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Differences in Species of Iodochlorhydroxyquin Absorption, Metabolism, and Excretion[1]

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The differences in species for absorption, metabolism and excretion of iodochlorhydroxyquin (I) were investigated by the separate determination method of unmetabolized I (free form) and its conjugated metabolites, *i.e.*, glucuronide (I-G) and sulfate (I-S) in urine and bile. As for urinary excretion ratio in rat, the order of the conjugates was I-S>I-G, while in guinea-pig and rabbit, I-G>I-S, and in man, I-G≫I-S. Moreover, the order of biliary excretion ratio in guinea-pig was characteristically I-S≫I-G differently from rat, in which it was I-G≫I-S. In all cases, unmetabolized I was of trace and neglected.

In addition to urinary and biliary excretion, as to blood concentration after oral administration, the complexed absorbability of I was shown. In small animals (rat and guinea-pig), the order of the concentration of unmetabolized I and the conjugates was I-G and I-S> unmetabolized I, but in beagle which was sensitive to the toxicity of I, unmetabolized I>I-G and I-S.

Furthermore, the biological stability of the conjugates of I was studied and the conversion of I-G⇌I-S was found to occur in rat. This result shows that the conjugates are hydrolyzed and reconjugation to glucuronide or sulfate occurs in rat.

Since species and strain differences of neurotoxicity of iodochlorhydroxyquin (chinoform, clioquinol or 5-chloro-7-iodo-8-quinolinol) (I) which caused SMON (subacute myelo-optico-neuropathy) were reported,[3-5] the difference of biopharmaceutical behavior of I has been interested to explain the difference of its neurotoxicity, to study the mechanism of SMON occurrence, to select an appropriate small animal in which SMON is induced experimentally, *etc.* In order to study biopharmaceutically, the present paper is to report the separate determination method of I and its metabolites, *i.e.*, iodochlorhydroxyquin glucuronide (I-G) and sulfate (I-S) in urine and bile. The method was standardized with synthesized I-G[6] and I-S.[7] And the species differences of absorption, metabolism and excretion of I determined with this method is also reported.

Experimental

Separate Determination of Conjugates——Immediately after sampling, biological sample, 1 ml of urine or 0.5 ml of bile, was heated for 30 sec in boiling water to inactivate native hydrolytic enzymes. After this, to 1 ml of urine sample was added 3 ml of ethylene dichloride (EDC), shaken for 15 min and centrifuged for 15 min at 3000 rpm. The EDC layer was used for unconjugated I (free form) determination. To 0.5 ml of urine layer was added 7 ml of the acetate buffer (0.4 M acetic acid–0.4 M sodium acetate, pH 4.5) containing 740 Fishman units of β-glucuronidase (Boehringer Mannheim, catalogue number 15472). The mixture was incubated at 38° for 30 min to hydrolyze I-G completely. Twenty-five ml of EDC was added to the mix-

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2) Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*

3) J. Tateishi, S. Kuroda, A. Saito, and S. Otsuki, *Lancet*, 1, 1289 (1972).
4) J. Tateishi, S. Kuroda, A. Saito, and S. Otsuki, *Lancet*, 2, 1263 (1971).
5) R. Brueckner, R. Hess, C. Pericin, and J. Tripod, *Arzneimittel-Forsch.*, 20, 575 (1970).
6) I. Matsunaga, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), 19, 190 (1971).
7) C.T. Chen, K. Samejima, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), 21, 911 (1973).

ture, shaken for 15 min and centrifuged for 15 min at 3000 rpm. The EDC layer was used for the determination of the liberated free form. To 5 ml of the buffer layer was added 2 ml of 1 N HCl and incubated for 24 hr at 38° to hydrolyze I-S. The liberated free form was extracted in 25 ml of EDC, which was used to determine free form. For this determination of free form in EDC layer, 2 ml or 20 ml of the EDC layer which had been kept for unmetabolized I and liberated free form determination, respectively, was evaporated, Modifying the method of Haskins, *et al.*[8] slightly, the residue was dissolved with 2.5 ml of methyl cellosolve, and 0.5 ml of $FeCl_3$ reagent solution[9] was added. Green I–Fe chelate was determined at 660 nm with Hitachi 124 Spectrophotometer. As for bile sample, to 0.5 ml of the sample was added 7 ml of distilled water and 30 ml of EDC. After shaken and centrifuged as described above, 5 ml of the bile phase was used for the determination of I-G. Other procedures and reagent volumes were the same with those described for the determination of urine sample. For plasma or serum sample, 0.05 to 0.5 ml of the sample was diluted to 1 ml with distilled water. I-G was hydrolyzed with 370 Fishman units of β-glucuronidase described previously at pH 4.5 and 38° for 1 hr and I-S was hydrolyzed with 1 N HCl at 38° for 5 hr. The other procedures were the same as the method of C.T. Chen, *et al.*,[10] which used gas chromatography (In this paper, gas chromatography was Shimadzu Seisakusho Ltd., GC-3BE type.) with electron capture detector.

Experimental Procedures for Small Animals——Male albino rats of Donryu (280—300 g) and male guinea-pigs of Hartley (400—450 g) were used. Both were fasted a night before experiment. After administration of I into a stomach directly with a stainless steel tubing through a mouth, urine sample was collected at appropriate times until 24 hr through a polyethylene tubing (HIBIKI No. 6 in rats and No. 9 in guinea-pigs) set into a bladder. Bile sample was also collected through a polyethylene tubing set into bile duct (*i.e.* bile fistula), and saline solution was infused into the duodenum with the automatic infusion pump (Natsume Seisakusho Ltd., KN-I type or Furue Science Co. Ltd., Microfeeder JP-W-100, 200G type) at the rate of 0.8 ml/hr in rats or 4.2 ml/hr in guinea-pigs until 24 hr after oral administration in order to prevent the water deficiency in the body. Femoral artery cannulation were used for plasma sample. I-G and I-S were administrated through femoral vein, respectively, with a view to studying the behavior of their conjugates, and urine and bile samples were collected at appropriate time intervals until 10 hr. After operation, both animals were kept in restraint cages and no foods were given, but water could be taken *ad libitum* during recovery and experimental periods.

In order to further investigate the urinary excretion in rabbit, it (about 2.5 kg) was fasted a night and I was orally administrated with a rubber catheter. It was kept in a metabolic cage in which only water could be taken *ad libitum*. Urine sample until 5 hr after administration was collected by putting a rubber catheter into a bladder.

Experimental Procedures and Regulation of Food for Man——Six hundred milligrams of I (usual doses in a day, Japanese Pharmacopoeia VIII) were pulverized in a motar with a pestle in the presence of 120 mg of CMC–Na and taken orally by five healthy adult volunteers. Urine samples were collected at appropriate time intervals until 24 hr. For food regulation, volunteers who were prohibited to take breakfast took I with 220 ml of water around at 10: 00 a.m. and took 100 ml of water and a light meal in 2 hr and 3 hr after the drug ingestion, respectively. They were not permitted to begin to take usual foods until 7 hr after the drug ingestion.

Materials——I was supplied from SMON Research Commission in Japan. Sodium carboxymethyl cellulose (CMC–Na) was obtained from Daiichi Pure Chemical Co. Ltd., and used without further purification. The other reagents were of special grade and purchased from Wako Pure Chemical Industries, Ltd. Aqueous suspension of I with CMC–Na was prepared by the insonater (Kubota Seisakusho Ltd., Model 200M). The concentration of CMC–Na used was 0.32 or 1.0% (w/v). I-G and I-S were synthesized and gifted from Prof. Z. Tamura.[6,7]

Determination of Dose——The dose of aqueous suspension administrated was determined as follows in order to correct a little amount of the suspension left in the syringe after administration. As for CMC–Na aqueous suspension, the samples of the same volume as the practical administration were extracted three times with EDC containing small amounts of ethyl alcohol and finally made 100 ml. And the proper quantity was determined by the Fe–chelate method described previously. On the other hand, aqueous suspension without CMC–Na was extracted completely only once with 25 ml of EDC.

Results and Discussion

Separate Determination of Glucuronide and Sulfate Conjugates

The β-glucuronidase used in this determination contains less than 2% of arylsulfatase and it was found that I-G was hydrolyzed rapidly with a small amount of the enzyme. There-

8) W.T. Haskins and G.W. Luttermoser, *Anal. Chem.*, 23, 456 (1951).
9) The reagent required is an aqueous solution of ferric chloride containing 1 g of ferric ion and 1 ml of concentrated hydrochloric acid per liter.
10) C.T. Chen, K. Samejima, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), 24, 97 (1976).

fore, the result of the enzyme hydrolysis, under the condition that I–G was hydrolyzed exclusively with keeping I–S as stable as possible, was shown in Fig. 1. The amount of the enzyme required for the above condition was 740 Fishman units. Consequently, it was found that I–S was hardly hydrolyzed (less than 4%) with 740 Fishman units during 30 min incubation at 38° in which the hydrolysis of I–G was completed. I–S could not be so completely hydrolyzed by the marketed enzymes, for example, arylsulfatase (Boehringer Mannheim Co. Ltd., catalogue number 15473), that I–S was hydrolyzed in acidic medium as described in the experimental part after the enzymatic hydrolysis of I–G. Thus, the separate determination was completed and the recovery ratios in this determination were 89.0% for I–G in urine, 82.2% for I–S in urine, 93.5% for I–G in bile, and 78.0% for I–S in bile.

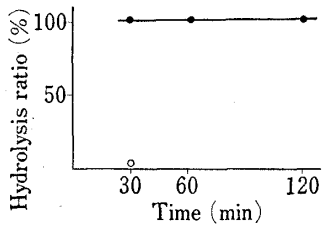

Fig. 1. Time Course of Hydrolysis of Iodochlorhydroxyquin Glucuronide and Sulfate in Urine 0.5 ml+pH 4.5 Acetate Buffer 7 ml Containing β-Glucuronidase 740 Fishman Units

●: glucuronide, ○: sulfate

Urinary and Biliary Excretion of I in Rat

The cumulative urinary excretion time course after oral administration of 1 ml of 0.32% (w/v) CMC–Na aqueous suspension (dose=15 mg) was shown in Fig. 2. The excreted amount of I–S was much greater than that of I–G, and the amount of unmetabolized I was of trace and neglected. The excretion ratio of the dose was about 12% until 24 hr. The excretion still continued after 24 hr and the total per cent of the dose amounted to about 20% until 72 hr.

In order to study biliary excretion after absorption of I, the same CMC–Na aqueous suspension was administrated orally in the rat with bile fistula. Cumulative urinary and biliary excretion of I were shown in Fig. 3. As seen from Fig. 3, the excreted conjugate in bile was exclusively I–G, and I–S was found very little (almost not detectable). And the

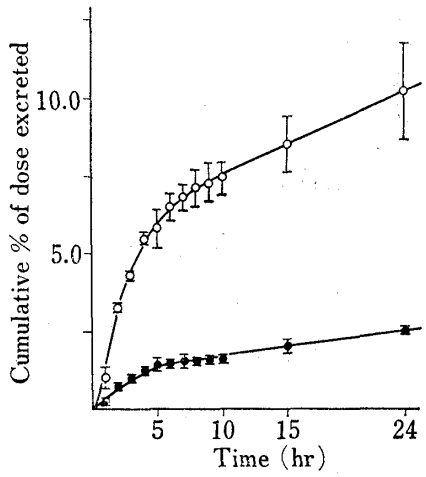

Fig. 2. Cumulative Curves of Urinary Eucretion Ratio after Oral Administration of Iodochlorhydroxyquin CMC aqueous Suspension in Rat

dose: 15 mg
—●—: glucuronide
—○—: sulfate
Circles and vertical bars indicated the mean values and ± standard deviations (±S.D.) of 3 rats, respectively.

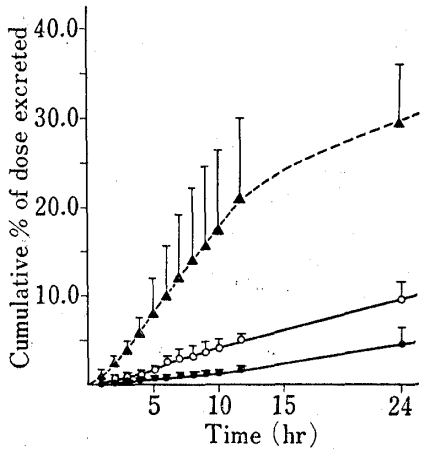

Fig. 3. Cumulatiue Curves of Urinary and Bilary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin CMC aqueous Suspension in Rat with Bile Fistula

dose: 15 mg
—●—: glucuronide in urine
—○—: sulfate in urine
---▲---: glucuronide in bile
Sulfate in bile was almost not detectable.
Each point and vertical bar represent the mean value and standard deviation (S.D.) in each direction of 3 rats, respectively.

biliary excretion as I–G after absorption of I was greater than the urinary excretion as I–G and I–S. The amount of unmetabolized I was of trace and neglected in both urine and bile. When the sum of the excreted ratio to the dose of I–G and I–S was regarded as the total excreted ratio of I since other metabolites than I–G and I–S are unreported at present and the excretion ratio of the unknown metabolites was very little,[11,12] the total per cent of the dose excreted was about 43% in urine and bile until 24 hr. But the excretion after 24 hr was also important regarding the problem of the deposition in body and therefore, urinary excretion after 24 hr and various factors affecting absorption of I will be discussed in the next paper.[13] The data in the rats with bile fistula as shown in the experimental part seemed to be fairly scattered, because bile which is probably one of the factors could not participate in the absorption of I in this experiment.

So far as comparing these data in the rats with and without bile fistula, the bile effect on urinary excretion after absorption of I in rats was not so clear. The bile effect and the contribution of the entero-hepatic circulation of I to absorption, which was neglected owing to bile fistula, should also be further studied.

Urinary and Biliary Excretion in Guinea-Pig

The total urinary excretion ratio until 24 hr in the normal guinea-pig without bile fistula after oral administration of 1.5 ml of 0.32% (w/v) CMC–Na aqueous suspension (dose=24 mg) was about 42%. The excreted amount of free form was as negligibly small as in rat. Contrary to the results in rat, it was observed that the amount of excreted I–G was larger than that of I–S in urine as shown in Fig. 4.

Cumulative urinary and biliary excretion time courses in the guinea-pig with bile fistula were shown in Fig. 5. As shown in Fig. 5, in bile, I–S was exclusively excreted. Total

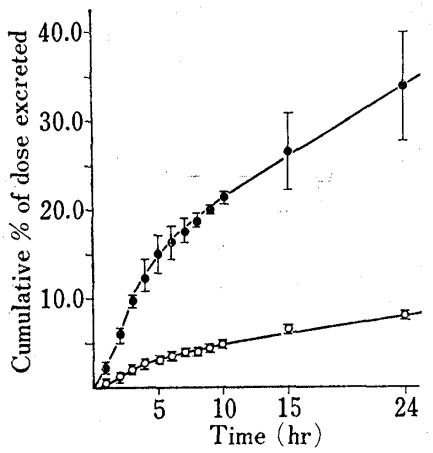

Fig. 4. Cumulative Curves of Urinary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin CMC aqueous Suspension in Guinea-Pig

dose: 24 mg
—●—: glucuronide
—○—: sulfate
Circles and vertical bars indicated the mean values and ± standard deviations (±S.D.) of 3 guinea-pigs, respectively.

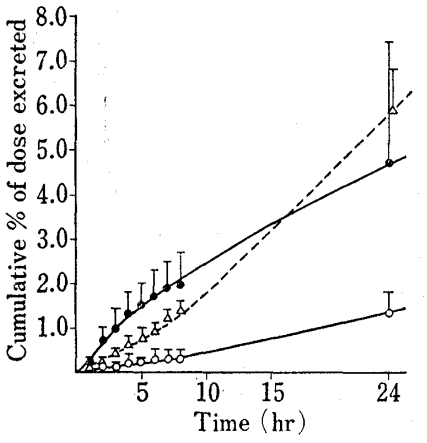

Fig. 5. Cumulative Curves of Urinary and Biliary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin CMC aqueous Suspension in Guinea-Pig with Bile Fistula

dose: 24 mg
—●—: glucuronide in urine
—○—: sulfate in urine
---△---: sulfate in bile
Glucuronide in bile was almost not detectable.
Each point and vertical bar represent the mean value and standard deviation (S.D.) in each direction of 3 guinea-pigs, respectively.

11) K. Liewendahl, *Acta Endocrin, Suppl.*, **133**, 1 (1968).
12) C.T. Chen, K. Hayakawa, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **23**, 2174 (1975).
13) M. Hayashi, T. Fuwa, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **24**, 2603 (1976).

excreted ratio was largely lowered owing to bile fistula. These results showed that the bile effect on absorption of I in guinea-pig was much greater than rat.

As for urinary excretion in rabbit after oral administration of 3 ml of 1.0% (w/v) CMC–Na aqueous suspension (dose=140 mg), it was also observed that I–G was excreted in urine to a larger extent than I–S in the same way as in guinea-pig. In this case, the excretion ratio to the dose as I–G was 3.6% and as I–S was 0.5% (the mean values of two rabbits) in 5 hr.

Urinary Excretion in Man

As shown in the experimental part, 600 mg of I (usual dose in a day, Japanese Pharmacopoeia VIII) after mixed well with CMC–Na in a motar with a pestle was administrated orally in male volunteers.[14] Excreted amounts of free form in urine were negligibly small in all volunteers, but the excretion patterns of metabolites were rather scattered among them. Thus, only one representative datum was taken in the present report to show the species difference of metabolism, and shown in Fig. 6. In this cases, I–G was excreted up to 30% until 24 hr, while I–S was excreted very little (less than 1%). In other cases, I–G was also excreted mainly and the excretion of I–S was of trace. And the excretion was observed to continue still after 24 hr similarly with other species.

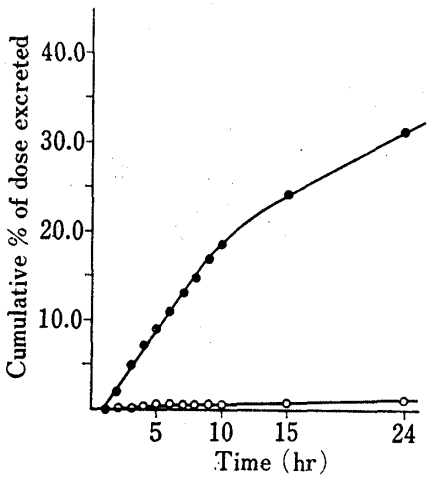

Fig. 6. Cumulative Curues of Urinary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin+CMC-Na in Man (Subject H)

dose: iodochlorhydroxyquin 600 mg +CMC-Na 120 mg
—●—: glucuronide
—○—: sulfate

Species Differences in Urinary and Biliary Excretion

The results for the species differences studied in the present report were listed together in Table I. Comparing the metabolism in the species studied here, it was found that urinary excretion patterns after

Table I. Cumulative Per Cent of Dose excreted until 24 hr after Oral Administration of Iodochlorhydroxyquin in Rat, Guinea-Pig and Man

			Rat[a]		Guinea-Pig[b]		Man[c]
Intact	urinary excretion ratio (%)	G	2.5±0.1	G	33.8±6.1	G	16.4±8.8
		S	10.2±1.5	S	8.1±0.3	S	—
		T	12.7±1.5	T	41.9±6.2	T	16.4±8.8
With bile fistula	urinary excretion ratio (%)	G	4.3±2.1	G	4.7±2.7		
		S	9.4±2.1	S	1.3±0.5		
		T	13.7±4.2	T	6.1±3.2		
	biliary excretion ratio (%)	G	29.7±6.2	G			
		S	—	S	5.9±0.9		
		T	29.7±6.2	T	5.9±0.9		

G: glucuronide, S: sulfate, T: total (G+S)
a) dose: 15 mg *p.o.* as CMC aqueous suspension
b) dose: 24 mg *p.o.* as CMC aqueous suspension
c) dose: 600 mg+CMC-Na 120 mg
—: The excretion was found very little and almost negligibly small.
In rat and guinea-pig, results are expressed as the mean value±standard deviation of 3 animals and in man, the mean value± standard deviation of 5 healthy male adults.

14) This experiment was primarily designed to study the CMC–Na effect on absorption of I in man, and the detail will be discussed in the next report,[13] in which the results on the cases of five healthy male adult volunteers who took 600 mg of I with and without 120 mg of CMC–Na as the cross-over test will be reported.

absorption of I seemed to be classified into three types. The first was the rat type, in which the excreted amount of I–S was much greater than that of I–G. The second was the guinea-pig type, in which contrary to the cases of the rat, the amount of excreted I–G was larger than that of I–S. A rabbit belonged to this type. And the third was the man type, in which I–G was mainly excreted and excreted I–S was very little. The characteristic point of the biliary excretion was that a guinea-pig excreted I–S more than I–G, in which I–G was found very little (less than 0.3%), while sulfate conjugates are said reportedly to be less significant in biliary excretion than glucuronide conjugates.[15]

The normal guinea-pig without bile fistula showed the highest excretion ratio in urine for 24 hr after oral administration. Although the biliary excretion ratio in the normal condition without bile fistula was not known, the biliary excretion ratio to the total excretion in the guinea-pig with bile fistula (5.9/(6.1+5.9)=0.49) was lower than in the rat with bile fistula (29.7/(13.7+29.7)=0.68). Since the contribution of biliary excretion after the absorption of I in the guinea-pig was smaller than in the rat, the guinea-pig could not be concluded to have the best absorbability of I among the species studied here from only the urinary excretion.

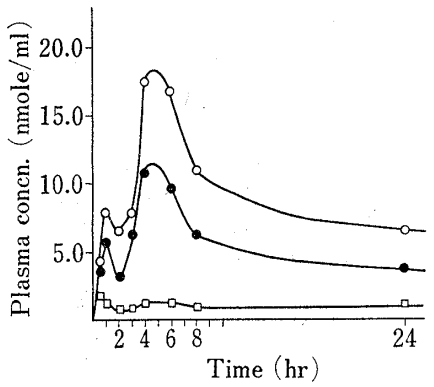

Fig. 7. Plasma Concentration of Unmetabolized (Free Form) and Conjugated Iodochlorxydrohyquin after Oral Administration of Iodochlorhydroxyquin CMC aqueous Suspension (Dose: 15 mg) in Rat

—□—: unmetabolized (free form)
—●—: glucuronide
—○—: sulfate

Species Differences in Blood Concentration

In addition to urinary and biliary excretion, species differences in blood concentration of unmetabolized and conjugated I were discussed comparing with other data.[16]

In rat, plasma concentration after oral administration of CMC–Na aqueous suspension showed rather scattered and in certain circumstances complexed two phase patterns. Thus, a representative datum was described in Fig. 7. As for metabolism of I, the order of the plasma concentrations of unmetabolized I and the conjugates was I–S>I–G>unmetabolized I.

On the other hand, in the case of guinea-pig the order was I–G>I–S>unmetabolized I as well as rabbit.[16]

Moreover, serum concentration of beagle was also investigated.[17] Consequently, whether 600 mg of I was administrated orally with a proper quantity of milk or three gelatin capsules, two phase patterns were shown similarly with rat. Concerning to metabolism of I, however, the order of the serum concentrations of unmetabolized I and the conjugates was unmetabolized I>I–S>I–G. Thus, our data supported the assertion of C.T. Chen, *et al.*,[16] in which species as man and dog that were sensitive to SMON disease had higher plasma concentration of unmetabolized I than I–G and I–S as shown in Fig. 8.

The complexed two phase patterns, which showed the complexed absorbability of I, seemed to be closely related with various processes of the absorption of I. For example, the gastric emptying time after oral administration and the following solubilization rate in the small intestine which was regarded as the main absorption site seemed to be one of the

15) R.L. Smith, *Progr. Drug Res.*, **9**, 299 (1966).
16) C.T. Chen, H. Kodama, Y. Egashira, K. Samejima, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 2007 (1976).
17) The study in beagle was carried out together with Professor Z. Tamura, Faculty of Pharmaceutical Sciences, the University of Tokyo and Associate Professor H. Ikeda, the Medical School Medicine, Okayama University.

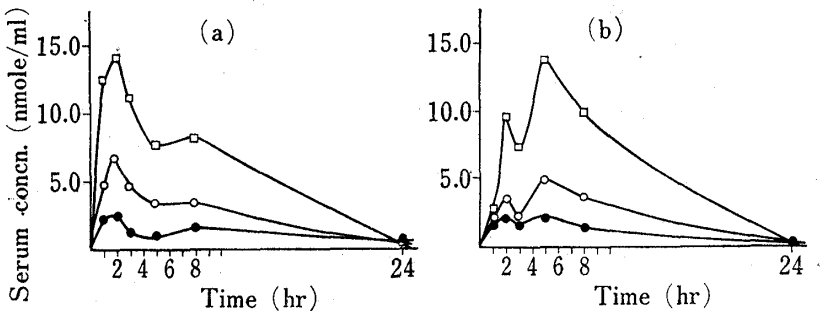

Fig. 8. Serum Concentration of Unmetabolized (Free Form) and Conjugated Iodochlorhydroxyquin after Oral Administration of Iodochlorhydroxyquin 600 mg+Milk (a) and Capsule (Gelatin) 200 mg×3 (b) inBeagle (Subject 611)[17]

—□—: unmetabolized (free form), —●—: glucuronide, —○—: sulfate

most important process, since the plasma concentration after intraduodenal administration in rat did not shown the two phase pattern. Of course, the reabsorption by the enterohepatic circulation might have a large effect, but the details are not yet known so far.

Metabolism of Conjugates

In order to study the biological stability of the conjugates, either I-G or I-S (3 mg per head equivalent to free form) was administrated intravenously in rat, and the excretion time courses of them were shown in Fig. 9 and 10. As shown in Fig. 9, when I-G was administrated, I-G itself was excreted exclusively in bile (37.6±8.1% of dose), but the excreted amount of I-S in urine (9.9±1.8% of dose) was more than that of I-G in urine (1.7±1.1% of dose). Similarly as shown in Fig. 10, when I-S was administrated, I-S was excreted in urine (44.4±

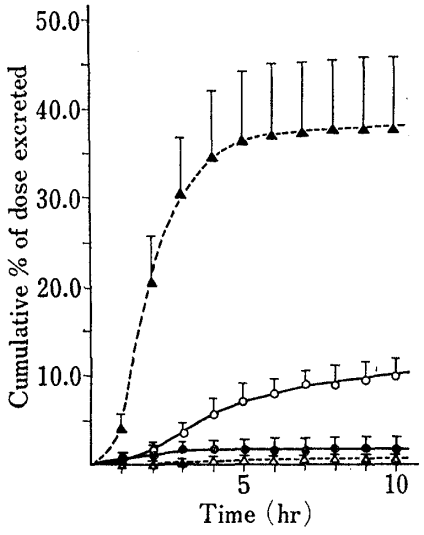

Fig. 9. Cumulative Curves of Urinary and Biliary Excretion Ratio after Intravenous Administration of Iodochlorhydroxyquin Glucuronide in Rat

dose: free iodochlorhydroxyquin equiv. 3mg *i.v.*
—●—: glucuronide in urine
—○—: sulfate in urine
---▲---: glucuronide in bile
---△---: sulfate in bile
Each point and vertical bar represent the mean value and standard deviation (S.D.) in each direction of 3 rats, respectively.

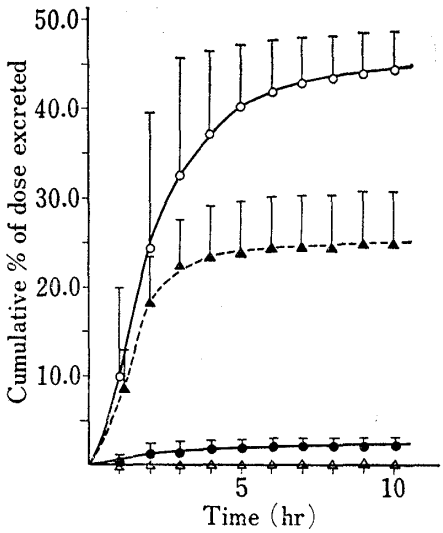

Fig. 10. Cumulative Curves of Urinary and Biliary Excretion Ratio after Intravenous Administration of Iodochlorhydroxyquin Sulfate in Rat

dose: free iodochlorhydroxyquin equiv. 3 mg *i.v.*
—●—: glucuronide in urine
—○—: sulfate in urine
---▲---: glucuronide in bile
---△---: sulfate in bile
Each point and vertical bar represent the mean value and standard deviation (S.D.) in each direction of 3 rats, respectively.

4.3% of dose) to a much greater extent than I–G (2.2±0.9% of dose), but the excreted conjugate in bile was almost exclusively I–G (24.9±6.0% of dose). The excretion of I–S in bile was almost negligibly small in both cases. These results show that the conjugates of I are hydrolyzed and reconjugation to glucuronide or sulfate occurs in rat. And that the excretion was almost ceased until 10 hr, but that the excretion ratio remained much less than 100% even after the conjugates were administrated intravenously, seem to show that I, the hydrolytic product of the conjugates, tends to be deposited in a body, although the deposit form is not clear. The hydrolysis and reconjugation of the conjugates seem to mean a complexed metabolic behavior of I. As the conjugates of 8-Hydroxyquinoline, the parent compound of I, was found hardly hydrolyzable in rat, as will be reported elsewhere, the halogenation effect is likely one of the interesting points of the metabolic study.

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