0090-9556/99/2701-0486–494$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 27, No. 1
Printed in U.S.A.

BIOTRANSFORMATION OF CURCUMIN THROUGH REDUCTION AND GLUCURONIDATION IN MICE

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(Received March 17, 1998; accepted December 10, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

Curcumin, the yellow pigment in turmeric and curry, has antioxidative and anticarcinogenic activities. In this study, we investigated the pharmacokinetic properties of curcumin in mice. After i.p. administration of curcumin (0.1 g/kg) to mice, about 2.25 μg/ml of curcumin appeared in the plasma in the first 15 min. One hour after administration, the levels of curcumin in the intestines, spleen, liver, and kidneys were 177.04, 26.06, 26.90, and 7.51 μg/g, respectively. Only traces (0.41 μg/g) were observed in the brain at 1 h. To clarify the nature of the metabolites of curcumin, the plasma was analyzed by reversed-phase HPLC, and two putative conjugates were observed. Treatment of the plasma with β-glucuronidase resulted in a decrease in the concentrations of these two putative conjugates and the concomitant appearance of tetrahydrocurcumin (THC) and curcumin, respectively. To investigate the nature of these glucuronide conjugates in vivo, the plasma was analyzed by electrospray. The chemical structures of these metabolites, determined by mass spectrometry/mass spectrometry analysis, suggested that curcumin was first biotransformed to dihydrocurcumin and THC and that these compounds subsequently were converted to monoglucuronide conjugates. Because THC is one of the major metabolites of curcumin, we studied its stability at different pH values. THC was very stable in 0.1 M phosphate buffers of various pH values. Moreover, THC was more stable than curcumin in 0.1 M phosphate buffer, pH 7.2 (37°C). These results, together with previous findings, suggest that curcumin-glucuronoside, dihydrocurcumin-glucuronoside, THC-glucuronoside, and THC are major metabolites of curcumin in vivo.

The use of medicinal plants or their active components in the prevention and treatment of chronic diseases is based on experience from traditional systems of medicine from various ethnic societies. During the past decade, a large number of natural products and dietary components have been evaluated as potential chemopreventive agents (Sharma et al., 1994). The application of chemopreventive agents to cancer prevention and control is attractive because conventional therapy alone has not been fully effective in combating either the high incidence or the low survival rate of several forms of cancer (Boone et al., 1990; Rao et al., 1995). Polyphenols, a class of dietary compounds, currently are being evaluated intensively as potential cancer chemopreventive agents (Stoner and Mukhtar, 1995). *Curcuma longa linn* is one of several medicinal plants to have attracted the interest of scientists. The powdered rhizome of this plant, turmeric, is used extensively to color and flavor foods. Its yellow color is imparted primarily by curcumin (diferuloyl methane), a polyphenolic pigment (Cooper et al., 1994) (Fig. 1). Although curcumin is thought to have chemopreventive properties, its disposition after ingestion has not been fully characterized.

Several studies have addressed the absorption, metabolism, and tissue distribution of curcumin after oral administration of 400, 80, and 10 mg of [^{3}H]curcumin in rats (Ravindranath and Chandrasekhara, 1980, 1981, 1982). Measurements of plasma levels with radioactivity have shown that curcumin is poorly absorbed from the gut. These studies indicated that curcumin is transformed during absorption from the intestines, and the transformed product(s), which is more polar and colorless than curcumin, enters the serosal side. Holder et al. (1978) reported that although some curcumin was found in bile after i.v. administration of 50 mg/kg [^{3}H]curcumin in rats, the majority of radioactivity in the bile was present in glucuronide conjugates of tetrahydrocurcumin (THC)[1] and hexahydrocurcumin. None of the previous studies, however, used HPLC to detect curcumin or its derivatives in plasma, so low levels might have been missed.

Curcumin is unstable at neutral and basic pH values and is degraded to ferulic acid ([4-hydroxy-3-methoxycinnamic acid]) and feruloylmethane (4-hydroxy-3-methoxycinnamoyl-methane) (Wang et al., 1997). In the course of our investigations, we found that more than 90% of curcumin decomposes rapidly in buffer systems at neutral-basic pH conditions. Oetari et al. (1996), using spectrophotometry,

This study was supported by grants from the National Science Council (NSC 86–2621-B-002–008-Z and NSC 86–2314-B-002–04), the National Health Research Institute (DOH 86-HR-403), and the National Research Institute for Chinese Medicine (NRICM-87102).

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[1] Abbreviations used are: THC, tetrahydrocurcumin; UGT, UDP-glucuronosyltransferase UDPGT, uridine diphosphoglucuronsyltransferase; CID, collision-induced dissociation; TLC, thin-layer chromatography; MS/MS, mass spectrometry/mass spectrometry.

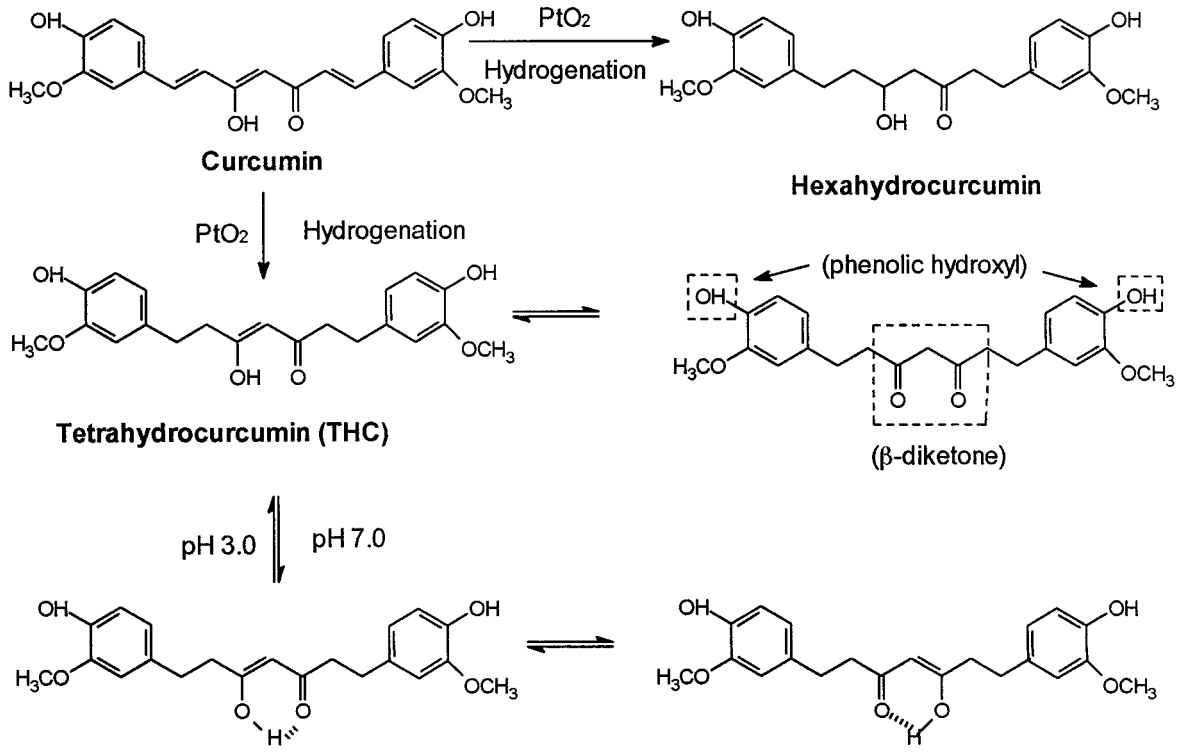

FIG. 1. *Preparation of THC and hexahydrocurcumin from curcumin by hydrogenation with PtO_2 as the catalyst and intramolecular hydrogen bonding of THC.*

also found that curcumin is unstable in phosphate buffer at pH 7.4. Although the exact mechanism of degradation is not clear yet, it appears to occur through an oxidative mechanism because the presence of antioxidants such as ascorbic acid, *N*-acetylcysteine, or glutathione completely blocks the degradation of curcumin at pH 7.4. Curcumin should be stable in the stomach and small intestines because the pH is between 1 and 6, and degradation of curcumin is extremely slow in these conditions (Wang et al., 1997). The effect of pH on metabolites of curcumin has not been investigated previously.

Thus, although the pharmacokinetic properties of curcumin have been investigated many questions remain as to its fate after administration. To gain further insight into its metabolism, we examined the biotransformation of curcumin and characterized the structures of its metabolites after oral or i.p. administration in mice. We used a sensitive HPLC assay technique so that even small amounts of curcumin and its metabolites could be detected. Because THC is a major metabolite of curcumin, we also investigated its stability in buffer solutions of various pH values.

Materials and Methods

Chemicals. Curcumin (from *Curcuma longa*, turmeric) and sulfatase-free β-glucuronidase (type IX-A from *Escherichia coli*) were purchased from Sigma Chemical Company (St. Louis, MO). HPLC grade methanol, acetonitrile, tetrahydrofuran, acetic acid, citric acid, dimethyl sulfoxide, and other chemicals used in buffer systems were purchased from E. Merck Chemical Co. (Darmstadt, Germany). Synthetic curcumin was provided by Yung Shin Pharmaceutical Ind. Co. (Ta-Cha, Taiwan). Buffer solutions of pH 3 to pH 10 were prepared as described previously (Stoll and Blanchard, 1990).

Animals. Female BALB/c mice (18–20 g, 6–7 weeks old) purchased from the Animal Center of National Taiwan University Hospital (Taipei, Taiwan, Republic of China) were used in this study. Unless otherwise stated, the standard food pellets (Laboratory Rodent Diet; PMI Feeds, Inc., St. Louis, MO) and tap water were given ad libitum. Mice were assigned to receive either oral or i.p. administration of curcumin and were sacrificed at various times after administration (five mice per group). For the oral administration experiments, curcumin was emulsified in 2.5% carboxymethyl cellulose and 20 ml/kg (1 g/kg) was administered by gavage. For the i.p. administration experiments, curcumin was dissolved in dimethyl sulfoxide and given to mice in a dose of 4 ml/kg (0.1 g/kg) i.p. injection.

Preparation of Curcumin Derivatives. Curcumin was converted to THC and hexahydrocurcumin by hydrogenation, with PtO_2 as the catalyst, according to the method of Uehara et al. (1987). Thin-layer chromatography (TLC) analysis on silica gel plates (Silica Gel 60 F_{254}, 20 × 20 cm; thickness, 2 mm; catalog 5717; E. Merck) was performed in a rectangular or cylindrical glass chamber covered with a glass plate. The inner side of the chamber contained Whatman no. 1 filter paper to prompt and ensure the saturation of solvent vapor in the chamber. After hydrogenation, THC was purified by preparative TLC (4% ethanol in $CHCl_3$, R_f = 0.83) and the appropriate fractions were collected and combined. The identity and purity of THC were confirmed by examining the mass spectrometry (MS), UV, and NMR spectra. THC appeared as off-white crystals with a melting point of 98–99°C. NMR analysis in $CDCl_3$ (^{1}H NMR) revealed: δ 2.57 (4H, *t*, *J* = 2 Hz), 2.81(4H, *t*, *J* = 2 Hz), 3.78 (6H, *s*, 2[time] OMe), 5.63 (1H, *s*), 6.64 (2H, *s*), 6.71 (2H, *s*), and 6.81 (2H, *q*, *J* = 7.2 Hz); analysis: calculated for $C_{21}H_{24}O_6$, C, 67.74; H, 6.45; found: C, 67.52; H, 6.68.

HPLC Analysis of Curcumin and THC. HPLC was performed with a Jasco liquid chromatograph equipped with a PU 980 intelligent pump and a variable-wavelength UV-975 UV/vis detector (JASCO Co., Tokyo, Japan). The recorder, a Waters 745B data module, and a Waters Nova-Pak C_{18} column (150 × 3.9 mm, 5-μm particle size) were used. The mobile phase consisted of 40% THF and 60% water containing 1% citric acid, adjusted to pH 3.0, with concentrated KOH solution (v/v), which is the procedure described by Cooper et al. (1994). The system was run isocratically at a flow rate of 1 ml/min. Sample detection was achieved at 420 nm, and injection volumes were 20 μl. Calibration curves over the range of 0.2 to 20 μM were established for the quantitation of curcumin. This HPLC method offered a detection limit of 5 ng/ml.

The concentrations of THC and the biotransformed derivatives of curcumin were estimated on a LiChrospher C_{18} column (250 × 4 mm, 5-μm particle size; Merck). The mobile phase consisted of 10% THF, 30% acetonitrile, and 1% acetic acid in water (v/v) and was adjusted to pH 3.0, 3.5, 4.0, 5.0, 6.0, or 7.0 with concentrated KOH solution. The system was run isocratically at a

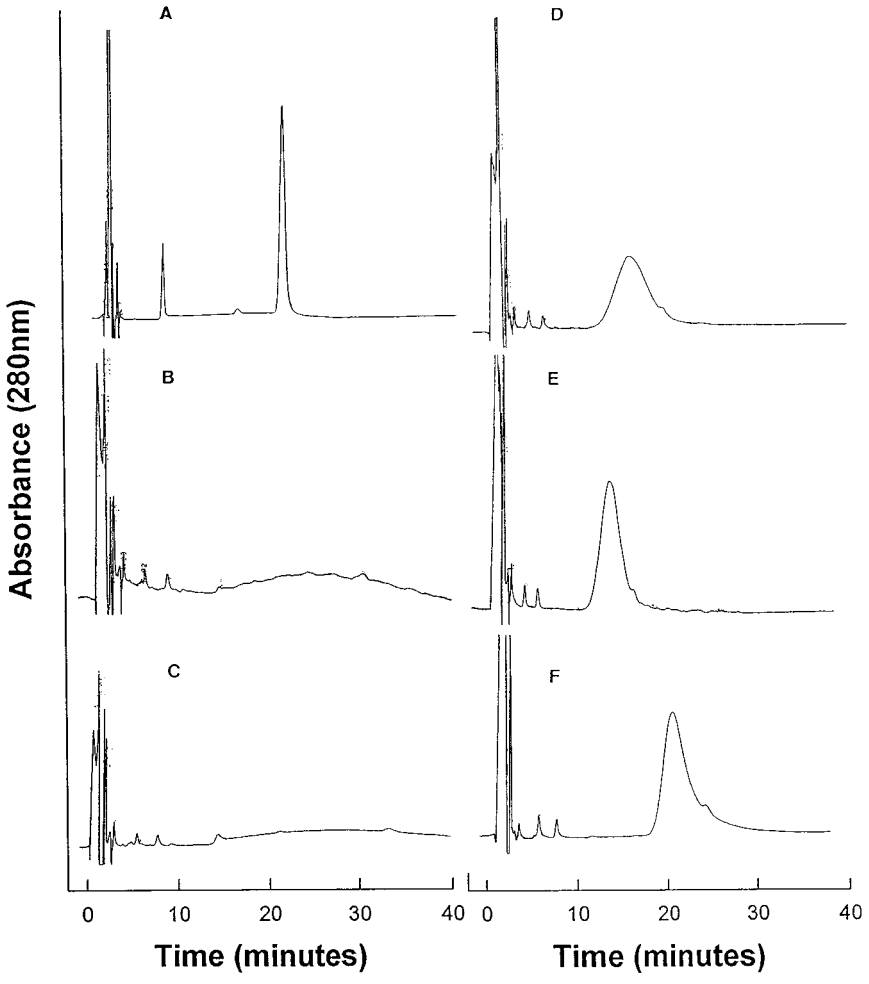

FIG. 2. *HPLC analysis of THC.*

THC concentration was estimated on a LiChrospher C_{18} column (250 × 4 mm, 5-μm particle size; Merck). The mobile phase was 10% THF, 30% acetonitrile, and 1% acetic acid in water (v/v) and was adjusted to pH 3.0 (A), 3.5 (B), 4.0 (C), 5.0 (D), 6.0 (E), and 7.0 (F), respectively, with concentrated KOH solution; flow rate, 1.0 ml/min.

flow rate of 1 ml/min. Sample detection was achieved at 280 nm, and injection volumes were 20 μl. Chromatographic peaks of incubation samples were identified by spiking with corresponding authentic standards.

Pharmacokinetic Studies in Mice. The pharmacokinetic properties of curcumin after a single oral or i.p. dose were studied in 6- to 7-week-old female mice. Curcumin was given orally or i.p. at a dose of 1 or 0.1 g/kg, respectively, and the mice were sacrificed at various intervals after administration. Plasma was obtained from blood (treated with 0.2 mg/ml heparin) by centrifugation at 4300*g* for 10 min, subsequently acidified to pH 3.0 with 6 N HCl, and extracted two times with equal volumes of ethyl acetate/propanol (9:1, v/v). The extraction recovery from plasma was approximately 95%. The plasma samples were separated by centrifugation at 5000*g* for 10 min in a desktop centrifuge and filtered through 0.22 μM polyvinylidene difluoride membrane filters. Sample detection was achieved at 420 nm, and injection volumes were 20 μl.

Tissue Distribution Studies. Tissues and blood samples of mice were obtained 1 h after i.p. administration of curcumin. The tissues were removed and then minced with scissors and homogenized in 3 volumes of phosphate buffer (pH 3.0) with a polytron. Plasma was collected from heparinized blood samples by centrifugation at 4300*g* for 10 min. An aliquot of homogenate or plasma then was transferred to a centrifuge tube with a glass stopper, acidified to pH 3.0 with 6 N HCl, and extracted with equal volumes of ethyl acetate/propanol (9:1, v/v) by mechanical shaking for 6 min. Samples were centrifuged at 5000*g* for 20 min, and the top layer was used for the determination of curcumin by HPLC. The extraction recovery from plasma was approximately 95%. The levels of curcumin in the kidneys, spleen, liver, brain, and intestine homogenates were analyzed similarly.

Metabolite Isolation and Identification. Curcumin was given i.p. at a dose of 0.1 g/kg in 1 h. Metabolites were identified by comparison of their HPLC retention times and MS spectra with those of known standards. Curcumin conjugates first were hydrolyzed by incubation of plasma with β-glucuronidase (1000 U) at 37°C for 30 min. Samples then were acidified to pH 3.0 with 6 N HCl and extracted with equal volumes of ethyl acetate/propanol (9:1, v/v) by mechanical shaking for 6 min. Samples then were centrifuged at 5000*g* for 20 min and the top layer was used for the determination. The extraction recovery from plasma was approximately 95%. Treatment of the curcumin conjugates with β-glucuronidase caused their HPLC peaks to shift to the positions of the unconjugated molecules, so the hydrolysis products could be identified by comparison with known standards. The chemical structures of the conjugates were also identified by MS, as described below.

MS Analysis of Prepared Plasma Samples. Mouse plasma samples were acidified to pH 3.0 with 6 N HCl and extracted two times with equal volumes of ethyl acetate/propanol (9:1, v/v). The organic layers were combined, dried under a stream of nitrogen, dissolved in CH_3OH/H_2O, (1:1, v/v) containing 1% acetic acid, and filtered through a 0.22-μm polyvinylidene difluoride membrane filter. The extraction recovery was approximately 95%. For the less abundant metabolites of curcumin, it was necessary to fractionate the enzyme-treated preparations by HPLC to obtain more homogenous and concentrated specimens for MS/MS analysis. MS/MS analysis of plasma was performed on a Finnigan TSQ 7000 tandem quadruple mass spectrometer (Finnigan MAT, San Jose, CA) operated in the positive ion electrospray mode. For off-line analysis, the sample was injected with an infusion carrier solution [CH_3OH/H_2O, containing 1% (v/v) CH_3CO_2H, 50/50], delivered at a flow rate of 3 μl/min by a syringe pump (Harvard Apparatus, South Natick, MA). The

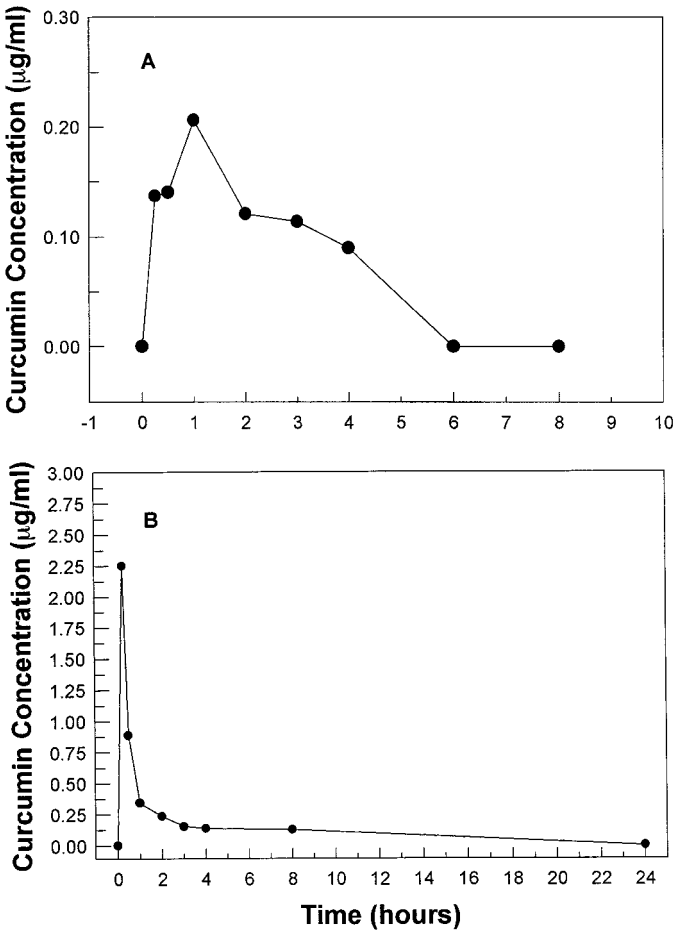

FIG. 3. *Mean plasma concentrations of curcumin in mice after single oral (1 g/kg) curcumin (A) and i.p. (0.1 g/kg) (B) administration of curcumin.*

The concentration of curcumin was determined with HPLC on a Nova-Pak C_{18} column (150 × 3.9 mm, 5-μm particle size; Waters). The mobile phase was 40% THF and 60% water containing 1% citric acid, adjusted to pH 3.0 with concentrated KOH solution (v/v). Each point is the mean of five mice.

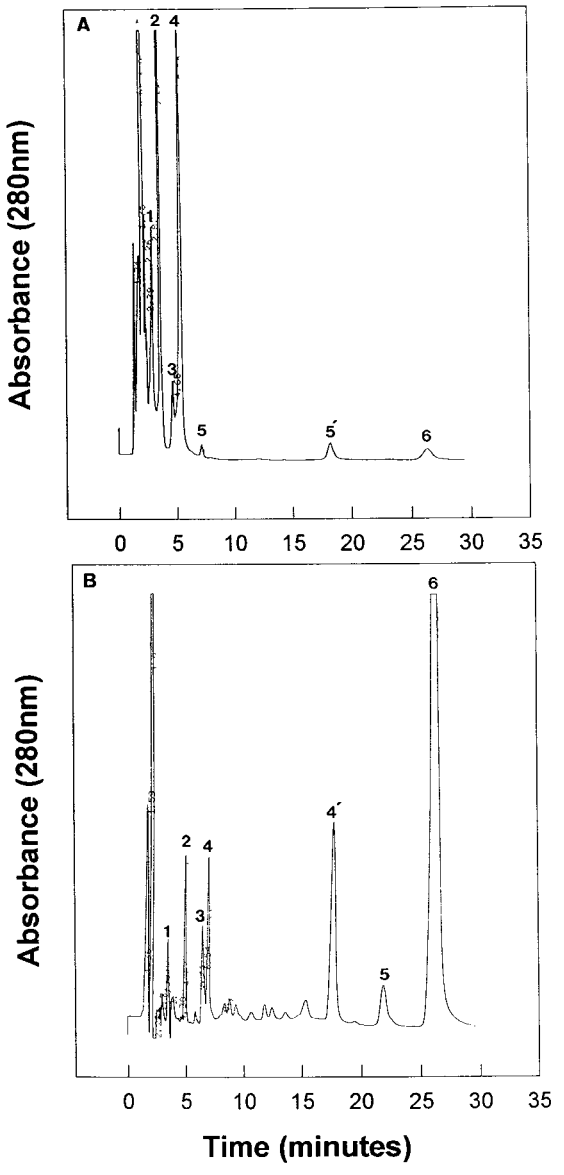

FIG. 4. *β-Glucuronidase hydrolysis of putative curcumin and THC conjugates 1 h after i.p. administration.*

A, plasma was acidified to pH 2.0, extracted twice with ethyl acetate/propanol, and analyzed by reversed-phase HPLC at 280 nm. P1, hexahydrocurcumin-glucuronoside; P2, THC-glucuronoside, P3, dihydrocurcumin; P4, curcumin-glucuronoside; P5 and P5′, THC; P6, curcumin. B, plasma was treated hydrolyzed with β-glucuronidase and extracted as described in A. P1, THC-glucuronoside; P2, curcumin-glucuronoside; P3, hexahydrocurcumin; P4 and P4′, THC; P5, dihydrocurcumin; P6, curcumin.

electrospray source was operated at a spray capillary voltage of 4.5 kV with N_2, at a pressure of 20 to 40 psi as the sheath gas. After detection of the metabolites of curcumin by MS/MS, specimens of plasma (or concentrated HPLC fractions of plasma) were introduced directly into the mass spectrometer and the MH^+ ion of each glucuronide conjugate was subjected to collision-induced dissociation (CID) to obtain a spectrum of product ions. Argon in the CID experiments, at a pressure of 1 to 4 × 10^{-3} Torr and a collision energy of 20 eV, was used as the collision gas. The resulting spectrum contained ions of the type $[M + nH]^{n+}$, where M is the molecular mass and nH^+ is the number of attached protons. This process of MS followed by CID experiments allowed us to obtain product ion spectra that could be used to characterize this class of conjugates.

Analysis of THC Stability by HPLC. To assess the effects of pH on the stability of THC, 20-μl aliquots of 5 mM THC (in methanol) were added to 980 μl of buffer solution, followed by incubation at 37°C for 5, 15, 30, 60, 120, or 240 min. The buffer system used was 0.1 M citrate phosphate buffer (pH 3), 0.1 M phosphate buffer (pH 6.0, 7.2, and 8.0), and 0.1 M carbonate buffer (pH 10). After incubation, 100 ml of each reaction mixture was added to 900 μl of HPLC mobile phase [10% THF, 30% acetonitrile, 1% acetic acid (v/v), pH 3.0]. Chromatography conditions were as described above.

Results

HPLC Chromatograms of THC. All olefinic protons in the ^1H NMR spectrum of curcumin were absent in the ^1H NMR spectrum of THC. HPLC analysis of THC revealed two peaks in the mobile phase (pH 3.0) (Fig. 2A). The HPLC profiles of THC varied with the pH of the THF mobile phase employed (Fig. 2), possibly because of the ability of the keto-enol structures of THC to form a six-membered ring through hydrogen bonding (Fig. 1). When THC was analyzed with a mobile phase of pH 3.5 to 4.0, HPLC revealed a broad, smeared peak (Fig. 2, B and C). This phenomenon might reflect rapid transition between the keto-enol β-diketone molecular species of THC. When the pH of the mobile phase was close to neutral (pH 6.0–7.0), the HPLC profiles of THC showed broadened peaks (Fig. 2, E and F), which suggested the appearance of two hydrogen-bonded, six-membered ring structures. This may be due to either the keto-enol tautomeric structure of THC or the ability of THC to form a β-diketone moiety, as shown in Fig. 1.

Pharmacokinetic Properties of Curcumin. When administered orally (1.0 g/kg), curcumin could be detected in the mouse plasma after 15 min (0.13 μg/ml), reached the maximum concentration at 1 h (0.22 μg/ml), and declined to below the detection limit (5 ng/ml) within 6 h (Fig. 3A). When administered i.p. (0.1 g/kg), the plasma concentration of curcumin reached maximum (about 2.25 μg/ml) at 15 min and declined rapidly within the first 1 h (Fig. 3B). The HPLC profiles of the plasma samples after hydrolysis with β-glucuronidase (Fig. 4) suggest that about 99% of curcumin and more than 85% of THC in the plasma is conjugated with glucuronide. Maximum concentration values were considerably higher after i.p. injection than after oral administration, indicating that administration of curcumin by gavage results in very low absorption into the blood.

Distribution of Curcumin in Tissues. The distribution of curcumin in the mouse plasma, liver, kidneys, spleen, brain, and intestines 1 h after i.p. administration is shown in Table 1. Plasma and brain tissue showed only traces of curcumin. Correspondingly, the concentration of curcumin after 1 h was highest in the intestines. The concentrations of curcumin in the liver and spleen were estimated to be 26.9 and 26.06 μg/g, respectively.

***β*-Glucuronidase Hydrolysis of Putative Curcumin Conjugates.** Figure 4 shows the reversed-phase HPLC profiles of plasma before and after treatment with β-glucuronidase. The two major metabolites of curcumin (P2 and P4) were detected at 3.30 and 5.18 min. A representative HPLC profile of the metabolites of curcumin after hydrolysis with β-glucuronidase is shown in Fig. 4B. In the presence of β-glucuronidase, P2 and P4 were hydrolyzed effectively to THC (P4, P4′) and curcumin (P6).

HPLC revealed both free and conjugated forms of curcumin in the plasma 1 h after i.p. administration. Curcumin and its metabolites were isolated and collected by HPLC. To characterize these metabolites by MS/MS, specimens of plasma (or concentrated HPLC fractions from enzyme-treated plasma) were introduced directly into the mass spectrometer and MH^+ ions were subjected to CID to obtain a spectrum of product ions. The metabolites of curcumin in the HPLC profile shown in Fig. 4A were as follows: P1, hexahydrocurcumin-glucuronoside; P2, THC-glucuronoside; P3, dihydrocurcumin-glucuronoside; P4, curcumin-glucuronoside; P5 (P5′), THC; and P6, curcumin. The resulting solution, containing the putative conjugates, was treated with β-glucuronidase, and the liberated products were analyzed by HPLC and MS/CID (Fig. 4B). In Fig. 4B, P1 is a THC-glucuronoside, P2 is a curcumin-glucuronoside, P3 is a hexa-curcumin (MH^+ at *m/z* 375.2), P4 and P4′ are THC, P5 is a dihydro-curcumin (MH^+ at *m/z* 371.1), and P6 is a free form of curcumin.

TABLE 1

Tissue distribution after i.p. administration of curcumin in mice

Sample or Tissue	Curcumin	Total Curcumin	Organ or Plasma[a]
	μg/g	*μg/total organ*	
Plasma	0.60 ± 0.03[a]	0.52 ± 0.03	0.86 ± 0.01
Kidneys	7.51 ± 0.08	3.00 ± 0.03	0.40 ± 0.07
Spleen	26.06 ± 1.06	2.61 ± 0.11	0.10 ± 0.01
Liver	26.90 ± 2.58	33.09 ± 3.17	1.23 ± 0.08
Brain	0.41 ± 0.01	0.18 ± 0.01	0.43 ± 0.04
Intestine	117.04 ± 6.86	319.52 ± 18.73	2.73 ± 0.27

Values are mean ± S.E.M. of five mice. Curcumin (0.1 g/kg) was administered i.p. to each mouse. Animals were killed 1 h after dosing.

[a] Organ weight is given in g. Plasma is given in ml.

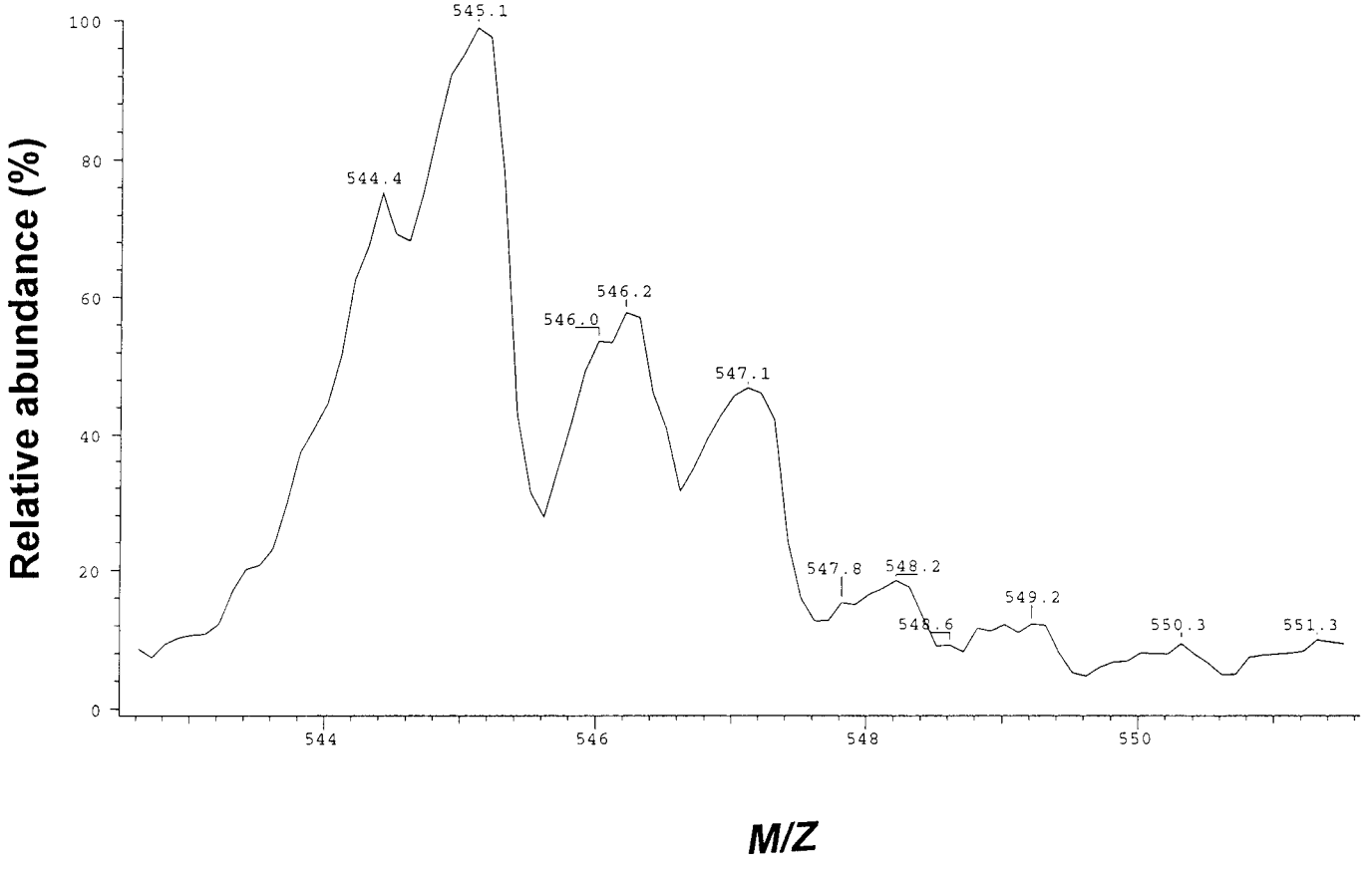

FIG. 5. *Mass spectra of glucuronide conjugates of curcumin and their hydrogenated derivatives before β-glucuronidase hydrolysis.*

MS/MS analysis of metabolites of curcumin was performed as described in *Materials and Methods.*

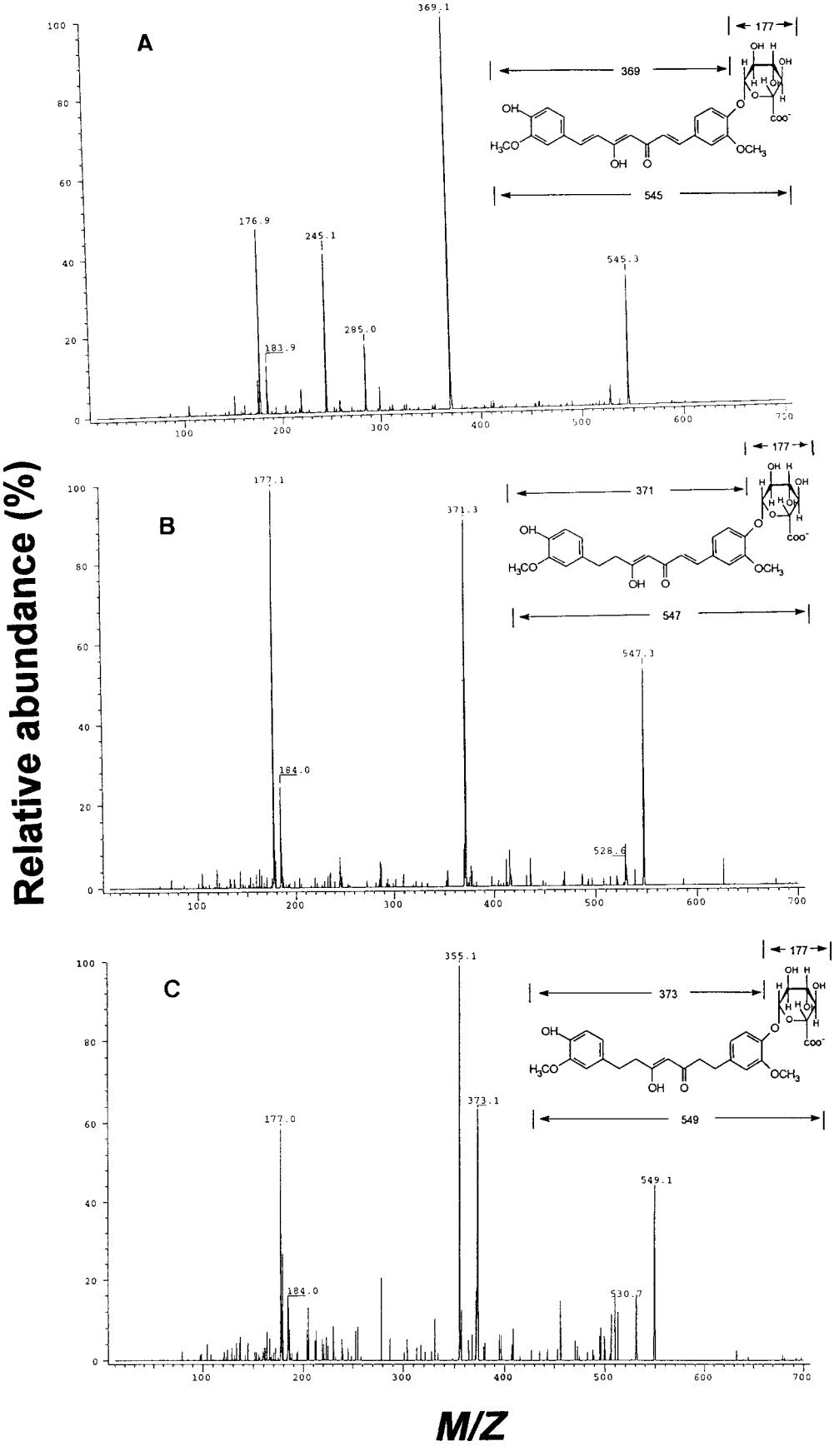

FIG. 6. *Mass/mass spectra analysis of biotransformation of curcumin.*

Mass/mass analysis of the plasma was performed as described in *Materials and Methods.*

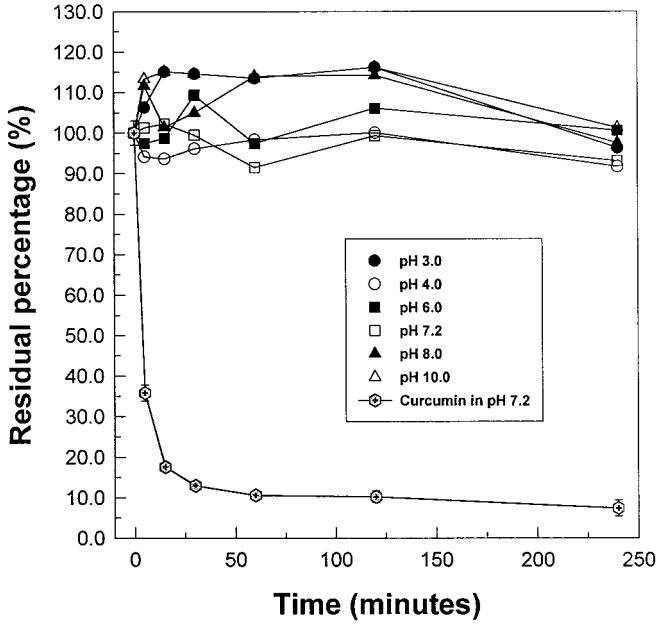

FIG. 7. *Effect of pH on the stability of THC.*

THC levels were measured with HPLC after incubation in buffer solutions of various pH levels (37°C) for 5, 15, 30, 60, 120, and 240 min. The buffer systems were 0.1 M citrate phosphate buffer (pH 3), 0.1 M phosphate buffer (pH 6.0, 7.2, and 8.0), and 0.1 M carbonate buffer (pH 10). The stability of curcumin at pH 7.2 is also shown.

Thus, one of the major metabolites in the plasma was the glucuronide conjugate of curcumin. THC-glucuronoside was present as a minor metabolite.

Identification of Glucuronide Conjugates of Curcumin in Plasma. To gain further insight into the glucuronide conjugation at the phenolic sites of curcumin, plasma samples collected from mice were analyzed directly by MS/MS. With this approach, one major glucuronide adduct together with three minor conjugates were detected (Fig. 5). Analysis of the data from MS/MS of the plasma revealed the *m/z* values of the $[M + H]^+$ ions of these candidate glucuronide conjugates. MS/MS gave an ion of *m/z* 545.3 for curcumin-monoglucuronoside, which was fragmented further to ions of *m/z* 176.9 and 369.1 under CID conditions (Fig. 6A). In the case of the other conjugate, the 547.3*m/z* product ion of dihydrocurcumin-monoglucuronoside was fragmented further to product ions of *m/z* 177.1 and 371.3 (Fig. 6B). In addition, the ion at *m/z* 549.1 for THC-monoglucuronoside was fragmented further to a product ion of *m/z* 177.0 and 373.1 (Fig. 6C). The common included ion at *m/z* 177 could be identified as the $[M + H]^+$ ion of the glucuronide moiety, whereas the individual ions at *m/z* 369.1, 371.3, and 373.1 could be identified as the molecular ions of curcumin, dihydrocurcumin, and tetrahydrocurcumin, respectively. The conjugated metabolites of curcumin produced in mice can be characterized, according to these product ion spectra, as curcumin-monoglucuronoside, dihydrocurcumin-monoglucuronoside, and THC-monoglucuronoside.

Studies of THC Stability. Reversed-phase HPLC was used to examine the stability of THC at 37°C in phosphate buffers of various pH (Fig. 7). THC was not decomposed after incubation in 0.1 M phosphate buffer for 8 h, regardless of pH. We also compared the stability of curcumin with that of THC in 0.1 M phosphate buffer at pH 7.2. Under these conditions, more than 90% of curcumin was degraded after incubation at 37°C for 4 h; in contrast, THC was very stable.

Discussion

Early studies on the tissue distribution of curcumin as well as in vitro studies of its absorption by everted intestinal sacs indicated that curcumin is transformed during absorption (Ravindranth and Chandrasekhara, 1982). When administered orally at a dose of 1 g/kg (suspension in arachis oil) in rats, about 75% of curcumin was excreted in the feces whereas only traces appeared in the urine (Wahlstrom and Blennow, 1978). Oral administration of [^{3}H] curcumin (0.6 mg/rat) led to about 89% of radioactivity being excreted in the feces and 6% being excreted in the urine. After i.p. administration, fecal excretion accounted for 73% of the radioactivity, whereas 11% was found in the bile (Holder et al., 1978). In view of the highly lipophilic character of the curcumin molecule, one would expect the body fat to contain a high proportion of bound curcumin. The poor absorption from the intestine, coupled with the high degree of metabolism of curcumin in the liver and its rapid elimination in the bile, makes it unlikely that high concentrations of the substance would be found in the body long periods of time after ingestion. In this study, we confirmed these pharmacokinetic properties of curcumin by means of a new and sensitive HPLC technique.

We also demonstrated the effect of pH on the peak separation of THC by using reverse-phase HPLC (Fig. 2). Reversed-phase HPLC of THC yielded two peaks of identical molecular weights (M^+, 372.2), one of which may have represented the β-diketone moiety of THC (Fig. 1). The structure of curcumin, as shown in Figs. 1 and 8, is the keto-enol conformation. This is probably how it exists in polar solvents, and increasing solvent polarity would be expected to favor the keto-enol tautomer. In nonpolar solvents, however, the diketo tautomer would be expected to predominate (Fig. 1).

In most organisms, glucuronidation is the most widely utilized conjugation pathway for converting environmental chemicals, drugs, and endogenous substrates to water-soluble metabolites. Experimental evidence indicates that some activities of the phase I and phase II enzyme systems are coordinately expressed and that the balance of these activities is an important determinant in preventing the accumulation of drugs or toxic intermediates to harmful levels (Bock et al., 1990). In this investigation, we found that most of the curcumin administered was reduced by an endogenous reductase system in a stepwise manner (Fig. 8) and subsequently glucuronidated by UDP-glucuronosyl transferases. Only traces of hexahydrocurcumin-glucuronoside were observed in plasma. The tentative structure of the hexahydrocurcumin-glucuronoside conjugate is shown in Fig. 8, although the exact site of glucuronylation remains to be elucidated. Studies on the absorption and metabolism of curcumin have reported that the main biliary metabolites of curcumin are glucuronide conjugates of THC and hexahydrocurcumin (Holder et al., 1978). Intraperitoneal administration of curcumin gave similar results in our study, indicating that curcumin is readily absorbed from the peritoneal cavity.

THC is one of the major metabolites of curcumin, although there is some controversy as to its biologic functions. Sugiyama et al. (1996) reported that THC is a stronger antioxidant than curcumin in vitro. They went on to suggest that THC also may exhibit the same physiologic and pharmacological activities as curcumin and may represent one of the biologically action forms of its parent compound. As we mentioned above, a major fraction of curcumin is reduced by endogenous reductase systems in a stepwise manner and subsequently glucuronidated by UDP-glucuronosyl transferase (Fig. 8). Sugiyama et al. (1996) suggested that the β-diketone moiety of THC must exhibit antioxidative activity by cleavage of the C—C bond at the

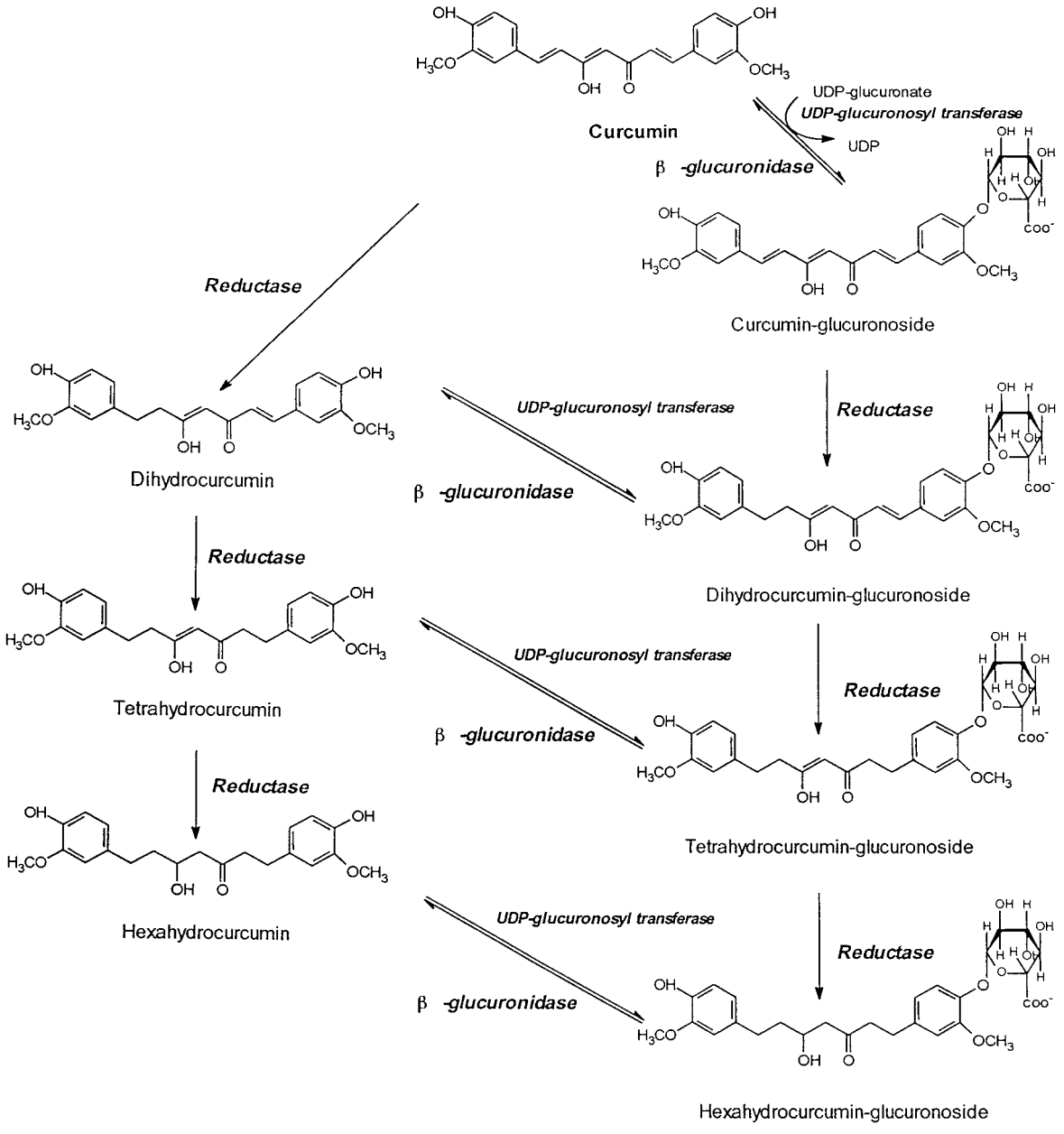

FIG. 8. *Proposed biotransformation and metabolites of curcumin in mouse plasma.*

active methylene carbon between the two carbonyls. On the other hand, Huang et al. (1995) found commercial curcumin, pure curcumin, and demethoxycurcumin to strongly inhibit TPA-induced tumor promotion, whereas bisdemethoxycurcumin and THC were less active. In contrast to the suggestion of Sugiyama et al., these findings indicate that the diketone moiety is not involved in the antioxidative activity or that the antioxidative property is not important for inhibiting tumor promotion.

Although curcumin is known to inhibit mutagenesis, lipid peroxidation, and free radical generation (Holder et al., 1978; Halliwell and Gutteridge, 1985; Hall and Braughler, 1989), little is known about the fate and pharmacological products of glucuronated curcumin and THC in vivo. Our previous studies indicated that curcumin is more stable in 10% serum than in phosphate buffer solution (Wang et al., 1997). Interestingly, we found THC to be more stable than curcumin in buffer solutions of physiologic (pH 7.2) and basic pH. THC was also stable in the plasma (data not shown). This may suggest that derivatives such as glucuronated curcumin and THC serve as the available forms of curcumin in vivo.

In conclusion, the results of previous studies, together with the findings presented here, indicate that both the biotransformation of curcumin and the stability of THC play important roles in the biological effects of curcumin and the microsomal enzyme reactions such as reduction and glucuronidation may be considered as metabolic activation of curcumin. Further experiments testing this conclusion are underway.

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