typhimurium* and *E. coli*, respectively. Liu et al. (*11*) showed that CIN exhibits antitumoral activity against human malignant tumors, including melanoma, glioblastoma, and prostatic or pulmonary adenocarcinoma. CIN induces cytostasis and a reversal of the malignant properties of human tumor cells in vitro. A 50% reduction in the level of cell proliferation was achieved by concentrations ranging from 1 to 4.5 mmol/L (*11*). It was also shown that phenolic compounds have potentially protective effects against heart diseases because of their ability to increase the resistance of low-density lipoproteins (LDLs) to cholesterol oxidation, lipid peroxidation, and the oxidative modification of apoprotein B_{100} (*12*−*15*).

While the metabolism (*4*, *16*, *17*) and health benefits (*9*, *11*−*13*) of phenolic acids have been widely documented, far less is known about their absorption, in particular, that of CIN and COU. Substantial absorption of phenolic acid was indirectly shown by Nutley et al. (*17*), who described a rapid increase in the level of hippuric acid and some minor phenolic compounds in the urine of rats and mice after oral or intraperitoneal CIN administration. Fahelbum and James (*16*) concluded that CIN was rapidly and extensively absorbed in rabbits and rats and then excreted in urine in the form of hippuric acid, its major metabolite. However, in these studies, the absorption site of CIN was not investigated. Ader et al. (*18*) and Wolffram et al. (*19*) followed CIN uptake by the jejunal mucosa of rats using radioactively labeled CIN and an in vitro mucosal uptake technique. In addition to diffusional uptake, they reported that a Na^+-dependent, saturable transport mechanism was involved in the movement of CIN across the jejunal brush-border membrane. Konishi et al. (*20*, *21*) demonstrated the active absorption of CIN and COU across Caco-2 cell monolayers via a proton-coupled carrier. However, neither the absorption of

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CIN throughout the digestive tract nor the sites of absorption and the renal elimination of COU have been studied.

The aim of this study was to investigate the sites of absorption of CIN and COU across membranes of the digestive tract organs in rats (stomach, duodenum, jejunum, ileum, cecum, and colon) and their urinary elimination. The experiments were conducted using in vitro gastrointestinal sac techniques and following urinary excretion after in vivo oral administration.

MATERIALS AND METHODS

Chemicals. Krebs Henseleit modified buffer, *p*-aminobenzoic acid, and NaCl were purchased from Sigma Chemical Co. (St. Louis, MO); diethyl ether, glucose, and hydrochloric acid were supplied by Acros Organics (Morris Plains, NJ). CIN, COU, and disodium hydrogen citrate sesquihydrate buffer were provided by Fluka Chemical Corp. (Ronkonkoma, NY).

Animals. Adult male Wistar rats ($n = 30$; Elevage Dépré, St. Doulchard, France) weighing 300 ± 20 g at the beginning of the experiment were used. They were housed for an acclimation period of 6 days in a temperature-controlled room (22 ± 3 °C) and maintained on a 12 h light/12 h dark cycle (lights on from 8:00 a.m. to 8:00 p.m.). Animals had free access to a commercial pelleted standard diet (A04, lot 21206, UAR, Epinay-sur-Orge, France) and tap water. All care and handling of animals were performed with the approval of the Institutional Authority for Laboratory Animal Care.

In Vitro Studies. *Gastrointestinal Sac Preparation*. Twenty animals were fasted for 24 h and anesthetized with an intraperitoneal injection of ketamine (Imalgene 1000, Merial laboratory, Lyon, France) at a dose of 0.15 g/kg of body weight and a subcutaneous injection of lidocaine (Xylocaïne, AstraZeneca laboratory, Rueil-Malmaison, France) at a dose of 0.04 g/kg of body weight. Different parts of the digestive tract were quickly removed: stomach, duodenum (between pylorus and ligament of Treitz), a segment of 10 cm from the mid-jejunum, ileum, cecum, and a segment of 10 cm from the colon beyond the cecum. The mucosal and serosal sides of each organ were washed with 10 mL of 0.9% NaCl (maintained at 37 °C), dried with absorbent paper, and weighed. One end of each organ was ligated, and glucose, CIN, and COU alone or supplemented with *p*-aminobenzoic acid were introduced in 2 mL of disodium hydrogen citrate sesquihydrate buffer (0.1 mol/L, pH 5.5, 37 °C) on the mucosal side through the other end. The second end was then ligated. Gastrointestinal sacs were immediately transferred into a tissue chamber containing 10 mL of warmed (37 °C), oxygenated (95% O_2/5% CO_2) Krebs Henseleit modified buffer [KHMB, composition in millimoles per liter, 118.1 NaCl, 4.7 KCl, 2.2 $CaCl_2 \cdot 2H_2O$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 glucose (pH 7.5)]. During all experiments, the chamber was closed with a rubber stopper to prevent any evaporation. After a 180 min incubation period, the gastrointestinal sacs were immersed in 5 mL of 0.9% NaCl for 1 min to remove the incubation solution that had remained adherent to the serosal side. They were then dried and weighed, and the mucosal fluid was collected. The organ lumen was rinsed with 5 mL of fresh KHMB.

Viability of the Gastrointestinal Preparations. To verify the integrity and viability of the gastrointestinal sacs, 2 mL of disodium hydrogen citrate sesquihydrate buffer containing glucose (11.1 mmol/L) was introduced on the mucosal side ($n = 4$). The glucose content was measured at 0, 60, 120, and 180 min in both serosal and mucosal fluids. The concentrations of glucose were determined using a Glucose RTU kit (Biomerieux sa, Marcy-l'Etoile, France) according to the manufacturer's instructions. Absorbance was measured at 505 nm with a Beckman Coulter instrument (UV-DU-640, Fullerton, CA).

CIN and COU Absorptions. CIN ($n = 5$) or COU ($n = 5$) were introduced (7 μmol in 2 mL of disodium hydrogen citrate sesquihydrate buffer) on the mucosal side. Samples (0.3 mL) were collected on the serosal side at 0, 15, 30, 60, 90, 120, and 180 min. At the end of experiment, the mucosal fluid was collected and the organ lumen was rinsed. Fluid collections were stored at −20 °C before high-performance liquid chromatography (HPLC) analysis.

The amount of CIN or COU in the organ wall was measured after an extraction step. Briefly, the organs were homogenized in 3 mL of 0.9% NaCl/g of tissue (Ultratturax, 24 000 rpm/min, 5 min, 0 °C). Aliquots of 0.5 mL were acidified to pH 1−2 with 100 μL of 1 mol of hydrochlorhydric acid/L and extracted twice with 8 mL of diethyl ether. The mixture was agitated (2750 rpm/min, 5 min), centrifuged (5000*g* for 10 min at 4 °C), and finally frozen (20 min at −20 °C). The ethereal fraction was removed and dried in a speed-vac (depression 15 Hpa, ramp 3, 35 min, 45 °C). The dried extracts were resuspended with 1 mL of an ethanol/diethyl ether (95/5, v/v) solution before HPLC analysis.

The distribution of CIN or COU among the three compartments (mucosal, serosal, and inside wall) is expressed as the rate of the amounts of CIN or COU recovered at the end of the experiment in each compartment.

Effect of p-Aminobenzoic Acid on CIN and COU Uptake. To investigate the involvement of a MCT-mediated transport, we studied the effect of *p*-aminobenzoic acid on absorption of CIN ($n = 3$) and COU ($n = 3$). CIN or COU solutions (7 μmol in 2 mL of disodium hydrogen citrate sesquihydrate buffer) were supplemented with *p*-aminobenzoic acid (175 mol/L) before introduction on the mucosal side of the various digestive organs. Samples (0.3 mL) were collected on serosal and mucosal sides at 180 min.

In Vivo Experiments. After the acclimation period, 10 animals were housed in individual metabolic cages for 3 days. On the fourth day, food was withheld. After a 24 h fasting period, a single oral dose of CIN (0.233 mmol/kg of body weight diluted in 2 mL of disodium hydrogen citrate sesquihydrate buffer at 0.1 mol/L and pH 5.5) was given to rats. Urine samples were collected every 4 h for 24 h before oral administration and then every 2 h for 8 h and every 4 h for 16 h after oral administration. Upon completion of the collection period, the rats were fed again for 3 days, after which they were deprived of food for 24 h before receiving a single oral dose of COU (0.233 mmol/kg of body weight diluted in 2 mL of disodium hydrogen citrate sesquihydrate buffer at 0.1 mol/L and pH 5.5). Urine was collected as previously described above. Urine samples were stored at −20 °C until they were required for HPLC analysis.

Sample Preparation and HPLC−Diode Array Detection (DAD) Analysis. CIN and COU were measured by HPLC according to a method previously described by Blanquet et al. (*22*). Before HPLC analysis, samples were filtered (GHP membrane, 0.45 μm pore size, Pall Corp., Port Washington, NY). Ten microliters of the filtrate was analyzed on a Lichrospher 100 RP-18 (5 μm) column (125 × 4 mm inside diameter; Merck, Darmstadt, Germany). Elution was performed with a flow rate of 1 mL/min and a gradient of two solvents, A and B, composed of water, methanol, and acetic acid (94.9/5/0.1, v/v/v) and acetonitrile, methanol, and acetic acid (94.9/5/0.1, v/v/v), respectively. The HPLC analysis was started with 90% solvent A and 10% solvent B. After 16 min, solvent B was added, reaching 20% within 2 min. These conditions were maintained for 14 min, and initial conditions were then recovered within 2 min. CIN and COU were detected by UV absorbance at 280 and 314 nm, respectively, and quantified by using standard curves established in 0.9% NaCl (absence of a matrix effect).

Statistical Analysis. Values are presented as means ± the standard error of the mean. Comparisons between groups were performed using the Student's *t* test. All statistical evaluations were performed on a computer using SAS (version 8.1. SAS Institute Inc., Cary, NC). The level of statistical significance was set at $P < 0.05$.

RESULTS

In Vitro Studies. Before studying CIN and COU absorption, we checked the viability of the gastrointestinal preparations. The ratio of the glucose contents in the serosal fluid to those in the mucosal fluid of the stomach, duodenum, jejunum, ileum, cecum, and colon increased with an incubation time of up to 180 min (**Table 1**), showing the viability of the gut sac tissues.

CIN Absorption. Absorption of CIN by the different parts of the rat digestive tract is presented in **Figure 1**. After incubation for the first 15 min, CIN was detected on the serosal side of each organ, showing its rapid absorption by all the digestive compartments. Comparison of absorption profiles testified to the existence of two different behaviors. In the stomach, the

Table 1. Viability of the Gastrointestinal Preparations[a]

incubation time (min)	ratio of glucose concentrations					
	stomach	duodenum	jejunum	ileum	cecum	colon
0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
60	1.2 ± 0.1	2.3 ± 0.2	1.9 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
120	1.4 ± 0.1	2.4 ± 0.2	2.1 ± 0.1	1.2 ± 0.0	1.4 ± 0.1	1.4 ± 0.1
180	1.7 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	1.4 ± 0.0	1.6 ± 0.1	1.8 ± 0.3

[a] Two milliliters of disodium hydrogen citrate sesquihydrate buffer (0.1 mol/L, pH 5.5, 37 °C) containing glucose (11.1 mmol/L) was introduced onto the mucosal side. The glucose content was measured at 0, 60, 120, and 180 min in both the serosal and mucosal fluids. Values represent the means ($n = 4$) ± the standard error of the mean of the ratio of serosal to mucosal glucose concentrations.

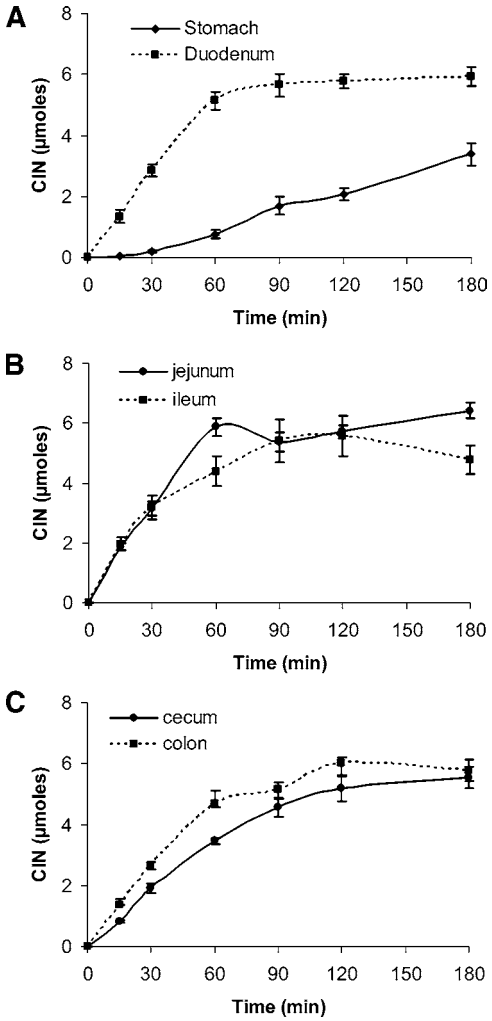

Figure 1. Absorption of CIN across membranes of digestive organs of the rat. CIN (7 μmol) in 2 mL of disodium hydrogen citrate sesquihydrate buffer (0.1 M, pH 5.5, 37 °C) was introduced onto the mucosal side of stomach and duodenum (A), jejunum and ileum (B), and cecum and colon (C). Gastrointestinal sacs were immediately transferred to a tissue chamber containing 10 mL of warmed (37 °C), oxygenated (95% O_2/5% CO_2) Krebs Henseleit modified buffer. Samples (0.3 mL) were collected on the serosal side at 0, 15, 30, 60, 90, 120, and 180 min. Values represent the means ($n = 5$) ± the standard error of the mean.

serosal content increased slowly and linearly over time without reaching a steady state. In all the other organs, an increase in CIN content was observed on the serosal side until it stabilized around 6 μmol. In the duodenum, jejunum, and colon, the steady state was reached 60 min after the beginning of the experiment, versus 90 min in the ileum and cecum. At the end of the experimental period, the ratio of the concentration of CIN in the serosal fluid to the mucosal fluid (**Table 2**) was always greater than 1 (from 1.8 for cecum to 3.4 for colon) except for that for the stomach (0.4). The ratio was reduced by *p*-aminobenzoic acid supplementation (**Figure 2**).

Table 2. Ratio of CIN or COU Concentrations in the Serosal Fluid to the Mucosal Fluid after a 180 min Incubation Period[a]

	ratio of concentrations	
	CIN	COU
stomach	0.4 ± 0.1	0.1 ± 0.0
duodenum	2.7 ± 0.2	0.5 ± 0.1
jejunum	3.2 ± 0.6	0.9 ± 0.2
ileum	2.8 ± 0.4	0.7 ± 0.1
cecum	1.8 ± 0.2	0.4 ± 0.1
colon	3.4 ± 0.3	0.4 ± 0.0

[a] Values represent the means ($n = 5$) ± the standard error of the mean.

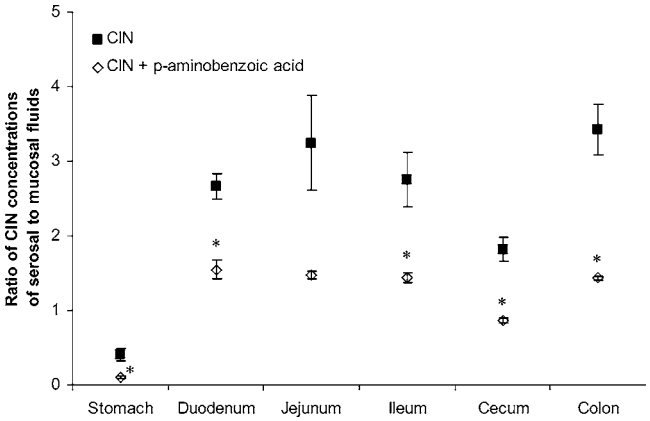

Figure 2. Effect of *p*-aminobenzoic acid on CIN absorption. CIN alone (7 μmol in 2 mL of disodium hydrogen citrate sesquihydrate buffer at 0.1 mol/L, pH 5.5, and 37 °C; $n = 5$) or supplemented with *p*-aminobenzoic acid (175 mol/L; $n = 3$) was introduced onto the mucosal side of the various digestive organs. Samples (0.3 mL) were collected on serosal and mucosal sides at 180 min. Values represent the means ± the standard error of the mean of the ratio of concentrations of serosal to mucosal fluids. Asterisks denote values significantly different from that for CIN alone ($P < 0.05$).

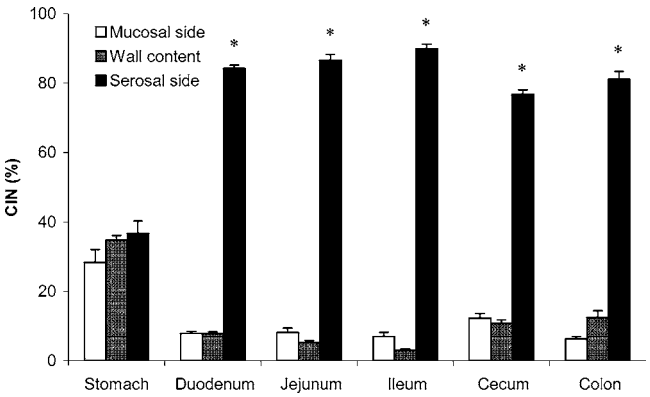

Figure 3. CIN recovered on the mucosal side, on the serosal side, and in wall of digestive organs after a 180 min incubation period. The experimentation was conducted as described in the legend of **Figure 1**. Values represent the means ($n = 5$) ± the standard error of the mean of the rate of the amounts of CIN recovered in the three compartments at the end of the experiment. Asterisks denote values significantly different from mucosal side and wall content of the same organ ($P < 0.05$).

The distribution of CIN among the mucosal side, the serosal side, and the organ wall is presented in **Figure 3**. After a 180 min incubation period, CIN was similarly distributed among

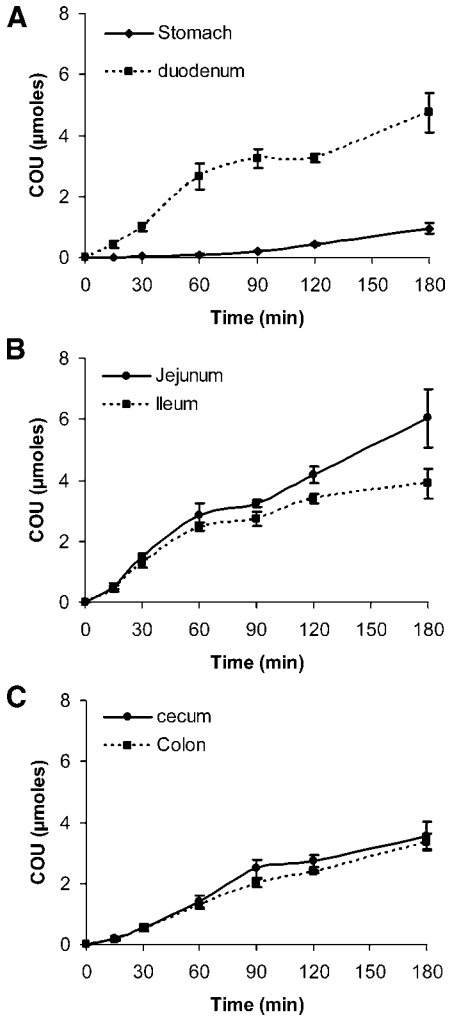

Figure 4. Absorption of COU across membranes of digestive organs of the rat. COU (7 μmol) in 2 mL of disodium hydrogen citrate sesquihydrate buffer (0.1 M, pH 5.5, 37 °C) was introduced onto the mucosal side of stomach and duodenum (A), jejunum and ileum (B), and cecum and colon (C). Gastrointestinal sacs were immediately transferred to a tissue chamber containing 10 mL of warmed (37 °C), oxygenated (95% O_2/5% CO_2) Krebs Henseleit modified buffer. Samples (0.3 mL) were collected on the serosal side at 0, 15, 30, 60, 90, 120, and 180 min. Values represent the means ($n = 5$) ± the standard error of the mean.

the three compartments of the stomach (28.4 ± 3.8% on the mucosal side, 34.9 ± 1.3% in the organ wall, and 36.7 ± 3.6% on the serosal side). In contrast, in all the other organs, CIN was more extensively (>75%) recovered on the serosal side ($P < 0.05$).

COU Absorption. COU was detected on the serosal side 15 min after its introduction into all organs except for the stomach, where absorption began only after 30 min (**Figure 4**). In the ileum, a steady state was reached 1 h after the beginning of the experiment; in all the other organs, we observed a progressive increase in the amount absorbed without reaching a plateau. As previously observed for CIN, the stomach was the weakest absorption site of COU.

After a 180 min incubation period, the ratio of the concentrations of COU in the serosal fluid to the mucosal fluid (**Table 2**) was approximately 4-fold lower than that of CIN (from 0.1 to 0.9). The ratio decreased when *p*-aminobenzoic acid was added (**Figure 5**).

COU detected on the serosal side (**Figure 6**) accounted for only 10% of the recovered dose for the stomach and more than

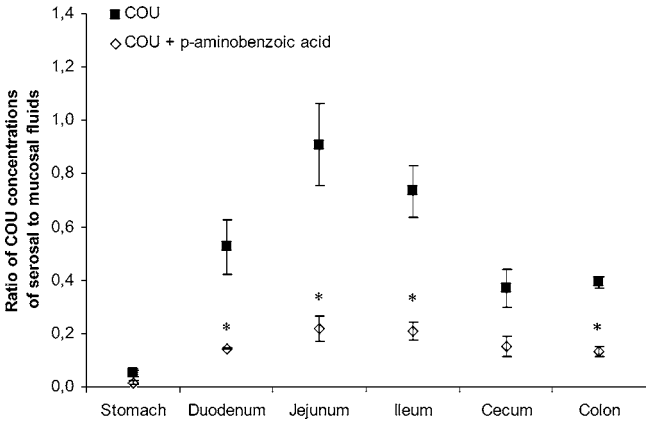

Figure 5. Effect of *p*-aminobenzoic acid on COU absorption. The experimentation was conducted as described in the legend of **Figure 2**. Values represent the means ($n = 3$) ± the standard error of the mean of the ratio of concentrations of serosal to mucosal fluids. Asterisks denote values significantly different from that for COU alone ($P < 0.05$).

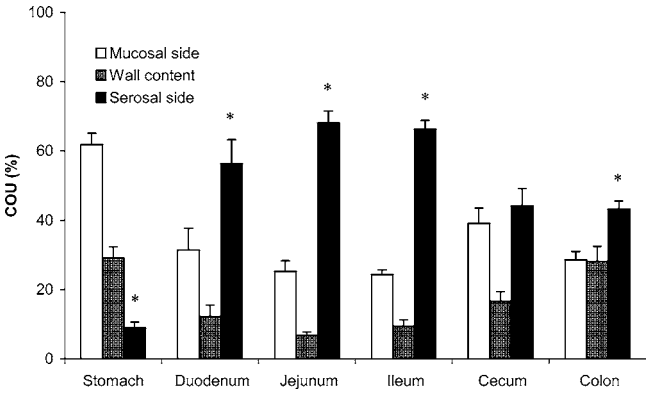

Figure 6. COU recovered on the mucosal side, on the serosal side, and in wall of digestive organs after a 180 min incubation period. The experimentation was conducted as described in the legend of **Figure 3**. Values represent the means ($n = 5$) ± the standard error of the mean of the rate of the amounts of COU recovered in the three compartments at the end of the experiment. Asterisks denote values significantly different from the mucosal side and wall content of the same organ ($P < 0.05$).

40% for all the other organs. Regardless of the organ that was studied, at the end of the experiment, the rates of COU recovered on the serosal side (**Figure 6**) were lower ($P < 0.05$) than those of CIN (**Figure 3**).

In Vivo Experiments. No urinary trace of CIN and COU was detected before the oral administration of either acid (**Figure 7**).

Single Oral Dose of CIN. Following its oral administration to fasted rats, CIN was rapidly excreted and extensively detected in urine samples collected during the first 8 h; thereafter, the amount of CIN decreased until it became undetectable after 20 h (**Figure 7A**). The excreted quantities over 24 h represented only 0.3% of the CIN administered by gavage.

Single Oral Dose of COU. After oral administration, COU was found in the urine (**Figure 7B**) and it was abundantly eliminated during the first 2 h of collection (16.3 ± 2.3 μmol/2 h). Afterward, its rate of excretion decreased quickly (3.2 ± 1.9 μmol eliminated between 2 and 4 h), and COU became undetectable 6 h after gavage. The rate of urinary excretion of COU was much higher than that of CIN representing 24% of the amount administered over 24 h.

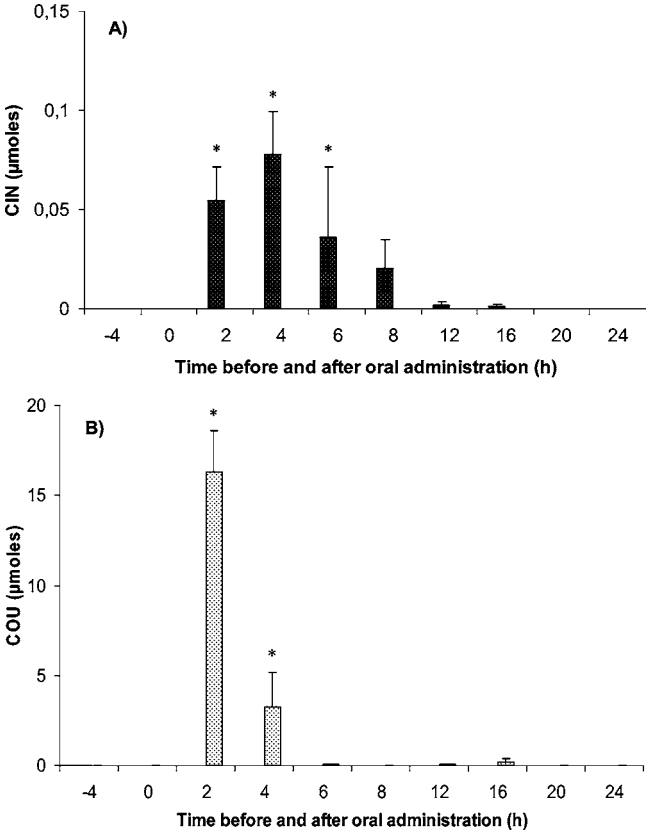

Figure 7. Urinary excretion of CIN (A) and COU (B) after their oral administration to rats. After a 24 h fasting period, a single oral dose of CIN or COU (0.233 mmol/kg of body weight) was given to rats. Urine samples were collected every 4 h for 24 h before oral administration and then every 2 h for 8 h and every 4 h for 16 h after gavage. Values represent the means ($n = 10$) ± the standard error of the mean. Asterisks denote values significantly different from time zero values ($P < 0.05$).

DISCUSSION

Because of their health benefits, the metabolism of phenolic acids, in particular, that of CIN, has been widely studied (*4*, *17*, *23*). Nevertheless, the sites of gastrointestinal CIN absorption are poorly known, and there are few works on COU. In this paper, we investigated the gastrointestinal absorption in rats of CIN and COU using an in vitro technique and their urinary excretion after in vivo oral administration.

In Vitro Intestinal Absorption Studies. The main drawback of in vitro gastrointestinal sacs is tissue viability in the incubation medium. Barthe et al. (*24*) and Chan et al. (*25*) showed that the viability can be maintained for periods of up to 120 min. In our study, the glucose concentrations inside and outside the gastrointestinal sacs were monitored to check sac viability and integrity. The results (**Table 1**) showed that, with an incubation period from 0 to 180 min, the ratio of the concentration of glucose in the serosal fluid to the mucosal fluid increased. The gastrointestinal sacs were thus viable and functional during all the experiments. Indeed, as glucose is actively transported in both the small (*26*) and large intestines (*27*), there is an increase in the external concentration compared to the internal concentration, and hence a concentration gradient. CIN and COU at the tested concentrations did not induce toxicity in the gut sac tissue since the ratio of glucose contents in the serosal side to that in the mucosal side was the same when glucose was introduced alone or supplemented with either CIN or COU (data not shown).

CIN Absorption. The results show a difference between two groups according to the presence or absence of a steady phase in the CIN absorption profile (**Figure 1**). In the first group, composed only of the stomach, the amount of CIN on the serosal side increased throughout the experiment. Gastric absorption was slower and more prolonged than in the other digestive absorption sites. Although only a small amount of acid crossed the gastric wall, we conclude that the stomach, an organ commonly considered a minor absorption site because of its thick wall, does absorb CIN. There have been recent reports of the gastric absorption of various flavonoids (*28*) and especially of ferulic acid (*29*), another hydroxycinnamic acid. At the end of the experimental period, CIN was similarly distributed ($P > 0.05$) among the serosal side, the mucosal side, and the gastric wall (**Figure 3**), but the concentration on the serosal side was much lower than that of mucosal fluid (**Table 2**). A carrier-mediated mechanism seems to be involved since the ratio of CIN concentrations in the serosal fluid to the mucosal fluid was significantly decreased (0.4 vs 0.1, $P < 0.05$) by *p*-aminobenzoic acid supplementation (**Figure 2**). *p*-Aminobenzoic acid is a typical monocarboxylic acid transporter (MCT) substrate (*30*), and our result suggests that CIN is transported via a proton-coupled MCT in the stomach. It has been previously demonstrated that ferulic acid is transported via MCT in the rat stomach (29) and that MCT is expressed in the mouse stomach (*31*).

The second group was composed of all the other digestive organs where CIN was accumulated on the serosal side (**Table 2** and **Figure 3**), suggesting an extensive absorption of CIN by the duodenum, jejunum, ileum, colon, and cecum. Using mucosal uptake techniques, Ader et al. (*18*) and Wolffram et al. (*19*) reported CIN absorption in rat jejunum. Our results demonstrate that the intestinal absorption of CIN may be extended from the duodenum to the colon. We speculate that an active transport process seems to be involved because, after incubation for 180 min, CIN was extensively recovered on the serosal side, whereas only ~10% was found on the mucosal side and in the organ wall ($P < 0.001$). The authors mentioned above established that uptake of CIN by rat jejunum is a Na^+-dependent saturable transport mechanism. However, according to Konishi et al. (*20*), CIN is absorbed across Caco-2 cell monolayers by an active Na^+-independent transport. These authors demonstrated that cinnamic acid is transported across Caco-2 cells via MCT. In our study, the ratio of the concentrations of serosal to mucosal fluids was significantly ($P < 0.05$, except for the jejunum for which $P = 0.08$) decreased by *p*-aminobenzoic acid supplementation (**Figure 2**), an indication that MCT may play a part in CIN absorption. Moreover, in rats, this transporter is distributed throughout the upper and lower intestines (*32*). The aim of our study was to localize the various gastrointestinal absorption sites of CIN and COU rather than to investigate the mechanisms that are involved. Our results underline the likely involvement of an active transport process all along the digestive tract, with the exact active uptake mechanisms in the different parts of the intestine remaining to be investigated.

COU Absorption. COU is absorbed by all the digestive organs. Only a small amount of this acid was detected on the serosal side of the stomach (**Figure 4**), indicating that the stomach is a minor absorption site. Moreover, the amount of COU detected on the gastric serosal side was approximately 3-fold lower ($P < 0.001$) than that of CIN. It is possible to speculate that COU is transported via MCT since the ratio of the concentrations of serosal to mucosal fluids was decreased

(however, not significantly; 0.016 vs 0.052, $P = 0.07$) by *p*-aminobenzoic acid supplementation (**Figure 5**).

In all the other organs, more substantial amounts of COU were recovered on the serosal side after a 180 min incubation, indicating that this acid is absorbed throughout the digestive tract. However, the serosal concentration was lower than that on the mucosal side (**Table 2**), suggesting the existence of a passive transport process which does not achieve equilibrium within 180 min. Nevertheless, the involvement of an active transport process cannot be ruled out. Indeed, Konishi et al. demonstrated that COU is absorbed according to passive and active transports, either in vitro across Caco-2 cell monolayers (*21*) or in vivo by the rat gastrointestinal tract (*33*). In our study, the ratio of the concentrations of serosal to mucosal fluids was significantly decreased ($P < 0.05$, except for the cecum for which $P = 0.07$) by *p*-aminobenzoic acid supplementation (**Figure 5**), indicating that MCT may be involved in COU absorption.

At variance with the results obtained with CIN, the absorption of COU was not completed at the end of the experiment. After a 180 min incubation, the rates for COU on the serosal side (**Figure 6**) were statistically ($P < 0.01$) lower than those for CIN (**Figure 3**). We therefore conclude that the absorption of COU is slower and less efficient than that of CIN in all the organs that were studied. The partition coefficient of dietary polyphenols seems to govern their penetration across the gastrointestinal wall by passive diffusion (*20*). The partition coefficient of COU (1.79) reported by Sangster (*34*) and that of CIN (2.13) reported by Hansch et al. (*35*) suggest a greater solubility in the aqueous environment for COU and, hence, a lower passive absorption rate. Moreover, there are wide variations in the transepithelial active transport of various phenolic acids in Caco-2 cell monolayers. Konishi et al. (*20*) investigated the effects of various derivatives of cinnamic acid on the uptake of fluorescein, a compound transepithelially transported via MCT in Caco-2 cell monolayers. They reported that COU inhibits the transport of fluorescein by only ~15% compared to controls, whereas cinnamic acid inhibits fluorescein permeation by 88% (*20*). Hydroxylation may considerably influence the affinity of various phenolic acids for the carrier, and COU has a lower affinity for MCT than CIN (*20*).

In Vivo Experiments. *Single Oral Dose of CIN*. Following its oral administration to fasted rats, CIN was quickly recovered in urine samples, and its excretion was achieved within 16 h. The amount of CIN recovered in urine was very small, representing only 0.3% of the administered dose. A similar phenomenon has been reported in rats and mice after an oral or an intraperitoneal [^{14}C]CIN administration (*17*). The authors showed that the excretion of radioactivity was rapid and that most of the administered dose was present in the 0−24 h urine. Moreover, unchanged CIN accounted for no more than 0.3% of the radioactivity recovered. The major metabolite was hippuric acid, which accounted for up to 77% of the administered dose. In a number of mammalian species such as rats (*36*) and also rabbits (*16*), dogs (*37*), and humans (*38*), CIN is rapidly and largely converted to hippuric acid before its urinary excretion. This metabolite has no antioxidant activity because it lacks the hydroxyl group (*39*).

Single Oral Dose of COU. As with CIN, we found that after its oral administration, COU was eliminated from blood by renal excretion. In rats, Konishi et al. (*33*) have recently shown that orally administered COU passes rapidly from the gastrointestinal tract into the bloodstream and is distributed to the whole body by the systemic circulation and then eliminated by the liver. For the first time, our results also demonstrate that COU is consistently removed from plasma by the kidney.

The main difference between the urinary excretion of CIN and that of COU resides in the high percentage of the latter recovered in urine. As previously reported (*40*), the quantities of polyphenols found intact in urine vary from one phenolic compound to another, ranging in humans from 1 to 25%. Even if COU urinary excretion is shorter (between 0 and 4 h), the urinary recovery rate of COU is 77 times higher than that of CIN (23% vs 0.3% of the administered dose). We cannot conclude that the bioavailability of CIN is low on the basis of slow urinary elimination alone. However, we indirectly speculate that, in rats, COU is far less metabolized and therefore might be relatively more bioavailable than CIN.

In conclusion, our results indicate that CIN and COU are absorbed by the rat digestive tract and recovered in urine. For the first time, we demonstrate that two phenolic acids are absorbed by all the gastrointestinal organs: stomach, duodenum, jejunum, ileum, cecum, and colon. The lowest rate of absorption was in the stomach. Regardless of the organ that was studied, the rate of COU absorption was lower than that of CIN. In all the digestive organs, we suggest that CIN and COU are partially absorbed across the epithelium via a carrier-mediated transport process, the proton-coupled MCT. After oral administration, the urinary recovery rate of COU was 77 times higher than that of CIN, suggesting that, in rats, COU is less metabolized than CIN. Despite their similar chemical structure, the absorption and metabolism of CIN and COU appear to be different. The physiological importance of phenolic acids in terms of antimicrobial, antifungal, antitumoral, and antioxidant activities depends on their bioavailability for intestinal absorption and their subsequent interaction with target tissues. Because of their broad therapeutic spectrum and their significant absorption across the digestive epithelium, CIN and COU could be used in the future to prevent or treat various diseases. COU could emerge as a better candidate than CIN, since despite weaker gastrointestinal absorption, it seems to be much less well metabolized than the latter.

ABBREVIATIONS USED

CIN, *trans*-cinnamic acid; COU, *p*-coumaric acid; MCT, monocarboxylic acid transporter.

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