

## The Pharmacokinetic Study on the Fate of 8-Hydroxyquinoline in Rat<sup>1)</sup>

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The fate of 8-hydroxyquinoline (Oxine) (OX) in rats, which is the parent compound of iodochlorhydroxyquin (chinoform, 5-chloro-8-hydroxy-7-iodoquinoline) (I) suspected to cause SMON, was investigated. Although both OX and I are metabolized to glucuronide and sulfate, the differences obtained from the excretion data are as follows: 1) After the intravenous administration of OX, glucuronide was more excreted (about 65% of dose) than sulfate (about 25% of dose) in urine in contrast to the case of I. 2) In bile, only 10% as glucuronide was excreted. The difference from I was considered due to the difference of molecular weight caused by the halogen substitution. 3) Both OX glucuronide and sulfate were stable in the body, and it was excreted as the administered form without exchanging to the another form after the intravenous administration of each conjugate.

Accordingly the fate of these compounds in the body was shown to be well influenced by the halogenation.

Furthermore, the blood concentration of free and conjugated OX, which was determined with synthesized <sup>14</sup>C-OX, was analyzed by the application of numerical deconvolution technique to the precursor-successor relationship between OX and each conjugate. Consequently, the usefulness of the technique was demonstrated and some pharmacokinetic features for OX were obtained.

**Keywords**—pharmacokinetics; numerical deconvolution method; two compartment model; non-linear curve fitting; 8-hydroxyquinoline; iodochlorhydroxyquin; glucuronide; sulfate

8-Hydroxyquinoline (Oxine) (OX) is the parent compound of iodochlorhydroxyquin (chinoform, 5-chloro-8-hydroxy-7-iodoquinoline) (I), which was used widely in Japan as an antimicrobia in the intestinal lumen. Now as I is suspected to cause SMON (subacute myelo-optico-neuropathy),<sup>3-5)</sup> it is important to investigate the fate of these compounds in the body and effect of halogen substitution to discuss the relationship between the chemical structure and the fate in the body. Thus, this study was carried out to get the basic information for the behavior in the body.

OX and its halogenated derivatives are metabolized mainly to glucuronide and sulfate, and excreted to urine and bile. In this study, the data on metabolism and excretion of OX in the rat were compared with I.<sup>6)</sup>

Moreover, for the purpose of pharmacokinetic analysis, <sup>14</sup>C-labelled OX was synthesized, and the blood concentrations of OX and its conjugated metabolites in the rats were also analyzed.

- 1) This work was presented at the 93th and 96th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1973, and Nagoya, April 1976, respectively. It was in partial fulfilment of Master of Pharmaceutical Science degree requirement of Hiroshi Kiwada to the Graduate School, University of Tokyo.
- 2) Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
- 3) T. Tsubaki, Y. Toyokura, and H. Tsukagoshi, *Japanese J. Med.*, **4**, 181 (1965).
- 4) Many researches are seen in "Collective List of References for SMON," ed. by SMON Research Committee in Japan (1974).
- 5) J. Tateishi, H. Ikeda, A. Saito, S. Kuroda, and S. Otsuki, *Neurology*, **22**, 702 (1972).
- 6) M. Hayashi, T. Fuwa, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **24**, 2589 (1976).

## Experimental

**Materials**—i) Sulfate of OX (Sul): Sul was synthesized by the method of Czapek<sup>7)</sup> with slight modification as follows. Pyridine was used as reaction medium in place of chloroform containing small amounts of pyridine. And the product was recrystallized three times from water.

ii) Glucuronide of OX (Glu): Glu was extracted from rabbits urine in which OX was given orally, according to the method for phenylglucuronide<sup>8)</sup> with some modification as follows. Oral dosage form was aqueous suspension with sodium carboxymethyl cellulose. Filtration was substituted for centrifugation to facilitate the extraction process. And the product was recrystallized three times from water.

iii) <sup>14</sup>C-OX: By Skraup reaction,<sup>9)</sup> 2,4-<sup>14</sup>C-OX was synthesized from 1,3-<sup>14</sup>C-glycerol according to the method of Urakubo, *et al.*<sup>10)</sup> After the product was extracted by steam distillation, the pale yellow fine needles were recrystallized from H<sub>2</sub>O-MeOH mixture. This product was confirmed by melting point and IR spectrum, comparing with authentic sample, 8-hydroxyquinoline (Tokyo Kasei, G.R.). Radiochemical purity was more than 99.0% and specific activity was 301  $\mu$ Ci/mmol.

**Methods of Separate Determination**—Firstly, both urine and bile sample were diluted to 10 ml with distilled water. And to 2 ml of each diluted sample was added 6 ml of ethylene dichloride (EDC), shaken for 15 min, and centrifuged for 15 min at 3000 rpm. EDC layer was used for unconjugated OX (Free) determination. Secondly, to 0.5 ml of aqueous layer was added 0.5 ml of pH 6.2 acetate buffer (0.2 M sodium acetate-0.2 M acetic acid), containing 1250 Fishman units of arylsulfatase (Boehringer Mannheim Co., Ltd., catalogue number 15473) and 1 mg of 1,4-saccharolactone (SLNA) for inhibition of contaminated glucuronidase activity. The mixture was incubated at 38° for 24 hr to hydrolyze Sul completely, and then, 6 ml of EDC was added, shaken and centrifuged in the same way. This organic layer was used for the determination of Sul. To another 0.5 ml of aqueous layer of the first extract was added 0.5 ml of pH 6.2 acetate buffer containing 2500 Fishman units of arylsulfatase and 1.0 ml of pH 4.5 acetate buffer containing 1500 Fishman units of  $\beta$ -glucuronidase (Boehringer Mannheim Co., Ltd., catalogue number 15472). The mixture was incubated at 38° for 48 hr to hydrolyze Glu and Sul completely. And then, 6 ml of EDC was added, shaken and centrifuged similarly. This organic layer was used for the determination of total conjugates (Glu+Sul).

Then, the determination for each liberated free form in EDC layer was as follows. To the 5.0 ml of the EDC extract layer from urine or bile sample was added 5.0 ml 1 N-NaOH, shaken for 15 min, and centrifuged for 15 min at 3000 rpm. The aqueous layer was determined at 254 nm UV absorption with Hitachi 139 spectrophotometer.

For blood samples, 0.1 ml of each sample was diluted to 2 ml with distilled water. And this aqueous solution was used for the separate determination similarly as urine and bile. However, benzene was used for extraction instead of EDC, and to 5 ml of this benzene layer was added 15 ml of liquid scintillator (0.4 mg POPOP and 6.5 g DPO/1000 ml toluene) and determined with Aloka LSC-601 liquid scintillation counter.

**Experimental Procedure**—Male albino rats of Donryu (280–300 g) were used. Propylene glycol solution for OX, 0.05 N-NaHCO<sub>3</sub> solution for Glu, or aqueous solution for Sul were administered intravenously through the femoral vein, respectively. Bile and urine samples were collected at appropriate time intervals until 8 hr through polyethylene tubes set into bile duct, *i.e.* bile fistula, and bladder, respectively. After <sup>14</sup>C-OX in propylene glycol solution 4.86 mg containing 10.1  $\mu$ Ci per head was administered intravenously, blood samples were collected from femoral artery through polyethylene cannula at appropriate time intervals until 4 hr.

**Data Analysis**—The non-linear least square method was applied for kinetic analysis of blood concentration data. The deconvolution was carried out numerically with the program reported previously.<sup>11)</sup>

## Results

### Excretion of OX and Its Conjugates

The urinary excretion time-courses after OX was administered intravenously are shown in Fig. 1, where the shoulders were observed around 3 to 5 hr. In contrast, Fig. 2 shows that the shoulders disappeared when bile was taken out by cannulation of the bile duct. Since the excretion almost ceased by 8 hr as shown in Fig. 2, the excretion ratios until 8 hr are listed in Table I with the previous results of I for comparison. The excreted metabolites

7) E. Czapek, *Monatsh. Chem.*, **35**, 641 (1914).

8) J.M. Porteous and R.T. Williams, *Biochem. J.*, **44**, 46 (1949).

9) A. Das and S.L. Mukherjee, *Science and Culture*, **16**, 477 (1951).

10) G. Urakubo and Y. Kido, *Radioisotopes*, **24**, 48 (1975).

11) H. Kiwada, K. Morita, M. Hayashi, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **25**, 1312 (1977).

of OX were Glu and Sul in urine and only Glu in bile, while unmetabolized OX was hardly detected both in urine and bile. Although these tendencies are consistent with I, Glu was excreted in urine to a larger extent than Sul, in contrast to I of which glucuronide was excreted to a less extent than its sulfate.

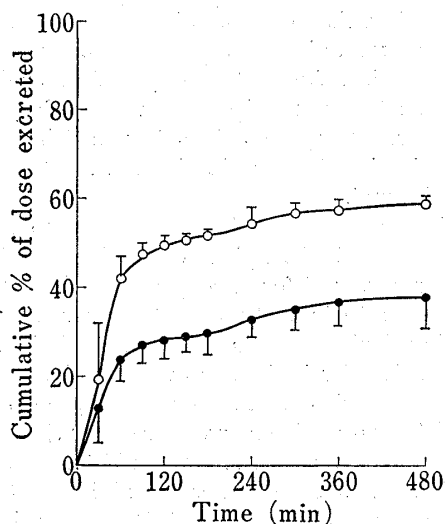


Fig. 1. Urinary Excretion Time-Courses of Conjugates after Intravenous Administration of 8-Hydroxyquinoline (OX) in Rat without Bile Fistula

○: glucuronide.  
●: sulfate.

Dose: 5.02 mg/head.

Lines are drawn by visual approximation. Circles shown are the mean values of 3 rats and standard deviations are expressed with vertical bars in each direction.

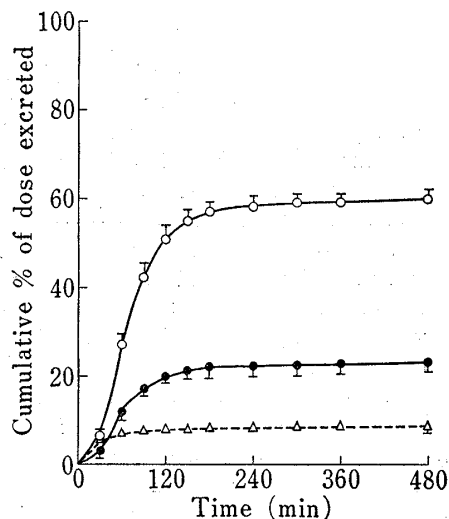


Fig. 2. Urinary and Biliary Excretion Time-Courses of Conjugates after Intravenous Administration of 8-Hydroxyquinoline (OX) in Rat with Bile Fistula

○: glucuronide in urine.  
●: sulfate in urine.  
△: glucuronide in bile.

Dose: 5.02 mg/head.

Lines are drawn by visual approximation. Points are the mean values of 3 rats and standard deviations are expressed with vertical bars in each direction. But only glucuronide in bile is expressed as the mean values of 2 rats except the final point.

TABLE I. Comparison between 8-Hydroxyquinoline (OX) and Iodochlorhydroxyquin (I)<sup>6)</sup> on Conjugation Metabolism and Stability of their Conjugates in Rat

Administered Form ( <i>i.v.</i> )	Excreted Form	OX		I	
		Urine	Bile	Urine	Bile
F <sup>a)</sup>	G	59.9±1.9	8.7±0.2	6.4±2.1	32.3±0.5
	S	22.9±2.1	—	34.6±2.4	—
G <sup>b)</sup>	G	91.2±4.9	10.5±1.0	1.7±1.1	37.6±8.1
	S	—	—	9.9±1.8	—
S <sup>c)</sup>	G	—	—	2.2±0.9	24.9±6.0
	S	96.7±5.4	—	44.4±4.3	—

F: free form G: glucuronide S: sulfate —: not detectable (less than 1%).

a) Dose: 5.02 mg for OX and 4.53 mg for I.

b) Dose: 3.24 mg as Free OX and 2.97 mg as Free I.

c) Dose: 1.40 mg as Free OX and 2.97 mg as Free I.

Free forms of OX and I were administered intravenously as propylene glycol solution and CMC aqueous suspension, respectively, and other dosage forms were aqueous solutions. Excretion Ratios until 8 hr for OX and until 10 hr for I are expressed as per cent of dose, but excretion of free form was negligibly small in all cases. Results are expressed as the mean ± value standard deviation of three rats.

### Enterohepatic Circulation

When the bile of one rat to which OX had been given intravenously was infused into the duodenum of the other rat, the urinary excretion of the conjugates was observed as shown in Fig. 3. These results confirmed the enterohepatic circulation which was suggested by the shoulder in Fig. 1 and its disappearance in Fig. 2. And the results also shows that OX was reabsorbed after the hydrolysis of excreted Glu in bile, since the urinary excretion of Sul which was not excreted in bile was observed in Fig. 3.

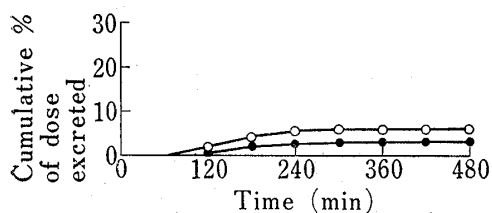


Fig. 3. Urinary Excretion Time-Courses of Conjugated 8-Hydroxyquinoline (OX) in the Rat, when the Bile of the Other Rat Administered OX Intravenously was Infused into the Duodenum

○: glucuronide.  
●: sulfate.  
Dose: 5.02 mg/head.

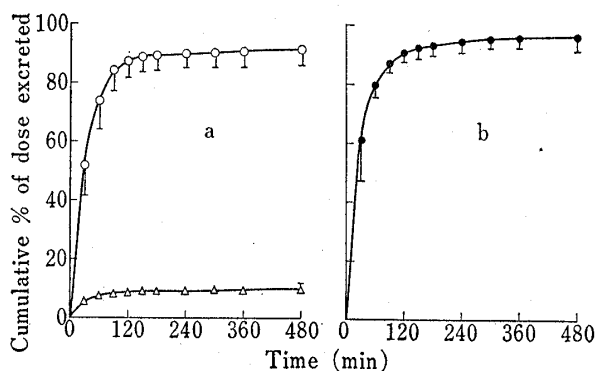


Fig. 4a,b. Urinary and Biliary Excretion Time-Courses of Conjugated 8-Hydroxyquinoline (OX) after Intravenous Administration of Conjugated OX in Rat

a) Glucuronide administration (Dose: 3.24 mg/head).  
○: glucuronide in urine. △: glucuronide in bile.  
b) Sulfate administration (Dose: 1.40 mg/head).  
●: sulfate in urine.

Points are the mean values of 3 rats and standard deviations are shown with vertical bars in the downward direction. Only glucuronide in bile are expressed as the mean values of 2 rats except the final point. All lines are drawn by visual approximation.

### Fate of the Conjugates *in Vivo*

When Glu was administered intravenously in the rat, about 90% and 10% of the dose (3.24 mg as OX per head) were excreted as Glu in urine and bile, respectively, while neither Free nor Sul was detected (Fig. 4-a, Table I). And when Sul was administered intravenously, more than 95% of the dose (1.40 mg as OX per head) was excreted in urine as Sul, but neither Free nor Glu was detected (Fig. 4-b, Table I).

These results which show that no hydrolysis of the conjugates of OX occur *in vivo* present a remarkable contrast to the previous results where both conjugates, *i.e.* glucuronide and sulfate of I were hydrolyzed and reconstituted *in vivo* (Table I).

### Blood Concentration Time-Courses of OX and Its Conjugates

The blood concentration time-courses of OX and its conjugates after intravenous administration of OX (4.86 mg containing 10.1  $\mu$ Ci) in the rat with bile fistula are shown in Fig. 5. Since the semilogarithmic plots of OX concentration data were not linear, the data were analyzed by the routine program for two compartment model.<sup>12)</sup> The good fitness was observed as shown in Fig. 5-a.

### Pharmacokinetic Analysis of Blood Concentration Time-Courses

In order to analyze the conjugates data, it was necessary to determine how many compartments are needed in the kinetic model of each conjugate to describe sufficiently the time

12) S. Awazu, M. Hanano, H.H. Moon, T. Fuwa, T. Iga, and H. Nogami, "Absorption, Metabolism, and Excretion of Drugs," based on the Second Symposium on Drug Metabolism and Action ed. by K. Kakemi, Hirokawa Publishing Co., Tokyo, 1971, p. 301.

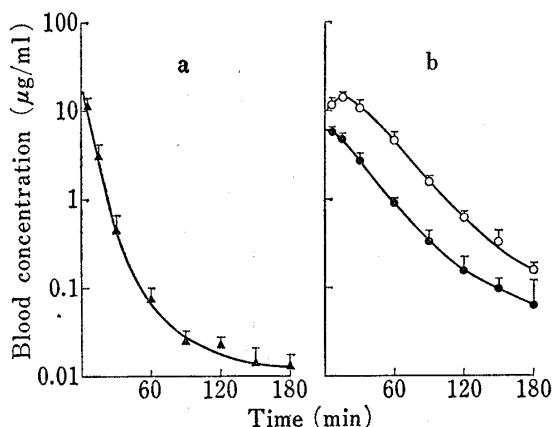


Fig. 5a,b. Semilogarithmic Blood Concentration Curves of 8-Hydroxyquinoline (OX) and Its Conjugates after Intravenous Administration of OX in the Bile Fistula Rat

▲: free. ○: glucuronide. ●: sulfate.

Dose: 4.86 mg/head.

Points are the mean values of three rats and standard deviations are expressed with vertical bars in the upward direction. Data of OX blood concentrations were fitted to two compartment model by non-linear least square method and results were shown in Fig. 5a.

Lines in Fig. 5b are drawn by visual approximation.

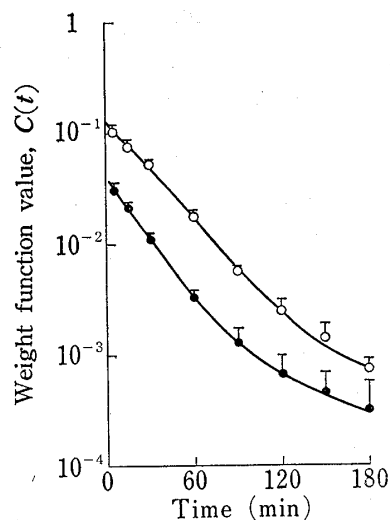


Fig. 6. Weight Function Values Calculated by Deconvolution with Blood Concentration Data of Free 8-Hydroxyquinoline (OX) and Its Conjugates after Intravenous Administration of Free OX

○: glucuronide. ●: sulfate.

Circles are the mean values of deconvoluted values for each run ( $n=3$ ), and vertical bars are their standard deviations. Lines were calculated by non-linear least square method fitting.

course. And further, initial approximate kinetic parameters were also necessary to fit the data to the kinetic model by non-linear least square method. The simple way to determine these requisites is to analyze the data after each conjugate was administered intravenously. But to do so in the present study,  $^{14}\text{C}$ -labelled compounds were necessary, and it was too much time consuming and too expensive work to obtain such compounds. In order to overcome these difficulties, the precursor-successor rule (see Appendix) and the numerical deconvolution technique which is reported elsewhere were used.<sup>11)</sup>

Since there is a precursor-successor relationship between OX and each conjugate, the following equation can be given:

$$B(t) = \int_0^t c(t) \cdot A(t-\theta) d\theta \quad (1)$$

where  $A$  and  $B$  are blood concentration of OX and its conjugates, *i.e.* Glu or Sul, respectively, and  $C$  is a weight function between  $A$  and  $B$ . The practical meaning of  $C$  is given as;

$$C'(t) = \frac{D}{V_1 \cdot k} C(t) \quad (2)$$

where  $D$ ,  $V_1$  and  $k$  are dose, distribution volume of OX and conjugation rate constant from OX to a corresponding conjugate, respectively, and  $C'$  is blood concentration of a corresponding conjugate when the conjugate is administered intravenously as a bolus.

$C$  was calculated by the numerical deconvolution technique for each run, and the mean values were shown in Fig. 6. The calculated points of  $C$  were fitted well to two compartment model for each metabolite. From these findings that Glu and Sul given as a bolus are eliminated from blood by two compartment model as well as OX, the pharmacokinetic model of OX including its conjugates was determined as shown in Chart 1. Then, all kinetic parameters were determined by non-linear least square method starting from the initial values which were

determined from the respective two compartment model and are shown in Table II and Fig. 7.

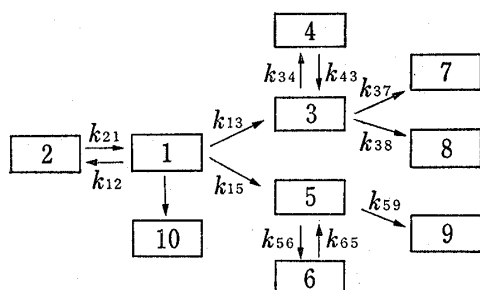


Chart 1. The Pharmacokinetic Model on the Fate of 8-Hydroxyquinoline (OX) in the Rat

Compartment 1: free OX in blood,  
3: glucuronide in blood,  
5: sulfate in blood,  
7: glucuronide in urine,  
8: glucuronide in bile,  
9: sulfate in urine.

Compartment 2, 4 and 6 mean the free, glucuronide, and sulfate which distributed slowly in tissue from plasma, respectively. Compartment 10 means the unknown metabolite or deposit.

$k$ : rate constant.

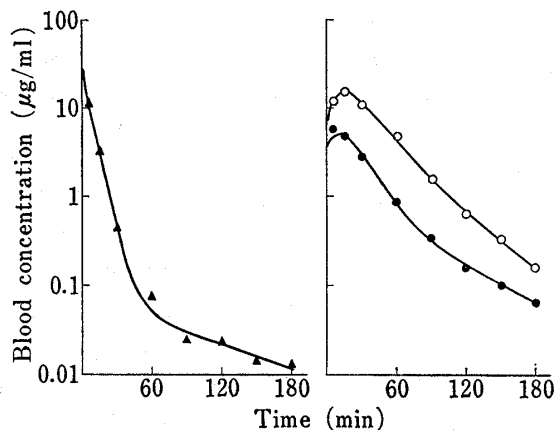


Fig. 7. Calculated Semilogarithmic Blood Concentration Curves and Observed Data Plots of Free and Conjugated 8-Hydroxyquinoline (OX) after Intravenous Administration of OX in the Bile Fistula Rat

▲: observed data of free.  
○: observed data of glucuronide.  
●: observed data of sulfate.

Lines are calculated curves by non-linear least square method fitting to the model in Chart 1.

TABLE II. The Calculated Pharmacokinetic Parameters of OX on the Basis of the Pharmacokinetic Model Described in Chart 1

$k_{12} = 0.00470 \pm 0.00062$	
$k_{21} = 0.01179 \pm 0.00219$	
$k_{10} = 0.01081 \pm 0.00078$	
$k_{13} = 0.08837 \pm 0.00637$	
$k_{15} = 0.02975 \pm 0.02975$	
$k_{34} = 0.000919 \pm 0.002375$	$k_{56} = 0.00915 \pm 0.00468$
$k_{43} = 0.01924 \pm 0.03597$	$k_{65} = 0.02214 \pm 0.00595$
$k_{37} = 0.03227 \pm 0.00344$	$k_{59} = 0.05410 \pm 0.00792$
$k_{38} = 0.00323 \pm 0.00034$	
$V_1 = 211.4 \pm 37.1$	
$V_3 = 127.3 \pm 16.6$	
$V_5 = 97.4 \pm 16.5$	

$k$ : rate constant,  $\text{min}^{-1}$ .  $V$ : distribution volume, ml.  
Values represent pharmacokinetic parameters  $\pm$  standard errors.

## Discussion

Although the structural difference between I and OX is only two substituted halogen atoms, Cl and I in 5 and 7 position, respectively, their pharmacokinetic behaviors are remarkably different. The conversion between the conjugates of I, *i. e.* the glucuronide and sulfate, occurs *in vivo*, while such a conversion does not occur between the conjugates of OX, *i. e.* Glu and Sul. More than 90% of Glu and Sul as well as OX are excreted by 8 hr after intravenous administration. In contrast, in the case of administration of I and its conjugates, about 30% or more are not excreted even after 10 hr (Table I). These results show the complexity of the pharmacokinetic behavior of I.

The distribution rate constants of OX and its conjugates are rather small, compared to their elimination constants. The reason of this characteristic feature can not be given yet. Another characteristic feature is that the urinary excretion rate constant of Glu ( $k_{37}$ ) is smaller than the conjugation rate constant OX ( $k_{13}$ ). This is not consistent with the customary assumption that the excretion process of a water soluble conjugate is not a rate determining in an elimination process of a lipophilic compound. But this is not the first case of an exception for the above assumption. For example, the excretion rate constant of N-acetyl-*p*-aminophenyl glucuronide is smaller than the rate constant of its conjugation.<sup>13)</sup> Accordingly, it is suggested that there should be case to take such assumption when glucuronide formation and excretion are analyzed.

The present pharmacokinetic analysis shows the usefulness of the numerical deconvolution technique. To make the analysis possible, it is assumed *a priori* that the glucuronide and sulfate conjugation occur in the compartment which includes blood. Since liver in which the conjugations occur is a well perfused organ which is usually classified as a central compartment,<sup>14)</sup> the assumption is reasonable. And the other necessary assumption that no conversion occurs between OX and its conjugates is supported with the results of Table I and with that the data when bile was taken out to prevent the enterohepatic circulation of Glu excreted in bile were analyzed.

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## Appendix

### Precursor-Successor Rule

Assume that a precursor is described by the (1 to  $m-1$ ) compartment system and its successor is described by the ( $m$  to  $n$ ) compartment system. And further assume that a precursor is introduced to compartment 1 by the rate of  $I(t)$  which is an arbitrary function of  $t$ , and that the compartments in each system are connected reversibly or irreversibly by a rate constant of  $k_{ij}$  but the precursor is connected irreversibly to the successor only by  $k_{1m}$ . Then the amounts of the precursor in compartment 1 ( $X_1$ ) and of its successor in compartment  $m$  ( $X_m$ ) are given in the Laplace transform as;

$$x_1 = \frac{(-1)^{1+1} \Delta_{1:1}}{\Delta} i \quad (\text{A-1})$$

$$x_m = \frac{(-1)^{1+m} \Delta_{1:m}}{\Delta} i \quad (\text{A-2})$$

where

$$\Delta = \begin{vmatrix} s+K_1 & -k_{21} & \cdots & -k_{m-1,1} & 0 & 0 & \cdots & 0 \\ -k_{12} & s+K_2 & \cdots & -k_{m-1,2} & 0 & 0 & \cdots & 0 \\ \cdot & \cdot & \cdots & 0 & \cdot & \cdot & \cdots & \cdot \\ -k_{1,m-1} & -k_{2,m-1} & \cdots & s+K_{m-1} & 0 & 0 & \cdots & 0 \\ -k_{1m} & 0 & \cdots & 0 & s+K_m & -k_{m+1,m} & \cdots & -k_{nm} \\ 0 & 0 & \cdots & 0 & -k_{m,m+1} & s+K_{m+1} & \cdots & -k_{n,m+1} \\ \cdot & \cdot & \cdots & \cdot & \cdot & \cdot & \cdots & \cdot \\ 0 & 0 & \cdots & 0 & -k_{m,n} & -k_{m+1,n} & \cdots & s+K_n \end{vmatrix} = \Delta^1 \Delta^2 \quad (\text{A-3})$$

13) J. Shibasaki, R. Konishi, Y. Takeda, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1800 (1971).

14) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 117 (1968).

and  $\Delta_{j,l}$  is the determinant obtained from  $\Delta$  by elimination of the  $j$ -th row and  $l$ -th column, and  $x$  and  $i$  are the Laplace transform of  $X$  and  $I$ , respectively. Subscripts denote each compartment, and  $K_i = \sum_j k_{ij}$ . And

$$\Delta^1 = \begin{vmatrix} s+K_1 & -k_{21} & \cdots & -k_{m-1,1} \\ -k_{12} & s+K_2 & \cdots & -k_{m-1,2} \\ \cdot & \cdot & \cdots & 0 \\ -k_{1,m-1} & -k_{2,m-1} & \cdots & s+K_{m-1} \end{vmatrix} \tag{A-4}$$

$$\Delta^2 = \begin{vmatrix} s+K_m & -k_{m+1,m} & \cdots & -k_{nm} \\ -k_{m,m+1} & s+K_{m+1} & \cdots & -k_{n,m+1} \\ \cdot & \cdot & \cdots & \cdot \\ -k_{mn} & -k_{m+1,n} & \cdots & s+K_n \end{vmatrix} \tag{A-5}$$

Then,

$$\Delta_{1:1} = \Delta_{1:1}^1 \Delta^2 \tag{A-6}$$

$$\Delta_{1:m} = (-1)^{1+m} k_{1m} \Delta_{1:1}^1 \Delta_{1:1}^2 \tag{A-7}$$

From Eqs. (A-1), (A-2), (A-6) and (A-7),

$$x_m = k_{1m} \frac{\Delta_{1:1}^2}{\Delta^2} x_1 \tag{A-8}$$

Then,

$$c_m = \left( \frac{k_{1m}}{V_m} V_1 \right) \left( \frac{\Delta_{1:1}^2}{\Delta^2} \right) c_1 \tag{A-9}$$

where  $c$  is the Laplace transform of concentration  $C$ . Accordingly, deconvolution between  $c_1$  and  $c_m$  gives the weight function of  $G$  as,

$$G = \left( \frac{k_{1m}}{V_m} V_1 \right) L^{-1} \left( \frac{\Delta_{1:1}^2}{\Delta^2} \right) \tag{A-10}$$

where  $L^{-1}$  represents the inverse Laplace transform. And the concentration time course of the successor after its bolus administration to compartment  $m$  is described as,

$$\begin{aligned} C_{m, \text{bolus}} &= \left( \frac{1}{V_m} \right) L^{-1} \left( \frac{\Delta_{1:1}^2}{\Delta^2} \right) \\ &= \frac{G}{k_{1m} V_1} \end{aligned} \tag{A-11}$$

This corresponds to Eq. (2) in the text.