Absorption of Chlorogenic Acid and Caffeic Acid in Rats after Oral Administration

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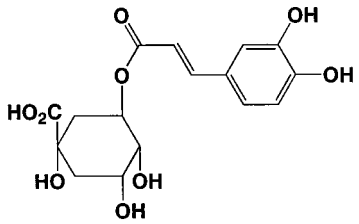
Absorption of orally administered chlorogenic acid (5-caffeoylquinic acid) and caffeic acid in rats was studied to obtain plasma pharmacokinetic profiles of their metabolites. Rats were administered 700 μmol/kg body weight of chlorogenic or caffeic acid, and blood was collected from the tail for 6 h after administration. Ingested caffeic acid was absorbed from the alimentary tract and was present in the rat blood circulation in the form of various metabolites. On the other hand, only traces of metabolites, supposedly caffeic and ferulic acids conjugates, were detected in rat plasma for 6 h after chlorogenic acid administration. Chlorogenic acid and small amounts of caffeic acid were found in the small intestine for 6 h after chlorogenic acid administration. These results suggest that chlorogenic acid is not well absorbed from the digestive tract, unlike caffeic acid, and subject to almost no structural changes to the easily absorbed forms.

Keywords: *Absorption; chlorogenic acid; caffeic acid; rats; blood plasma; metabolites*

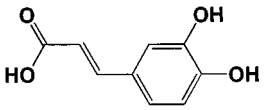
INTRODUCTION

Flavonoids and other phenolics, such as hydroxycinnamic and hydroxybenzoic acid compounds, are widely distributed in the plant kingdom. In recent years, their physiological functionality has attracted much attention and has been studied by many researchers. It has been shown that a number of these phenolics act as antioxidants, with plural mechanisms involving free radical scavenging and metal ion chelation (Terao et al., 1993; Rice-Evans et al., 1995; Meyer et al., 1998). Their antioxidative action could prevent oxidative damages in vivo, relating to various diseases such as cancer, cardiovascular diseases, and diabetes.

Hydroxycinnamic acids, such as *ρ*-coumaric acid (4-hydroxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid, Figure 1), and ferulic acid (4-hydroxy-3-methoxycinnamic acid), are largely derived from fruits, vegetables, grains, and coffee, and may occur in esterified form with quinic acid or glucose (Herrmann, 1989). The most familiar of these are monocaffeoylquinic and dicaffeoylquinic acids, with chlorogenic acid (5-caffeoylquinic acid, Figure 1) being predominant, in particular in fruits. Our recent studies showed that the major antioxidant of *Corchorus olitorius* L. leaves, a vegetable with high antioxidative activity, is chlorogenic acid. The chlorogenic acid content was as high as 380 ± 20 mg/100 g of fresh weight (Azuma et al., 1999a,b). Previously, it was suggested that these hydroxycinnamic acids inhibit carcinogenesis and increase the antioxidant defense system in vivo (Tanaka et al., 1993; Nardini et al., 1997). For chlorogenic acid, inhibitory effects on carcinogenesis in the large intestine, liver, and tongue and protective effects on oxidative stress in vivo have been reported (Mori et al., 1986; Tanaka et al., 1990, 1993; Tsuchiya et al., 1996).



Chlorogenic acid
(5-Caffeoylquinic acid)



Caffeic acid

Figure 1. Chemical structures of chlorogenic acid and caffeic acid.

There is increasing information on the absorption and metabolism of flavonoids in vivo. On the other hand, limited studies have been concerned with the hydroxycinnamic acids, despite their high content in fruits and some vegetables. In particular, very few reports on absorption and metabolism are known for chlorogenic acid, although some studies concerned with caffeic and ferulic acids have been reported (Jacobson et al., 1983; Gumbinger et al., 1993; Bourne and Rice-Evans, 1998; Chesson et al., 1999; Choudhury et al., 1999). The purpose of this study was to investigate the absorption of chlorogenic acid and its related compound, caffeic acid, to provide phamacokinetic profiles of parent compounds and their metabolites in rats.

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MATERIALS AND METHODS

Chemicals. Chlorogenic acid was purchased from Nacarai Tesque Co. (Kyoto, Japan) and caffeic acid from Wako Pure Chemicals (Osaka, Japan). β-Glucuronidase, sulfatase type VIII, and sulfatase type H-5 were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of analytical or HPLC grade.

Animals and Diets. Nine-week-old Wistar male rats weighing 190−200 g were supplied by Japan SLC, Inc. (Hamamatsu, Japan). The animals were kept in an environmentally controlled animal facility operating on a 12 h dark/light cycle at 23 °C and 55% humidity for 6−7 days before experiments, with free access to tap water and standard MF diet (Oriental Yeast Co., Ltd., Japan). All rats were fasted for 14−15 h prior to caffeic or chlorogenic acid administration. Three rats were orally administered 700 μmol/kg caffeic acid in 2 mL of propylene glycol by direct stomach intubation. A second group of three rats was given 700 μmol/kg chlorogenic acid in 2 mL of water, and a third group was given 700 μmol/kg chlorogenic acid in 2 mL of 3 M propylene glycol. Before (control) and after administration, blood samples were collected from the tail vein at each time point, and blood plasma was immediately prepared by centrifugation for 20 min at 4 °C and 1000 *g*. For comparison, 700 μmol/kg chlorogenic acid in 2 mL of 3 M propylene glycol was injected intraperitoneally (ip), and blood plasma samples were prepared as described.

Determination of Chlorogenic, Caffeic, and Ferulic Acids in Plasma. Chlorogenic, caffeic, and ferulic acids were determined by high-performance liquid chromatography (HPLC) after extraction from the blood plasma. To 50 μL of plasma were added 50 μL of 0.1 M sodium acetate buffer, pH 5.0, and 900 μL of methanol/acetic acid (100:5, v/v). The mixture was vortexed for 30 s, sonicated for 30 s, again vortexed for 30 s, and centrifuged for 5 min at 4 °C and 5000 *g*. The supernatant was diluted with water (1:1, v/v), and 20 μL was injected onto an HPLC column. The HPLC conditions for plasma samples after caffeic acid administration were as follows: column, TSKgel ODS-80Ts (5 μm, 4.6 × 150 mm, TOSOH, Japan); mobile phase, methanol/water/acetic acid (25:73:2, v/v/v) containing 50 mM lithium acetate; and flow rate, 0.8 mL/min (analysis A). Plasma samples after chlorogenic acid administration were analyzed under the following conditions: column, CAPCELL PAK C18 MG (5 μm, 4.6 × 150 mm, Shiseido, Japan); mobile phase, methanol/water/acetic acid (20:78:2, v/v/v) containing 50 mM lithium acetate; and flow rate, 0.8 mL/min (analysis B). Elution was monitored with an electrochemical detector (Coulochem II, ESA, Bedford, MA) with the first electrode potential of +100 mV and second electrode potential of +800 mV. Chlorogenic acid (retention volume: 4.8 mL in analysis B), caffeic acid (6.4 mL in analysis A, 9.1 mL in analysis B), and ferulic acid (14.0 mL in analysis A, 20.7 mL in analysis B) were quantitatively determined by an external standard method. The detection limits for chlorogenic, caffeic, and ferulic acids were 0.05, 0.05, and 0.1 μM, respectively, with linear detector response up to 20 μM. When necessary, samples were diluted with mobile phase before HPLC analysis.

Enzymatic Hydrolysis of Conjugates of Chlorogenic, Caffeic, and Ferulic Acids and Determination of Their Concentration in Plasma. *Glucuronides.* Plasma (50 μL) was mixed with 50 μL of β-glucuronidase solution in 0.1 M sodium acetate buffer, pH 5.0, containing 50 units of enzyme. The mixture was incubated at 37 °C for 2 h. Released compounds were extracted and determined by HPLC as already described. The difference in chlorogenic, caffeic, and ferulic acids content after and before incubation was assumed to be the amount of respective glucuronide conjugates in the sample.

Sulfates. Plasma (50 μL) was mixed with 50 μL of sulfatase type VIII solution in 0.1 M sodium acetate buffer, pH 5.0, containing 25 units of enzyme. To prevent hydrolysis by β-glucuronidase present in the sulfatase preparation, 21 mmol/L of D-saccharic acid 1,4-lactone was added to the enzymatic solution as a β-glucuronidase inhibitor (Hackett and Griffiths, 1982). The mixture was incubated at 37 °C for 4 h. The difference in chlorogenic, caffeic, and ferulic acids content after and before incubation was assumed to be the amount of respective sulfate conjugates in the sample.

Sulfate/Glucuronides. Plasma (50 μL) was mixed with 50 μL of sulfatase type H-5 solution in 0.1 M sodium acetate buffer, pH 5.0. This sulfatase preparation contained 500 units of β-glucuronidase and 25 units of sulfatase. The mixture was incubated at 37 °C for 50 min. The amount of chlorogenic, caffeic, and ferulic acids after subtracting those before incubation and released by β-glucuronidase and sulfatase type VIII from those during this incubation was defined as the amount of sulfate/glucuronide conjugates in the sample.

Isolation and Mass Spectral Analysis of Caffeic Acid Metabolites. Three rats were orally administered 1.2 mmol/kg of caffeic acid in 2 mL of propylene glycol by direct stomach intubation; 2 h later, blood was taken from the heart while the rats were under diethyl ether anesthesia, and plasma was prepared by centrifugation for 20 min at 4 °C and 1000 *g*. Caffeic acid metabolites in plasma were hydrolyzed with sulfatase H-5 as already described. Compounds released during incubation were extracted with methanol, and methanolic extracts were centrifuged for 10 min at 4 °C and 5000 *g*. Then, the supernatant was evaporated completely. Three fractions were trimethylsilylated with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetoamide at 60 °C for 30 min. The derivatized samples were analyzed using a HP 5989B MS-system equipped with a HP 5890 series II GC-system (Hewlett-Packard, Palo Alto, CA) fitted with a capillary column DB-1 (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA) attached with a retention gap column (5 m × 0.25 mm i.d., Hewlett-Packard) at an ionization voltage of 70 eV. Helium carrier gas flow rate was 1 mL/min, in the constant flow mode. The temperature program was started at 60 °C for 2 min, then raised to 210 °C at 30 °C /min, raised to 280 °C at 2 °C /min, and finally held at 280 °C for 5 min. Temperatures of the injection port, the separator, the ion source, and the analyzer were 220, 280, 250, and 100 °C, respectively.

Determination of Chlorogenic Acid and Its Metabolites in Small Intestine. Rat small intestine 1, 2, 4, and 6 h after oral administration of 700 μmol/kg chlorogenic acid was taken from three rats per one group. These specimens were cut in 15 mL of physiological saline and homogenized with an Ultra-Turrax T25 homogenizer (Janke & Kunkel IKA-Labortechnik, Germany) at 4 °C for 1 min. After centrifugation at 5000 *g* for 10 min, chlorogenic acid and its metabolites in the supernatants were quantitatively determined by HPLC as already described.

RESULTS

Quantitative Changes of Caffeic Acid and Its Metabolites in Rat Plasma. A typical chromatographic profile of rat plasma is shown in Figure 2. Enzymatic treatment released two compounds that eluted at 8.0 min (compound **I**) and 17.5 min (compound **II**). Based on a comparison of mass spectra and retention times with those of authentic compounds on gas chromatography−mass spectrometry (GC−MS) analysis, **I** and **II** were identified as caffeic acid and ferulic acid, respectively. Retention times and characteristic ions of these trimethylsilylated derivatives were as follows: Compound **I**: retention time, 12.6 min, *m/z* 396 (100) (M^+), 381 (28), 327 18), 307 (12), 293 (7), 249 (9), 219 (91), 191 (14), 145 (9), 132 (14), 129 (13), 117 (28), 75 (21), 73 (73); compound **II**: retention time, 11.7 min, *m/z* 338 (100) (M^+), 323 (69), 308 (63), 293 (42), 279 (16), 249 (48), 219 (19), 191 (12), 146 (21), 75 (16), 73 (62). These compounds have the same chromatographic properties as the authentic compounds in HPLC analysis.

By using a combination of enzymatic hydrolysis, free caffeic acid and its six metabolites were quantified. The

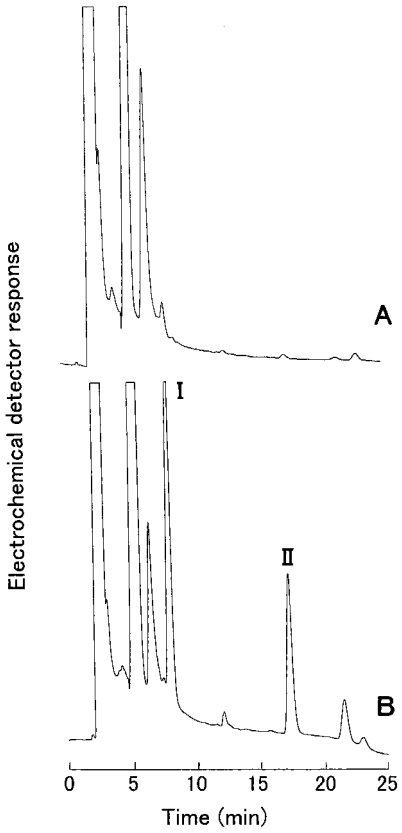

Figure 2. Representative HPLC chromatograms of rat plasma extracts of blood taken (A) before caffeic acid administration and (B) 2 h after caffeic acid administration and hydrolyzed with sulfatase/β-glucuronidase.

metabolites were grouped according to their conjugated moieties, because the number and position of conjugated moieties could not be distinguished (Figure 3). All metabolites reached their maximum concentrations within the first 2 h after administration, and then their plasma level decreased. Free caffeic acid and ferulic acid showed the lowest maximum concentrations of all metabolites; 1.2 ± 0.2 and 1.6 ± 0.2 μM, respectively (Figure 3A). Caffeic acid glucuronides became the main plasma metabolites 2 h after administration, with a concentration of 26.1 ± 3.5 μM, corresponding to 41% of all of the metabolites determined at this time (Figure 3B). The second major metabolite was caffeic acid sulfate/glucuronide conjugates (Figure 3D). All kinds of ferulic acid conjugates were present at relatively low maximum concentrations <10 μM (Figure 3B−D); their total concentration 2 h after administration was ~50% of the total concentration of caffeic acid conjugates. The proportion of ferulic acid conjugates of total metabolites increased to 47% after 6 h from 34% after 2 h.

Quantitative Changes of Chlorogenic Acid and Its Metabolites in Rat Plasma. In rat plasma after administration of 700 μmol/kg chlorogenic acid in 2 mL of water, only small amounts of three metabolites, supposedly caffeic acid glucuronides, caffeic acid sulfates/glucuronides, and ferulic acid sulfates/glucuronides, were observed. However, no chlorogenic acid and its conjugates were detected (Figure 4). These metabolites reached their maximum concentrations of 0.12−0.34 μM within the first 0.5−1 h after administration; then their plasma levels decreased. The oral administration using 3 M propylene glycol instead of water as a vehicle also gave similar results.

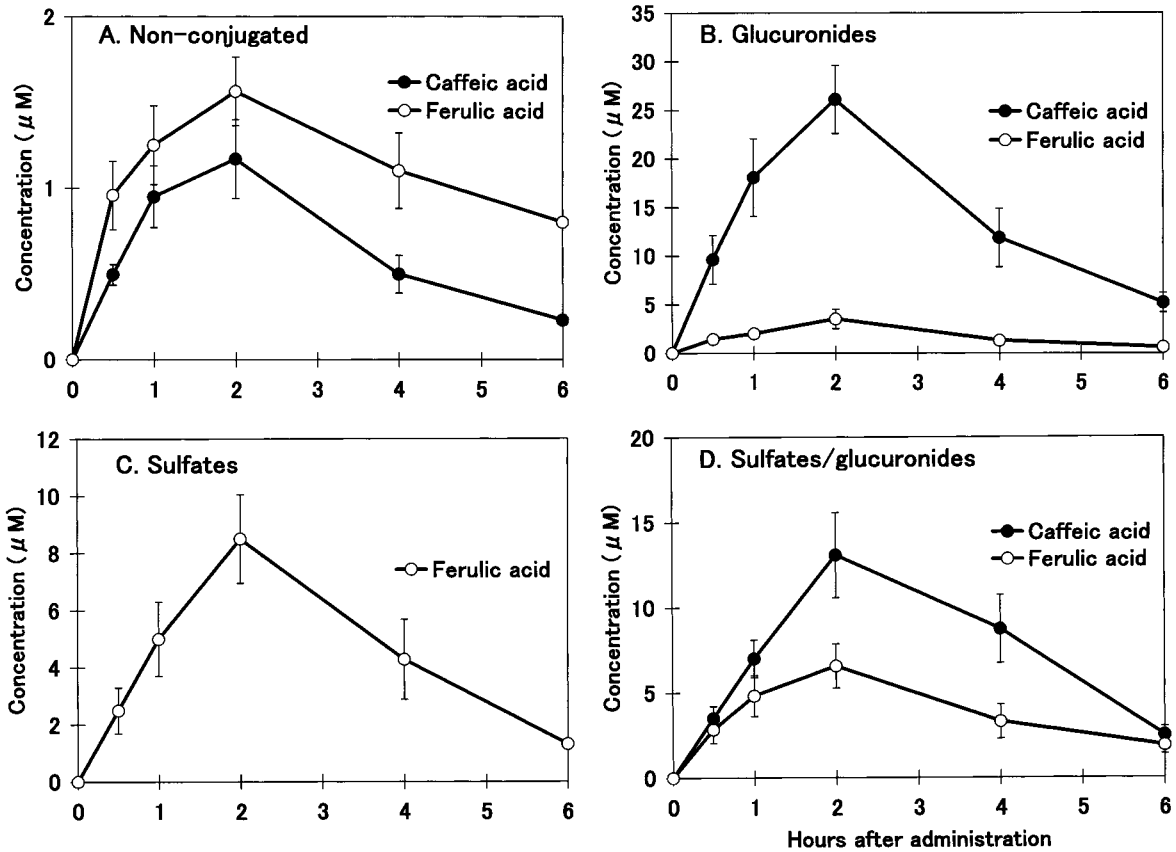

Figure 3. Caffeic acid metabolite concentrations in rat plasma after oral administration of 700 μmol/kg caffeic acid. Values are the mean ± SEM (n = 3).

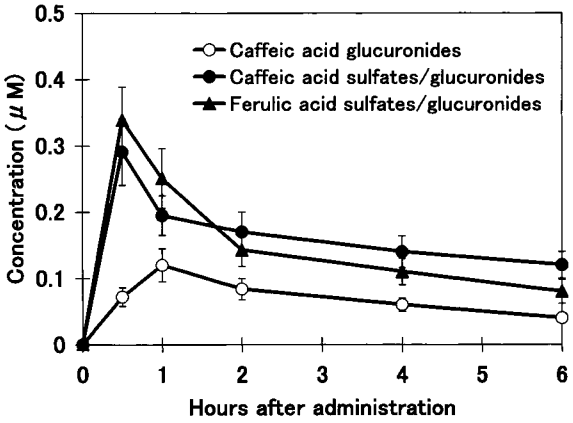

Figure 4. Chlorogenic acid metabolite concentrations in rat plasma after oral administration of 700 μmol/kg chlorogenic acid. Values are the mean ± SEM (n = 3).

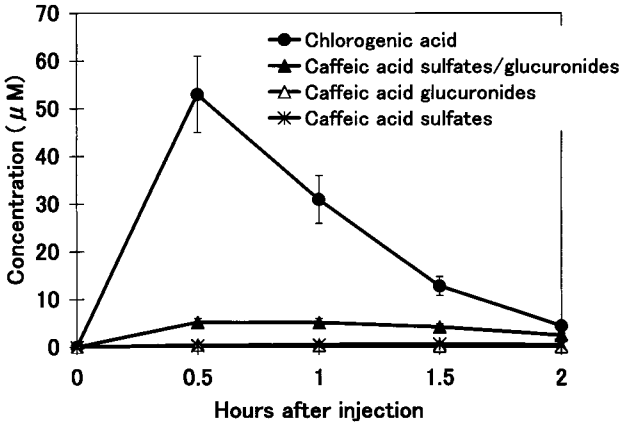

Figure 5. Chlorogenic acid metabolite concentrations in rat plasma after 700 μmol/kg chlorogenic acid was injected intraperitoneally. Values are the mean ± SEM (n = 3).

Table 1. Composition of Chlorogenic Acid and Its Metabolites in Rat Small Intestine after Chlorogenic Acid Administration

hours after administration	chlorogenic acid	caffeic acid
1	59.5 ± 7.1[a] (99.2%)	0.5 ± 0.1 (0.8%)
2	46.3 ± 6.5 (98.9%)	0.5 ± 0.1 (1.1%)
4	14.2 ± 2.6 (97.9%)	0.3 ± 0.1 (2.1%)
6	7.2 ± 1.3 (98.6%)	0.1 ± 0.0 (1.4%)

[a] Values are the mean ± SEM (n = 3) and are expressed in μmol.

On the other hand, chlorogenic acid was detected with a maximum concentration as high as >50 μM in rat blood plasma after the same amount of chlorogenic acid in 2 mL of 3 M propylene glycol was injected ip to rat (Figure 5). Three kinds of caffeic acid conjugates were also observed in plasma at much lower concentrations compared with chlorogenic acid. Among them, caffeic acid sulfate/glucuronide conjugates were predominant.

To investigate the behavior of chlorogenic acid in the digestive tract, the composition of chlorogenic acid and its metabolites in the small intestine 1, 2, 4, and 6 h after oral administration were determined (Table 1). Chlorogenic acid and small amounts of caffeic acid were detected throughout the experimental period, and the proportion of chlorogenic acid was as high as 98−99 mol %. Forty three percent of chlorogenic acid administered was recovered from the small intestine 1 h after administration.

DISCUSSION

A number of reports on absorption and metabolism of flavonoids have been published (Hollman et al., 1995; Gross et al., 1996; Manach et al., 1997; Hollman and Katan, 1998; Donovan et al., 1999; Miyazawa et al., 1999). Absorbed flavonoids are present in the common blood circulation in the form of glucuronide, sulfate, and methylate conjugates and are excreted via urine or bile. Piskula and Terao (1998a) proposed that the first step of conjugation of dietary flavonoid was glucuronidation, occurring in the intestinal mucosa. After the compound entered the common blood circulation in the glucuronized form, it was sulfated in the liver and methylated in the liver and kidney.

In this study, we investigated absorption of chlorogenic acid, as a nonflavonoid phenolic antioxidant, in comparison with that of caffeic acid. Almost all of the caffeic acid metabolites found in rat plasma were in the form of glucuronide, sulfate, and sulfate/glucuronide conjugates of caffeic acid or its methylated compound, ferulic acid (Figure 3). The presence of nonconjugated caffeic acid and ferulic acid might be due to overdosing of the animals, as discussed for quercetin absorption by Piskula and Terao (1998b). The quantitative changes of the particular metabolites were similar to pharmacokinetic profiles of quercetin and (−)-epicatechin metabolites, although there were some differences in absorption rate and composition of metabolites. (Piskula and Terao, 1998a,b). It is assumed that ingested caffeic acid, absorbed from the alimentary tract, may be metabolized through the same pathway as that proposed for flavonoids.

On the other hand, the results presented here (Figures 4 and 5) indicate that it might be very difficult to absorb chlorogenic acid from the alimentary tract, although this compound easily enters blood vessels after ip injection and is then partly metabolized. It is known that ingested flavonoid glycosides can be at least in part hydrolyzed to flavonoid aglycones by the intestinal microflora in the digestive tract before being absorbed. From our results that almost all of ingested chlorogenic acid remained intact in the small intestine (Table 1), it is suggested that the intestinal mucosa or intestinal microflora may not possess esterase activity that is able to cleave chlorogenic acid into caffeic acid and quinic acid. Previously, esterase activity has been observed in human fecal extracts, but not in human small intestine, liver, and plasma extracts (Plumb et al., 1999). There is a possibility that the hydrolysis of chlorogenic acid occurs by the action of esterases in colonic microflora and that the resulting caffeic acid can be absorbed in part. Although the reason intact chlorogenic acid may not be absorbed from the alimentary tract has not been clarified in this study, we suppose that the quinic acid moiety in chlorogenic acid structure could be one of factors inhibiting chlorogenic acid absorption.

There is increasing interest in chlorogenic acid because it is one of the important phenolic antioxidants that are widespread in plant food. Further investigations are needed to find the conditions for its absorption and to clarify its physiologic activity in the digestive organs.

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