PHARMACOKINETIC STUDY OF THE ENTEROHEPATIC CIRCULATION OF ACETAMINOPHEN GLUCURONIDE IN RATS

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(Received March 28, 1984)

A pharmacokinetic model of the enterohepatic circulation of acetaminophen glucuronide was investigated in rats with particular attention to a lag time between biliary excretion and reabsorption. The plasma drug data obtained after acetaminophen glucuronide injection into the various sites of the gut confirmed that there is a lag time in the enterohepatic circulation and that the lag time is due to the intestinal transit period of the conjugate to the site of the hydrolysis. The value of the lag time was fairly close to that reported previously in the rat. Based on the result, a compartment model with periodic trigonometric function for the enterohepatic circulation was built up and the urinary excretion data were fitted to this model. Same parameters which are considered to be common to other glucuronide conjugates were in good agreement with those reported previously, indicating that the model and those values are useful to study the enterohepatic circulation for glucuronides of other compounds in the rat.

Keywords — enterohepatic circulation; acetaminophen glucuronide; pharmacokinetics; compartment model; lag time; mean transit time; rat

INTRODUCTION

In a previous study,¹⁾ the drug concentration data in plasma, urine, and bile of bile duct-cannulated rats following intraduodenal administration of acetaminophen glucuronide (AG) or sulfate (AS) showed that these conjugates were hydrolyzed by the microflora in the gut and the liberated acetaminophen was reabsorbed, confirming enterohepatic circulation of the conjugates. On the other hand, the excretion of AS into the bile after acetaminophen injection was negligible in spite of its main metabolite in the rat, but a considerable amount of AG was excreted into the bile. With these results, the enterohepatic circulation of AG following acetaminophen injection was suggested in intact rats. This is also supported by the urinary metabolite excretion patterns between intact and bile duct-cannulated rats following acetaminophen injection.¹⁾ Thus, a further investigation of enterohepatic circulation of AG is necessary to complete the pharmacokinetic model of the fate of acetaminophen.

Although certain physiologically based pharmacokinetic models have included enterohepatic circulation,^{1,3)} classical compartment models with these characteristics have not been extensively developed.^{4–7)} Among them, two reports described the enterohepatic circulation by a compartment model with a finite lag time.^{4,6)} Although existence of the lag time is assumed after biliary excretion, before reabsorption can occur, the physiological meaning of the lag time has not been elucidated. Therefore, in the present study we investigated a pharmacokinetic model of the enterohepatic circulation of AG in rats with particular attention to the lag time between biliary excretion and reabsorption.

MATERIALS AND METHODS

Materials — AG was obtained as described previously¹⁾ and the other reagents were of reagent grade.

Animal Experiments — Male Wistar rats (250–280 g) were fasted overnight before use, but had free access to water. AG was adminis-

tered intravenously in normal saline solution. The conjugate or acetaminophen was also administered intraduodenally, intraileally (25 cm upper part from the caecum⁸⁾), or intracaecally into the respective tract over 30 s, after a midline abdominal incision had been made along the linea alba. Following each administration, the abdominal incision was closed with a suture and the rats were kept in a Ballman cage⁹⁾ for the remainder of the experiment. The total volume of drug solution administered was about 1 ml per head.

Blood samples were collected from the femoral artery through a polyethylene cannula at appropriate times. Bile and urine samples were also collected at appropriate times through polyethylene tubes cannulated into the bile duct and bladder, respectively. The bladder was rinsed with 0.5 ml of normal saline solution after urine collection in order to ensure complete drug collection from the urine. Normal saline solution (2 ml aliquots) was orally administered at intervals of 2 h for continuous bile and urine sample collections. For each operation, the animals were anesthetized lightly with ether at suitable intervals as necessary during surgery. New animals were used for each experiment.

Analytical Methods — Plasma, bile, and urine samples were assayed as described previously.¹⁾

Data Analysis — The data for the total cumulative fraction of the dose excreted in urine and bile were fitted to Eqs. 1–4 and Eqs. 5–7 by using a nonlinear least-squares analysis, solving the differential equations by the Runge-Kutta-Gill method, with weighting based on the reciprocal value of the square of the standard error for each datum. The computer program used was SALS¹⁰⁾ (Statistical Analysis with Least-Squares Fitting, University of Tokyo) for nonlinear regression analysis. The mean residence time (MRT) was calculated by using the area under the curve and the area under the moment curve from mean plasma drug concentration data.¹¹⁾

RESULTS

Enterohepatic Circulation of AG

Table I shows cumulative urinary and biliary excretions after intravenous administration of 452.4 $\mu\text{mol/kg}$ of AG into the intact or bile duct-cannulated rats. The fraction of dose eventually recovered as AG in the bile of bile duct-cannulated rats was about 7% and this was in accord with our previous data.¹⁾ There were significant differences between intact and bile duct-cannulated rats at the time above 2.5 h for the total fraction of dose recovered from the urine. This suggests the enterohepatic circulation of AG. There were also significant differences between intact and bile duct-cannulated rats for AG fraction recovered in the urine during the time between 2.5 to 8 h.

The excretion of AS in the urine of bile duct-cannulated rats after intravenous administration of AG was about 6%. The result shows that AG is hydrolyzed and re-conjugation to sulfate occurs in rats, consistent with our previous results.¹⁾

Plasma Drug Levels Following AG or Acetaminophen Injection into the Gut

The activities of β -glucuronidase in the gut are much higher in the distal region such as the caecum and colon than in the proximal region such as the duodenum.^{12,13)} It is also reported that cleavage of AG occurs in the distal region of the gut *in vitro* by microfloral incubations.¹⁴⁾ Based on these facts, plasma drug concentrations of bile duct-cannulated rats following intraduodenal, intraileal, or intracaecal administration of 452.4 $\mu\text{mol/kg}$ of AG are measured and shown in Fig.1. The dose of 452.4 $\mu\text{mol/kg}$ is an upper-limit dose that no dose-dependent effect on the urinary metabolite excretion patterns after the same dose of acetaminophen injection into rats is observed.¹⁾

There is evidently a lag period of approximately 2 h before acetaminophen and AS appear in the plasma after intraduodenal administration, while, there is no such lag period after intraileal or intracaecal administration. Plasma AS levels after AG administration into the gut predominantly appeared. This indicates that the reabsorbed acetaminophen is metabolized prefe-

TABLE I. Cumulative Urinary and Biliary Excretions^{a)} Following Intravenous Administration of 452.4 $\mu\text{mol/kg}$ of AG

Time h	Intact rats ($n=5$) In urine (%)			Bile duct-cannulated rats ($n=4$) In urine (%)			In bile (%)
	AG	AS	Total ^{b)}	AG	AS	Total ^{b)}	AG
0.5	22.98 $\pm$ 1.56	0.21 $\pm$ 0.10	23.19 $\pm$ 1.55	22.88 $\pm$ 2.77	0.47 $\pm$ 0.09	23.43 $\pm$ 2.88	2.06 $\pm$ 0.23
1.0	41.41 $\pm$ 2.62	0.91 $\pm$ 0.34	42.32 $\pm$ 2.29	36.24 $\pm$ 2.85	0.96 $\pm$ 0.13	37.31 $\pm$ 2.90	3.48 $\pm$ 0.28
1.5	54.27 $\pm$ 3.06	1.57 $\pm$ 0.51	55.84 $\pm$ 2.64	45.20 $\pm$ 4.84	1.71 $\pm$ 0.30	47.70 $\pm$ 4.80	4.51 $\pm$ 0.31
2.0	62.70 $\pm$ 2.80	2.30 $\pm$ 0.68	65.00 $\pm$ 2.28	52.95 $\pm$ 4.18	2.65 $\pm$ 0.32	56.02 $\pm$ 4.05	5.21 $\pm$ 0.28
2.5	*70.52 $\pm$ 2.70	3.18 $\pm$ 0.74	*73.70 $\pm$ 2.08	57.36 $\pm$ 3.51	3.15 $\pm$ 0.48	60.97 $\pm$ 3.53	5.71 $\pm$ 0.37
3.0	**75.91 $\pm$ 2.28	4.08 $\pm$ 0.81	**79.99 $\pm$ 1.57	61.20 $\pm$ 3.15	3.71 $\pm$ 0.59	65.46 $\pm$ 3.05	6.16 $\pm$ 0.18
3.5	**79.53 $\pm$ 2.02	4.79 $\pm$ 0.89	**84.33 $\pm$ 1.27	65.72 $\pm$ 2.24	4.26 $\pm$ 0.66	70.71 $\pm$ 2.02	6.45 $\pm$ 0.16
4.0	**82.29 $\pm$ 1.88	5.50 $\pm$ 0.94	**87.78 $\pm$ 1.08	70.11 $\pm$ 2.20	4.75 $\pm$ 0.69	75.59 $\pm$ 1.99	6.62 $\pm$ 0.14
4.5	**84.26 $\pm$ 2.12	6.10 $\pm$ 1.04	**90.36 $\pm$ 1.27	73.33 $\pm$ 2.14	5.17 $\pm$ 0.78	79.27 $\pm$ 1.84	6.71 $\pm$ 0.16
5.0	*85.65 $\pm$ 2.26	6.72 $\pm$ 1.10	**92.37 $\pm$ 1.40	75.84 $\pm$ 2.13	5.38 $\pm$ 0.80	81.99 $\pm$ 1.87	6.79 $\pm$ 0.17
5.5	*86.67 $\pm$ 2.22	7.27 $\pm$ 1.16	**93.94 $\pm$ 1.34	77.21 $\pm$ 2.10	5.60 $\pm$ 0.85	83.57 $\pm$ 1.91	6.84 $\pm$ 0.18
6.0	*87.41 $\pm$ 2.16	7.75 $\pm$ 1.22	**95.17 $\pm$ 1.28	78.63 $\pm$ 2.07	5.76 $\pm$ 0.88	85.16 $\pm$ 1.84	6.88 $\pm$ 0.20
6.5	*87.90 $\pm$ 2.15	8.14 $\pm$ 1.23	**96.03 $\pm$ 1.34	79.85 $\pm$ 2.20	5.98 $\pm$ 0.92	86.60 $\pm$ 1.90	6.92 $\pm$ 0.21
7.0	*88.30 $\pm$ 2.13	8.42 $\pm$ 1.30	**96.73 $\pm$ 1.38	80.69 $\pm$ 1.95	6.23 $\pm$ 0.92	87.69 $\pm$ 1.82	6.92 $\pm$ 0.22
7.5	*88.49 $\pm$ 2.08	8.66 $\pm$ 1.34	**97.15 $\pm$ 1.38	81.30 $\pm$ 1.91	6.31 $\pm$ 0.92	88.38 $\pm$ 1.78	6.93 $\pm$ 0.22
8.0	*88.74 $\pm$ 2.02	8.66 $\pm$ 1.34	**97.42 $\pm$ 1.43	81.75 $\pm$ 1.84	6.36 $\pm$ 0.93	88.88 $\pm$ 1.72	6.93 $\pm$ 0.22
20.0	88.74 $\pm$ 2.02	8.66 $\pm$ 1.34	**97.42 $\pm$ 1.43	82.17 $\pm$ 1.88	6.41 $\pm$ 0.90	89.35 $\pm$ 1.20	6.93 $\pm$ 0.22

a) Percent of dose recovered with SE.

b) The sum of excretion in the urine. Those values of bile duct-cannulated rats include a trace of acetaminophen recovered from the urine (not shown).

The significances of differences between intact and bile duct-cannulated rats for AG or total fraction recovered in the urine, using Student's *t* test, are as follows: * $p < 0.05$ and ** $p < 0.01$

FIG. 1. Time Courses of Drug Concentrations in the Plasma of Bile Duct-Cannulated Rats after Intraduodenal (A), Intraileal (B), or Intracaecal (C) Administration of 452.4 $\mu\text{mol/kg}$ of AG

○, acetaminophen; ●, AS; □, AG.

Each point represents the mean of 4 rats with SE.

rentially to sulfate conjugate, because acetaminophen has an intermediate hepatic extraction ratio in the linear dose range^{15,16)} and the sulfation process is a main route of metabolism of acetaminophen.¹⁾ These results indicate that cleavage of AG occurs in the distal region of the gut and the liberated acetaminophen was reabsorbed.

However, AG appearance in the plasma after intraduodenal administration was rapid in con-

trast to the cases of acetaminophen and AS appearance. This indicates that a direct absorption of AG from the proximal intestinal lumen occurred, as has been previously suggested by Grafström *et al.*¹⁷⁾

Fig. 2 shows the time courses of the drug concentrations in plasma of bile duct-cannulated rats following intraduodenal, intraileal, or intracaecal administration of 452.4 $\mu\text{mol/kg}$ of aceta-

FIG. 2. Time Courses of Drug Concentrations in the Plasma of Bile Duct-Cannulated Rats after Intraduodenal (A), Intraileal (B), or Intracaecal (C) Administration of Acetaminophen at 452.4 $\mu\text{mol/kg}$. ●, acetaminophen; ●, AS; ○, AG. Each point represents the mean of 2 rats.

TABLE II. Influence of Site of Administration into the Gut on the Excretion Patterns in Urine and Bile after 452.4 $\mu\text{mol/kg}$ of AG or Acetaminophen (AAP) Injection into the Bile Duct-Cannulated Rats

Substance site			In urine (%) ^{a)}		In bile (%) ^{a)}			Total ^{b)}
			AS	AG	AAP	AS	AG	
AG	Intraduodenal	(n=4)	67.89 ± 1.99	13.13 ± 1.54	1.34 ± 0.32	4.25 ± 1.05	6.45 ± 0.40	93.14 ± 1.36
	Intraileal	(n=4)	65.83 ± 6.95	7.25 ± 2.08	1.75 ± 0.63	5.76 ± 0.94	3.47 ± 0.56	84.07 ± 7.00
	Intracaecal	(n=4)	76.14 ± 2.89	4.22 ± 1.64	0.32 ± 0.31	8.59 ± 1.51	4.05 ± 0.52	93.32 ± 1.30
AAP	Intraduodenal	(n=2)	62.11	4.46	0.26	8.14	6.11	81.07
	Intraileal	(n=2)	51.42	10.76	2.18	8.76	16.05	89.16
	Intracaecal	(n=2)	52.01	6.43	2.13	8.12	8.30	76.98

a) The mean percent of dose excreted (with SE) during 24 h. In the case of AAP, the mean percent of dose excreted is shown.

b) The sum of excretions in the urine and bile.

minophen. The drug absorption was rapid irrespective of site of administration, indicating that there is no site difference in the gut for the absorption of acetaminophen. Further, the time course data after acetaminophen administration differed from those after AG administration; plasma acetaminophen levels predominantly appeared at the earlier periods in contrast to the case of AG administration. This indicates that a rapid absorption of acetaminophen caused saturable kinetics of AS formation in the first-pass effect in the liver¹¹ because a rapid absorption of acetaminophen resulted in a high concentration in the liver vein in contrast to the case of AG administration.

Table II shows the recoveries in the urine and bile of bile duct-cannulated rats after intraduodenal, intraileal, or intracaecal administration

of AG and acetaminophen, respectively. There is no marked difference between AG and acetaminophen administration for the excretion patterns in urine and bile, indicating that AG was hydrolyzed by the microflora in the gut and reabsorbed as acetaminophen. But the AS fractions of dose recovered from the urine after acetaminophen administration are rather smaller than those after AG administration and there was a statistically significant difference ($p < 0.01$) between intraduodenal and intracaecal administrations of AG for the recovery of AG in the urine. These suggested that a rapid absorption of acetaminophen injected into the duodenum, ileum, or caecum caused saturable kinetics of AS formation and/or a part of AG injected into the duodenum was directly absorbed from the proximal intestinal lumen, in accord with

TABLE III. Pharmacokinetic Parameters for Area under the Curve (AUC), Mean Residence Time (MRT), and Mean Transit Time (MTT) Calculated from Mean Plasma Acetaminophen Concentration Data after Administrations of 452.4 $\mu\text{mol/kg}$ of AG into the Gut at Various Sites

Site	AUC ($\mu\text{M} \cdot \text{h}$)	MRT (h)	MTT (h)
Intraduodenal	96.6 (532.9)	5.22 (5.77)	
Intraileal	149.1 (536.2)	3.35 (3.71)	1.87 (2.06)
Intracaecal	152.3 (485.1)	1.76 (2.65)	3.46 (3.12)

The values of parameters in parentheses were obtained from mean plasma AS concentration data.

FIG. 3. Pharmacokinetic Model of the Enterohepatic Circulation of Acetaminophen Glucuronide in Intact Rats (A) or in Bile Duct-Cannulated Rats (B)

Compartments 1, 2, and 5 are the blood, urine, and feces, respectively. Compartment 3 or 4 is the gut. The amount of drug is designated by X and k is the rate constant. In the present case, k_{45} was set to be zero because the total fraction of dose recovered from the urine of intact rats was nearly unity.

plasma data.

The mean residence time (MRT) was calculated from the mean plasma concentration of acetaminophen after intraduodenal, intraileal, or intracaecal administration of AG and shown in Table III. The mean transit time (MTT) in the gut was also calculated by subtracting MRT of intraileal or intracaecal administration from the MRT of intraduodenal administration.

Pharmacokinetic Model of the Enterohepatic Circulation

The semilogarithmic plots of sigma minus value calculated from the mean total fraction

data for urine and bile of bile duct-cannulated rats of Table I *versus* time were linear. These linear plots included even the earlier period for both urine and bile data, indicating that the disposition of drug in the blood is appropriately described by one-compartment model. The overall elimination rate constants obtained from the sigma minus plots were 0.494 h^{-1} for urine and 0.579 h^{-1} for bile and these values are fairly close to each other.

As the result of Fig. 2 suggested the trigonometric function for the enterohepatic circulation to be valid, the resultant model of the

FIG. 4. A• Relationships between Calculated and Observed Fraction of Dose Excreted into the Urine (■) and Bile (●) of Bile Duct-Cannulated Rats after AG Injection

Solid lines are calculated values and observed values are the mean of 4 rats with SE.

B• Relationships between Calculated and Observed Fraction of Dose Excreted in the Urine (■) of Intact Rats after AG Injection

Solid lines are calculated values and observed values are the mean of 5 rats with SE. The amount of X_4 for calculated values is not shown because of its very slight amount.

TABLE IV. Pharmacokinetic Parameters ^{a)} Calculated from Least-Squares Fit of Models to Observed Data

Parameter (h^{-1})	Bile duct- cannulated rats	Intact rats
k_{12}	0.4182	0.5586
k_{13}	0.0383	0.0226
k_{34}		0.3246
k_{41}		2.0502

a) Parameters for the models are shown in Fig. 3.

enterohepatic circulation of AG is shown in Fig. 3A. In this model, the rate constant for the hydrolysis of AG in the gut was omitted because AG is rapidly hydrolyzed into acetaminophen *in vitro* by the microforal incubations.¹⁴⁾

As in the present study the total fraction of dose recovered from the urine of intact rats was nearly unity (Table I), the excretion of the drug into the feces could be neglected. Therefore, the differential equation of amount of drug (X) in each compartment is given by Eqs. 1–4.

$$dX_1/dt = -(k_{12} + k_{13})X_1 + k_{41}X_4 \quad (1)$$

$$dX_2/dt = k_{12}X_1 \quad (2)$$

$$dX_3/dt = k_{13}X_1 - k_{34}X_3 \quad (3)$$

$$dX_4/dt = k_{34}X_3 - k_{41}X_4 \quad (4)$$

where k is rate constant between each compartment.

On the other hand, the model of bile duct-cannulated rats is shown in Fig. 3B and the differential equations are also given by Eqs. 5–7.

$$dX_1/dt = -(k_{12} + k_{13})X_1 \quad (5)$$

$$dX_2/dt = k_{12}X_1 \quad (6)$$

$$dX_3/dt = k_{13}X_1 \quad (7)$$

The data for urine and bile of bile duct-cannulated rats were fitted to Eqs. 6 and 7 simultaneously, solving the differential equations by the Runge-Kutta-Gill method. There was a good quality of fit of the observed data to the calculated data ($r=0.9992$, Fig. 4A). The result of the fitting is shown in Table IV.

Similarly, the data for urine of intact rats were fitted to Eq. 2, solving the differential equations from 1 to 4 simultaneously. In this fitting, the initial value of k_{34} was obtained from the MTT assuming that the intestinal transit process of AG in the gut is a first-order kinetics, and that of k_{41} was also obtained appropriately from the time course data after acetaminophen injection into the gut. There was also a good quality of fit of the observed data to the calculated data ($r=0.9999$, Fig. 4B). The results are summarized in Table IV. The parameters obtained from intact rats is in approximate agreement with those of bile duct-cannulated rats. The result indicates that the compartment model with periodic trigonometric function for the entero-

hepatic circulation is valid in the rat.

DISCUSSION

The sum of excretions in the urine and bile for bile duct-cannulated rats was above 96% (Table I) and this result suggested that the excretion of AG into the other routes could be neglected. The total fraction of dose recovered from the urine of intact rats was about 97%. This indicated that excreted AG into the bile is completely subjected to the enterohepatic circulation. Based on these facts, the present enterohepatic circulation with no compartment for non-renal and -biliary, and fecal eliminations may be valid. Further, although in the present study we assumed the blood to be one-compartment model based on the result of the sigma minus plots, the observed and calculated values based on this model agreed reasonably well for both urine and bile (Fig. 4), suggesting that the assumption is valid.

In the present study, acetaminophen and AS appearance in the plasma after AG administration into the gut was evidently different between intraduodenal and intraileal (or intracaecal) administration (Fig. 1). These drug appearance in the plasma after intraduodenal administration was delayed markedly and had a lag period of about 2 h. This confirmed that there is a lag time in the enterohepatic circulation, and that the lag time is due to the period of passage of the conjugate down the intestinal tract until the conjugate reaches the site of hydrolysis in the gut. The value of 2 h for the lag time was fairly close to that of 3.7 h reported by Colburn *et al.*⁴⁾ in the rat. This is because a lag time for biliary transport would be ruled out, at least in the rat, because the lack of a gallbladder permits only hepatic bile secretion.

Although in a previous study,¹⁾ AG concentrations in the plasma of 1 out of 6 rats after intraduodenal administration of AG were mostly noise level (limit of detection, 7 μM) so that averaged plasma AG concentrations were negligible, in the present study AG levels in the plasma were detected in all of 4 rats even in the

earlier period (Fig. 1A). Furthermore, although in the previous study acetaminophen and AS concentrations in the plasma were detected at 3 h after intraduodenal administration of AG, in the present study AS concentrations in the plasma were detected at 2 h. These scatters in the plasma AG levels and lag time between the previous and present studies are not clear but may be partly explained due to these drastic experimental procedures. However, it is certain that there is a lag time for acetaminophen and AS appearance in the plasma after intraduodenal administration of AG. Further, rapid appearance of AG in the plasma after intraduodenal administration of AG suggested the possibility that direct absorption of AG from the proximal intestinal lumen occurs,¹⁷⁾ as have been reported for glucuronides of other compounds.^{18,19)}

As shown in Table IV, the ratio of k_{13} to k_{12} for intact rats is rather smaller than that of bile duct-cannulated rats. This may be partly explained by the fact that a part of AG excreted into the gut via the bile was directly absorbed from the proximal intestinal lumen. Consequently, the value of the ratio for intact rats would decrease apparently.

In the present study we calculated the MTT from the time course data after AG administration into the various sites of the gut and approximately estimated the transit rate constant in the gut assuming that this transit process to be a first-order kinetics. Based on this assumption, the enterohepatic circulation model with periodic trigonometric function was built up, in accord with those reported previously.^{5,7)} The obtained values of k_{34} and k_{41} were 0.3246 and 2.0502 h^{-1} , respectively. These values correspond to those in the case of 3 compartments in the gut adopted by Koizumi *et al.*⁷⁾; 0.2911 h^{-1} for k_{34} (defined by k_t in their paper) and 1.8586 h^{-1} for k_{41} (defined by k_r). Although to describe the gut by only 2 compartments may be physiologically unnatural, the present model with periodic trigonometric function for the enterohepatic circulation may be useful from the point of view of simplicity for the model.

From the fact that AG is rapidly hydrolyzed into acetaminophen *in vitro*,¹⁴⁾ in the present study the rate constant for the hydrolysis of AG in the gut was omitted. However, the time courses of AS and acetaminophen concentrations in the plasma after intraileal or intracaecal administration evidently differed between AG and acetaminophen administration (Figs. 1 and 2), indicating that the rate of appearance of liberated acetaminophen in the plasma after AG administration was very slow in contrast to the case of acetaminophen administration itself. If the result of *in vitro* is also applicable *in vivo*, the slow rate of appearance of liberated acetaminophen in the plasma may be due to the rate of access of AG to the enzyme site or to the rate of release of liberated acetaminophen to the intestinal lumen. It may be also considered due to the ability of the enzyme because a large amount of AG was injected rapidly in the present study. Although the reason for slow appearance of liberated acetaminophen in the plasma is not clear, it is certain that the obtained value of 2.0502 h^{-1} for k_{41} may not represent the true value of acetaminophen absorption rate constant because a rapid absorption after acetaminophen administration was confirmed by the fact that the plasma peak time after acetaminophen administration appeared at the first sampling time of 15 min, as shown in Fig. 2. Based on the above considerations, the value of k_{41} may represent a rate-limiting rate constant between drug appearance in the site of the hydrolysis and reabsorption.

But as being $k_{41} \gg k_{34}$, the enterohepatic circulation of AG is strongly rate-limited by the intestinal transit rate constant (k_{34}). The present model and those values may be useful to study the enterohepatic circulation for glucuronides of other compounds in the rat as well as further study of the fate of acetaminophen. Furthermore, fairly good accordance of the lag time to that reported by Colburn *et al.*⁴⁾ suggests that when a compartment model with a finite lag time is applied for the enterohepatic circulation of other glucuronides in the rat, the present value of the lag time is also useful.

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