Biliary Excretion of Furosemide Glucuronide in Rabbits¹⁾

Hitoshi SEKIKAWA,^{*,a} Naomi YAGI,^a Katsuhiko ODA,^a Harumi KENMOTSU,^a Masahiko TAKADA,^a Hsue-fen CHEN,^b Emil T. LIN,^b and Leslie Z. BENET^b

Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido,^a Ishikari-Tobetsu, Hokkaido 061-02, Japan, and Department of Pharmacy, School of Pharmacy, University of California,^b San Francisco, CA 94143-0446, U.S.A. Received July 18, 1994; accepted December 6, 1994

Furosemide (F) was administered to rabbits intravenously and intraduodenally and the biliary excretion was studied. The major metabolite excreted in bile was furosemide glucuronide (FG). F and acyl migration isomers of FG (FG-iso) were also excreted in bile. The biliary excretion rates of total F (F + FG + FG-iso) following intraduodenal administration of F were much smaller than those following intravenous administration. The fraction of (F + FG-iso) in bile following intraduodenal administration of F were larger than those following intravenous administration.

Stability of FG or FG-iso in bile and supernatant solution of the duodenum homogenate of rabbits was studied. FG was unstable in both media and its degradation followed apparent first-order kinetics in both media. In bile, FG degraded to produce several FG-iso and F, while in the supernatant solution of the duodenum homogenate, it hydrolyzed immediately to F. FG-iso were hardly detected in the supernatant solution. These results indicated that FG excreted in bile degraded easily to FG-iso and F. FG might easily hydrolyze to F enzymatically in the duodenum, and the resultant F might be reabsorbed from the intestinal tract. Unabsorbed FG-iso and F might be excreted in the feces.

Key words furosemide; furosemide glucuronide; biliary excretion; acyl migration; enterohepatic recirculation; rabbit

Furosemide (F), 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid, is one of the most potent loop diuretics used in the treatment of hypertensive crises, particularly in patients with acute pulmonary edema^{2,3)} or renal failure,⁴⁾ even when the glomerular filtration is less than 5 ml/min.^{5,6)} Though pharmacokinetics/pharmacodynamics of F in human have been of interest,^{7–9)} pharmacokinetic parameters of F vary greatly in the literature.^{7–12)} The main elimination route of F is *via* kidney. Several studies reported the mean total clearance of F to be between 96–194 ml/min in healthy volunteers.⁹⁾ The renal clearance was reported to be 68–118 ml/min, whereas the non-renal clearance ranged from 29 to 69 ml/min.⁹⁾ The non-renal clearance of F was superior to renal clearance in some disease states such as nephrotic syndrome.⁹⁾ The non-renal route of F elimination has been postulated to be by biliary excretion. Rupp found about 46 and 12% of the dose in the feces in healthy volunteers following oral and intravenous administration of F, respectively.¹³⁾ He speculated that biliary excretion of F would be 12% of intravenous dose, because only unchanged F was excreted in the feces. He reported that over 60% of an intravenous dose of ³⁵S-furosemide was recovered in the feces of patients with impaired renal function. This meant that biliary excretion increased instead of renal excretion which was a major elimination route of F. At that time, the major metabolite of F was believed to be 4-chloro-5-sulfamoylanthranilic acid (CSA),¹⁴⁾ the hydrolyzed product of F. Spitznagle *et al.* reported CSA and F were excreted in bile in rabbits.¹⁵⁾ Prandota and Pruitt reported CSA, F and unknown metabolites were excreted in bile following intravenous administration of F to rats.¹⁶⁾ It might be that CSA cannot be excreted in bile because its molecular weight (251) was below the lowest limit (about 325) of excretion in rats.¹⁷⁾ Conjugation of F with glucuronic acid (FG) has been

proposed as the metabolite of F instead of CSA since the 1980s.^{9,18,19)} Smith *et al.* found FG as a major metabolite of F excreted in human urine.¹⁸⁾ They found no CSA in the urine, and speculated that CSA might be an artifact during the assay when the biological specimens were strongly acidified prior to the extraction into organic solvent. The FG fraction excreted in human urine varied greatly in groups in the literature,^{9,18,19)} one possible reason for this variation might be its instability. The degradation rate in the photodegradation of FG (*t*_{1/2} = 12 min under indirect sunlight) was larger than that of F.^{9,20)} Moreover, Sekikawa *et al.* in their report on intramolecular acyl migration and hydrolysis of FG in buffer solutions (pH between 1 and 10),²¹⁾ stated that FG was unstable in both neutral and alkaline solutions. FG degraded rapidly to form acyl migration isomers of FG (FG-iso) by hydroxy ion catalysis, and then FG-iso hydrolyzed to F in these media.

We studied the biliary excretion following intravenous and intraduodenal administration of F to rabbits. We handled the bile specimens carefully protecting FG from photodegradation and acyl migration to obtain the correct values of the excretions. We also studied the stability of FG in bile and supernatant solution of the homogenate of duodenum in rabbits.

MATERIALS AND METHODS

Materials Furosemide (F) tablets (Lasix® Tablet, 40 mg/tablet) and F injections (Lasix® Injection, 100 mg/10 ml) were obtained from Hoechst Japan, Ltd., Tokyo, Japan. F powder (lot No. 26F-0636) and β -glucuronidase (from bovine liver, 5000 Sigma units/ml, lot No. 126F-6171) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Piretanide (lot No. 700 L023) supplied by Hoechst Japan, Ltd. was dissolved in phosphate buffer

* To whom correspondence should be addressed.

at pH 7.4 and used as an internal standard. Pentobarbital sodium injections (Nembutal®) were obtained from Abbott Laboratories, North Chicago, IL, U.S.A. CSA was obtained from U. S. Pharmacopeia (Rockville, MD, U.S.A.). Acetonitrile and methanol were HPLC grade from Kanto Chemical Co., Inc., Tokyo, Japan. All other chemicals were of reagent grade.

Preparation and Purification of Furosemide Glucuronide (FG) Five healthy male volunteers (ages between 22 and 26) took Lasix® tablets (80 mg) together with 200 ml of water. Urine was collected for 4 h. pH of the urine was adjusted immediately to around 5 by addition of 7.5 N acetic acid and stored in a refrigerator. To 300 ml of urine, 150 ml of ethyl acetate was added and extracted twice. The pH of aqueous fraction was adjusted to 2 with 10% hydrochloric acid. Then, 150 ml of ethyl acetate was added to the aqueous fraction and extracted. The ethyl acetate fraction was dehydrated with magnesium sulfate. After concentration of this fraction to about 30 ml *in vacuo* at 40 °C, 15 ml of toluene was added. The obtained crude precipitate was dissolved in a minimum volume of acetonitrile. FG fraction was obtained by preparative HPLC. The combined eluate was concentrated and dried *in vacuo* and the residue was extracted into ethyl acetate. Ethyl acetate was evaporated to dryness *in vacuo*. The obtained crystals of FG were identified by FAB-MS (GCMS 9100-MK, Shimadzu Co., Kyoto, Japan). Cluster ions ($[M+Na]^+$) combined sodium ion with FG were seen at m/z 529 and m/z 531. FG was more than 98% pure. A single peak of FG was observed in the HPLC chromatogram, and disappeared completely by hydrolysis with β -glucuronidase.

Preparation of Acyl Migration FG-iso for Stability Study The mixtures of F, FG and FG-iso were obtained as follows: three μ g FG was dissolved in 10 ml of 0.01 N sodium hydroxide (resultant pH was 9). The solution was incubated at 37 °C for 80 min. The pH of the solution was adjusted to 4 by addition of acetate buffer solution; the ratios of F, FG and FG-iso were 16.3, 39.5 and 44.2%, respectively. The solution was kept at 4 °C in the refrigerator prior to the studies.

Biliary Excretion Studies Male albino rabbits weighing 2.5–2.9 kg were fasted for about 24 h prior to drug administration. The rabbits were anesthetized with sodium pentobarbital (25 mg/kg) by intravenous administration into the ear vein, and maintained under anesthesia for the duration of the experiment. After a 4–5 cm incision of the abdomen below the xiphoid process, the opened portion of the gallbladder was ligated. A polyethylene tube (PE-50, Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) was cannulated into the common bile duct. Bile was collected for 30 min prior to the administration of F. For intravenous or intraduodenal administration, F (Lasix® Injection) was injected into the ear vein or duodenum at a dose of 10 mg/kg body weight. Bile was led into a test tube cooled in ice. Bile was collected at designated time intervals for 8 h (intravenous administration) or 12 h (intraduodenal administration) post-administration. To prevent further acyl migration or hydrolysis of FG, acetate buffer solution (pH 5.0) was added immediately to 1 ml of bile to make up 10 ml. The

concentration of F, FG or FG-iso in the specimens was determined within 3 h after each collection.

Stability Studies of FG in Bile and Supernatant Solution of Duodenum Homogenate in Rabbits Bile was obtained by the method described above. Male albino rabbits weighing 2.6–3.2 kg were fasted for 24 h and were anesthetized by sodium pentobarbital. The duodenum segment (from 10 cm below the stomach, about 9 cm and 2.4 g) was cut off. Segments were washed with 0.9% sodium chloride solution and homogenated at 10000 rpm for 3 min with a homogenizer (AM-2, Nippon-Seiki Co., Tokyo, Japan). The homogenate was centrifuged at 3000 rpm for 10 min. The obtained supernate was diluted 20 times (5%) and 200 times (0.5%) with 0.9% sodium chloride solution. The supernatant solution or bile was transferred in a flask and maintained at 37 ± 0.1 °C in an incubator (M-100 D, Taiyo Kagaku Ind., Co., Tokyo, Japan). One ml of FG solution (in 0.1 M acetate buffer solution, pH 4) was added to the bile or supernatant solution, incubated at 37 °C and 60 strokes/min. The initial FG concentration was 1 μ g/ml. An aliquot (0.5 ml) was drawn from the solution at appropriate time intervals, then the pH was adjusted to around 5 by addition of acetate buffer. The supernatant solutions were centrifuged at 3000 rpm for 5 min and passed through a membrane filter (0.5 μ m, Nihon Millipore Kogyo Co., Ltd., Yonezawa, Japan). Internal standard solution (0.5 ml) was added to 1 ml of the solution and mixed. The FG concentrations were analyzed by HPLC. A stability study of FG was also made in isotonic phosphate buffer solutions at pHs 8.7 and 6.5 which were equivalent to pH values of the bile and supernatant solution, respectively.

Analytical Procedure for F, FG and Its Acyl Migration Isomers Analytical procedures for F, FG and FG-iso were based on F assay²¹⁾ after the enzymatic hydrolysis of FG and alkaline hydrolysis of FG and FG-iso. For F assay, 2 ml of a sample was added to 1 ml of phosphate buffer (pH 5.0) and the solution was passed through the membrane filter described above. To 1 ml of the solution, 0.5 ml of the internal standard solution (piretanide, 20 μ g/ml) was added. The mixture was injected into the HPLC system. For (F + FG) assay, 1 ml of β -glucuronidase solution (1000 Sigma units/ml, in 0.1 M acetate buffer, pH 5.0) was added to 2 ml of sample solution. For (F + FG + FG-iso) assay, 1 ml of 0.15 N sodium hydroxide was added to 2 ml of sample solution. Hydrolysis was done by incubating for (F + FG) and (F + FG + FG-iso) at 37 °C for 2 and 0.5 h, respectively. After the hydrolysis, the solution was passed through the membrane filter. Internal standard solution (0.5 ml) was added to 1 ml of the solution and the mixture was injected into the HPLC system. Each concentration of F, FG and FG-iso was calculated from these values.

Preparative HPLC for FG HPLC was performed on an Inertsil PREP-ODS, with a 10 μ m reversed phase column (250 $\times$ 20 mm i.d., Gasukuro Kogyo Inc., Tokyo, Japan) and a Shim-pack CLC-ODS, with a 30 μ m guard column (50 $\times$ 2.1 mm i.d., Shimadzu Co.). The column was at room temperature (21–24 °C). A spectrofluorometer, model RF-535 (Shimadzu Co.) was used as a detector. Excitation and emission wavelengths were 345 and 425 nm,

respectively. The mobile phases used were 20% acetonitrile (A) and 80% acetonitrile (B). A gradient program was made as follows: from 0 to 15 min, the mobile phase was 0% B; from 15 to 30 min, 50% B and from 30 to 35 min, 0% B. Sample solutions (200 μ l) were injected into the HPLC system with a run time of 35 min. The flow rate was 3.0 ml/min. The eluate of FG was obtained at around 17 min. During the elution, the detector was not used because of the rapid photodegradation of FG.

HPLC Conditions for F Assay The apparatus was the same as that described above except for the column and temperature. The column used was a Shim-pack CLC-ODS (150 $\times$ 6 mm i.d., particle size 5 μ m, Shimadzu Co.) maintained at $40 \pm 0.5^\circ\text{C}$ in the column oven (CTO-6A, Shimadzu Co.). Excitation and emission wavelengths were 345 and 415 nm, respectively. The mobile phases were 20% acetonitrile containing 0.3% acetic acid (A) and 80% acetonitrile containing 0.3% acetic acid (B). A gradient program was made as follows: from 0 to 3 min, the mobile phase was 10% B; from 3 to 10 min, 40% B; from 15 to 18 min, 60% B; from 18 to 20 min, 40% B and from 20 to 30 min, 10% B. Sample solutions (10–50 μ l) were injected into the HPLC system with a run time of 30 min. The detection limit of F in bile was 5 ng/ml.

HPLC Conditions for FG Assay For FG determination in bile or supernatant solution of duodenum, to distinguish a FG peak from FG-iso peaks or F peak, the following conditions were used: HPLC was performed on an Altex Ultrasphere ODS, with 5 μ m reversed phase column (250 $\times$ 4.6 mm i.d.) from Beckman Instruments, Inc., Berkeley, CA, U.S.A. The mobile phases used were 15% acetonitrile and 4% methanol containing 0.3% acetic acid (solvent A, pH 2.5) and 90% acetonitrile containing 0.3% acetic acid (solvent B, pH 4.0). Flow rate was 0.6 ml/min. The following gradient program was made: from 0 to 30 min, 6% B; from 30 to 50 min, 35% B; and from 50 to 70 min, 6% B. Excitation and emission wavelengths were 345 and 425 nm, respectively. Sample solutions (10–50 μ l) were injected into the HPLC system with a run time of

70 min. All other conditions were the same as the F assay. The detection limits of FG in bile and supernatant solution of duodenum homogenate were 1 ng/ml.

Protection from the Photodegradation During the study, the process was protected carefully to avoid the photodegradation of F, FG and FG-iso in a darkened room.²⁰⁾

RESULTS AND DISCUSSION

Metabolites Excreted in Bile Following intravenous and intraduodenal administration, several peaks were observed in the HPLC chromatogram of bile samples. Figure 1b shows those found 30 min after administration of F to a rabbit. Peak 3 was that of F. The retention time of peak 2 was that of FG. After the hydrolysis with β -glucuronidase, the peak area of 2 decreased (to 12% of the peak area in b) and the peak area of F increased (790% of the peak area in b). However, peak 2 still remained in the chromatogram. The peaks of 1 and 2 disappeared after the hydrolysis with sodium hydroxide (d). The peak area of F increased still more than that after the hydrolysis with β -glucuronidase (990% of the peak area in b). Peak 2 was therefore considered to be a mixture of FG and other metabolites. The compounds of peaks 1 and 2 shown in Fig. 1c were β -glucuronidase resistant. They were hydrolyzed to F with 0.15 N sodium hydroxide (Fig. 1d). Without adjusting the pH and allowing the bile samples to stand at room temperature for several minutes, peak 2 decreased and peaks 1 and F increased. Peaks 1 and 2 in Fig. 1c were thought to be the breakdown product of FG, that is, FG-iso. As mentioned later, it would not be practical to estimate the concentrations of F, FG and FG-iso directly from the HPLC chromatogram because the peaks of FG-iso overlapped each other or the peak of FG. Hydrolysis with β -glucuronidase and with 0.15 N sodium hydroxide gave each fraction. Rachmel *et al.*²²⁾ reported on the acyl migration of FG in aqueous solution. They purified FG from bile following intraperitoneal administration of F to rats, but did not mention what

Fig. 1. HPLC Chromatograms of F and Its Metabolites in Bile Following Intravenous Administration of F to Rabbits

(a) Blank bile; (b) bile, 30 min after administration; (c) bile, after hydrolysis with β -glucuronidase of (b); (d) bile, after hydrolysis with sodium hydroxide of (b). 1, FG-iso; 2, FG and FG-iso; 3, F; 4, piretanide (i.s.).

Fig. 2. Remaining Ratios of F and Its Metabolites in Bile Stored Frozen at -20°C for 20 d

(a) samples with pH adjusted to around 5; (b) samples without adjustment of pH. Each bar represents the mean $\pm$ S. D. of 11 determinations.

Fig. 3. Cumulative Amounts of F and Its Metabolites in Bile Following Intravenous (a) and Intraduodenal (b) Administration of F to Rabbits

O, F; ▲, FG; □, FG-iso. Each point represents the mean $\pm$ S.E. of three rabbits.

compounds were excreted in bile other than FG. In our study, F, FG and FG-iso were found, but CSA was not detected.

Figure 2 shows the remaining ratios of F, FG and FG-iso fractions in bile samples adjusted to pH 5 (a) and without pH adjustment (b) when stored at -20°C for 20 d. The initial values were obtained by immediate determination (within 3 h) after the collection of bile and adjustment of pH to around 5. The ratios of F, FG and FG-iso shown in Fig. 2a were almost equal to those determined immediately. Without adjustment of the pH, the ratio of FG decreased and the ratio of FG-iso increased; the ratio of F was almost equal. These results indicated that without adjustment of the pH of collected bile to around 5, FG easily broke down to FG-iso during storage in the freezer. We reported previously that the reaction of acyl migration was catalyzed by hydroxy ion and that it was accelerated at higher pH.²¹⁾ As the pH of bile is higher (about pH 8.7 in rabbit) than those of other body fluids, the rate of degradation of FG in bile might be much

larger. Without adjustment of the pH, total concentrations as F (F + FG + FG-iso) were somewhat smaller than initial values. The results might indicate the possibility of other pathways of degradation of these metabolites.

Figure 3 shows the cumulative amount of F, FG and FG-iso excreted in bile following intravenous (a) and intraduodenal (b) administration of F to rabbits. The mean cumulative amounts of F, FG and FG-iso for up to 8 h following intravenous administration were 130.5, 427.8 and 133.8 μg , respectively. Their excretion seemed to be almost completed within 8 h. Following intraduodenal administration on the other hand, excretion continued even beyond 8 h. The mean cumulative amounts of F, FG and FG-iso for up to 8 h following intraduodenal administration were 15.1, 35.6 and 18.4 μg , respectively, and those up to 12 h were 42.8, 72.1 and 43.7 μg .

Figure 4 shows the biliary excretion rates of F, FG and FG-iso following intravenous (a) and intraduodenal (b) administration of F to rabbits. The peaks of the excretion

Fig. 4. Biliary Excretion Rates of F and Its Metabolites Following Intravenous (a) and Intraduodenal (b) Administration of F to Rabbits. Symbols are the same as Fig. 3.

TABLE I. Biliary Excretion of F and Its Metabolites in Rabbits by 8 h

		Intravenous administration	Intraduodenal administration
		Total recovery (%) (as F)	Total recovery (%) (as F)
		2.53 ± 1.28	0.269 ± 0.036^a
Percent of each fraction against total excretion amount (as F)	F	18.89 ± 1.41	22.67 ± 4.53
	FG	61.74 ± 1.55	51.03 ± 3.77^b
	FG-iso	19.35 ± 2.39	26.30 ± 1.28^b

Results represent the mean $\pm$ S.E. of three rabbits. a) $p < 0.01$, b) $p < 0.05$ as compared with intravenous administration.

Fig. 5. Time Courses of the Degradation of FG in Bile of Rabbit and Buffer Solution (pH 8.7) at 37°C

○, bile; ●, buffer solution. Each point represents the mean $\pm$ S.E. of three determinations.

rates of F and FG were observed immediately after intravenous administration of F and decreased rapidly thereafter. The peak of FG-iso was observed at 22.5 min following intravenous administration. Following intraduodenal administration of F, however, none of the three excretion rates of F, FG or FG-iso showed a clear peak. The rates increased after 8 h and showed a secondary peak at 10.5 h. One possible reason might be the small

Fig. 6. HPLC Chromatograms of FG and Its Degradation Products in Bile of Rabbit at 37°C

(a) At 0 min; (b) after 40 min. 1–6, FG-iso; 7, FG; 8, F; 9, piretanide.

absorption rate of F, because the F ($pK_a = 3.8^{23}$) molecule might exist in an ionized form in the duodenum.

Table I summarizes the total recovery percentage of the dose and the percentage of each fraction against total excreted amount following the two means of administration in rabbits for up to 8 h. The mean total recovery percentage for up to 8 h following intraduodenal administration of F was only 0.269% and at 12 h only 0.615%. These values were significantly lower than those after intravenous administration ($p < 0.05$). The percentage of FG to total biliary excretion amounts were the largest of the three fractions following either means of F administration. However, after intraduodenal administration this percentage was significantly lower while those of

F and FG-iso were increased compared to those following intravenous administration.

Stability of FG in Bile Figure 5 shows the time course of the degradation of FG in bile and buffer solution (pH 8.7) at 37°C. In both solutions it followed apparent first-order kinetics: the apparent degradation rate constants of FG being 3.35×10^{-2} and $6.58 \times 10^{-2} \text{ min}^{-1}$, respectively. The half-life ($t_{1/2}$) of FG in bile and buffer solution was 19.5 and 10.5 min, respectively, that in bile being almost two fold that in buffer solution. This might be due to the stabilization by micelle formation of bile acids which acted as the biological surfactant.

FG degraded easily to FG-iso and F in both bile and buffer solution. Figure 6 shows the chromatograms of FG and its degradation products in bile after incubation of FG for 40 min at 37°C. Six peaks of FG-iso were observed in the chromatogram, some of them perhaps overlapping.²¹ Further improvement of the HPLC conditions may allow separation of the peaks. The peak of FG disappeared with the treatment with β -glucuronidase, while the peaks of FG-iso did not. The peaks of FG and FG-iso disappeared by the hydrolysis with sodium hydroxide.

Rachmel *et al.* reported the degradation of FG to FG-iso in buffer solutions between 6.0 and 9.0.²² They found larger rate constant of degradation at higher pH. Our study demonstrated that the reaction of the degradation of FG to FG-iso occurred in bile *in vitro*.

Stability of FG in Supernatant Solution of Duodenum Homogenate Figure 7 shows the time course of the degradation of FG in 0.5 and 5% of supernatant solution of duodenum homogenate and buffer solution at pH 6.5; in all cases the degradation followed apparent first-order kinetics. When FG was added to the duodenum homogenate solution, FG was hydrolyzed immediately to produced F. The $t_{1/2}$ of FG in 0.5 and 5% of supernatant solution of homogenate and buffer solution was 5.4, 1.2 min and 30 h, respectively, thus being much smaller in the supernatant solution than in the buffer solution at the same pH value. FG-iso were found in the chromatogram

Fig. 7. Time Courses of the Degradation of FG in Supernatant Solutions of Duodenum Homogenate and Buffer Solution (pH 6.5) at 37°C

○, 5% homogenate; △, 0.5% homogenate; ●, buffer solution. Each point represents the mean $\pm$ S.E. of three determinations.

of the products in buffer solution at pH 6.5, although no peaks were not detected in the supernatant solution. As the degradation rate of FG to F in homogenate solution was so large, FG-iso were hardly detectable in the supernatant solution of the homogenate. These results suggested that FG might be hydrolyzed by an enzyme existing in the duodenum.

Figure 8 shows the HPLC chromatogram of the solution of the mixture of FG, FG-iso and F (a), and the solution after their incubation in 5% supernatant solution of the duodenum homogenate at 37°C for 80 min (b). FG disappeared completely, while the peaks of FG-iso remained, suggesting that FG-iso were enzyme-resistant.

From these results, it was demonstrated that F, FG and FG-iso were excreted in bile following intravenous and intraduodenal administration of F to rabbits. Hirom *et al.* reported that for rabbits a molecular weight of more than 475 ± 50 was required to excrete in bile.¹⁷ As the molecular weight of F is 331, F itself might not be excreted in bile. FG which has a molecular weight is 507 might be excreted originally in bile and transformed into FG-iso or F by acyl migration or hydrolysis during its passage through the bile duct.

From these results, we speculated on the biliary excretion and developed a reabsorption model of F as shown in Fig. 9. FG might be an original major metabolite of F, perhaps degrading to FG-iso and F by acyl migration or hydrolysis in the liver and bile. As shown in Fig. 5, degradation of FG in bile was rapid. The production of FG-iso and F might increase during storage in the gallbladder or passage through the bile duct. The excreted

Fig. 8. HPLC Chromatograms of FG and Its Degradation Products in 5% Supernatant Solution of Duodenum Homogenate of Rabbits at 37°C

(a) At 0 min; (b) after 120 min. 2—6, FG-iso; 7, FG; 8, F; 9, piritanide.

Fig. 9. Schematic Model of the Hydrolysis of FG and Reabsorption of F in the Intestinal Tract via Biliary Excretion

F and resultant F by enzymatic hydrolysis in the duodenum might be reabsorbed from the duodenum. Part of the excretion thus might go into the enterohepatic recirculation in such a manner. On the other hand, as FG-iso was stable to enzyme (Fig. 8), it might not be absorbed from the intestinal tract. Unabsorbed FG-iso might be excreted in feces. Even if a part of the FG-iso fraction were hydrolyzed slowly to F non-enzymatically during its passage through the intestinal tract (pH value of intestinal tract is higher than the duodenum), the absorption of F might be negligible because most of the F molecule exists in an ionized form in this site.

It is known that the molecular weight required for the biliary excretion in humans is almost the same as that in rabbits.¹⁷⁾ In animal species which possess a gallbladder (e.g., humans and rabbits), bile accumulates temporarily in the gallbladder and is then excreted to the duodenum by a stimulus such as food intake. The period of storage in the gallbladder might determine the ratio of the FG degradation or the formation of F and FG-iso. Enterohepatic recirculation may be one reason for the inter- or intra-subject variations in the pharmacokinetic parameters of F.

This study confirmed that FG, FG-iso and F were excreted in bile of rabbit. FG was unstable in bile. The recovery of total F excreted was small in rabbits. Rupp reported that 12% of an intravenous dose was excreted in the feces in humans.¹³⁾ If the enterohepatic recirculation of F occurred in humans, the total amount of F excreted in bile might exceed 12%. A three-compartment open model was proposed by Rupp¹³⁾ and Hammarlund *et al.*²⁴⁾ for the plasma F concentration-time curve following intravenous administration. They explained the terminal phase as being due to the distribution of F in the deep peripheral tissue. We speculate there may be a contribution of enterohepatic recirculation of F to the terminal phase.

The amount of F excreted in bile should not be ignored in humans, and further pharmacokinetic/pharmacodynamic studies of F must be made especially following oral administration.

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