

## Various Factors affecting Intestinal Absorption of Iodochlorhydroxyquin in Rat and Man<sup>1)</sup>

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Various factors affecting the intestinal absorption of iodochlorhydroxyquin (I), which is remarked in relation to SMON (subacute myelo-optico-neuropathy), were investigated. From urinary excretion data in rats, it was found that the excretion ratio to the dose studied was larger in the case of the low dose, but it seemed that the sodium carboxymethyl cellulose (CMC-Na) effect was more effective for the high dose. And from plasma concentration data in rats, the CMC-Na effect at the high dose was also recognized. Therefore considering that the dose dependent effect of CMC-Na was due to that the endogeneous suspending agent in digestive fluid, probably bile component, was enough to suspend I of the low dose, but not of the high dose, the effect of bile on the absorption of I by the method of bile or saline solution infusion into the duodenum of the bile fistula rat was further investigated. The result was that bile was more effective on the absorption than CMC-Na, and so bile could be well regarded as the endogeneous suspending agent. But urinary data of man showed that there were two types of man, sensitive and insensitive for CMC-Na, and so its effect was not so obvious.

Iodochlorhydroxyquin (chinoxaline, clioquinol or 5-chloro-7-iodo-8-quinolinol) (I) had been used widely in Japan as an antimicrobia in an intestinal lumen before I was suspected to cause SMON (subacute myelo-optico-neuropathy).<sup>3)</sup> Since then, I has been investigated by many workers<sup>4)</sup> from various standpoints, such as metabolism,<sup>5-9)</sup> distribution,<sup>10)</sup> neurology,<sup>11)</sup> epidemiology,<sup>12-15)</sup> etc. From epidemiological studies, Tsubaki, *et al.*<sup>13,14)</sup> pointed out that the increase of SMON occurrence in Japan closely corresponded to the increase of I production. And Kanai *et al.*<sup>15)</sup> also pointed out that SMON began to occur after pharmaceutical products of I which contained suspending agents were produced in Japan. Sodium carboxymethyl cellulose (CMC-Na) or a surface active agent had been used as a suspending agent which was

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- 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) M. Yoshioka and Z. Tamura, *Igaku no Ayumi*, **74**, 320 (1970).
- 6) Z. Tamura, M. Yoshioka, T. Imanari, J. Fukaya, J. Kusaka, and K. Samejima, *Clin. Chim. Acta*, **47**, 13 (1973).
- 7) K. Nagasawa and H. Yoshidome, *Chem. Pharm. Bull.* (Tokyo), **21**, 903 (1973).
- 8) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 190 (1971).
- 9) C.T. Chen, K. Samejima, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **21**, 911 (1973).
- 10) J. Tateishi, S. Kuroda, S. Watanabe, S. Otsuki, and M. Ogata, *Folia Psychiat. Neurol. Japon.*, **26**, 159 (1972).
- 11) J. Tateishi, H. Ikeda, A. Saito, S. Kuroda, and S. Otsuki, *Neurology*, **22**, 702 (1972).
- 12) K. Nakae, S. Yamamoto, I. Shigematsu, and R. Kono, *Lancet*, **1**, 171 (1973).
- 13) T. Tsubaki, Y. Honma, and M. Hoshi, *Lancet*, **1**, 696 (1971).
- 14) T. Tsubaki, Y. Honma, and M. Hoshi, *Nippon Iji Shinpo*, No. 2448, 29 (1971).
- 15) M. Kanai and M. Kasai, *Igaku no Ayumi*, **79**, 365 (1971).

expected to help water insoluble I become wettable and suspendable in a gastrointestinal tract. While I was already reported to be absorbed<sup>16-20)</sup> in spite of its very low solubility, I has been further ascertained to be absorbed to a certain extent according to the study on I concerning SMON. Accordingly, the absorption time course and the availability of I, such a very insoluble drug, and the effect of suspending agent on its absorption have been an interesting problem in biopharmaceutics. The authors report in the present paper the effect of CMC-Na, one of the suspending agents added to a commercial product, on the absorption of I, using urinary and biliary excretion data after the oral administration in rat. Then, those effects of dose and bile as the endogeneous suspending agent on the absorption of I were further investigated. If necessary, plasma concentration data were also discussed.

Since it has been reported<sup>17-20)</sup> that I is metabolized mainly to its glucuronide (I-G) and sulfate (I-S) which are excreted in urine and bile, and that unmetabolized I (free form) and other metabolites are of trace, the total excreted amount of the conjugates (I-G and I-S) was used as the measure of the absorption in the present report.

### Experimental

**Materials**—All are same as described previously,<sup>21)</sup> in so far as not otherwise provided. Aqueous suspension of I with or without CMC-Na was prepared with sonication. The concentration of CMC-Na was 0.32% (w/v). Each 1 ml of both aqueous suspensions was administered into a stomach directly with the syringe attached a stainless steel tubing through a mouth in a rat. The concentrations of I for the low oral dose were 4.31 mg/ml and 3.98 mg/ml for CMC-Na aqueous suspension and pure aqueous suspension (control), respectively, as the result of the difference in the concentrations of I in suspension prepared with sonication. The dilution to adjust the concentrations was avoided to prevent any change of dispersing states in each suspension prepared. For the high oral dose, the concentrations were 15.3 mg/ml for CMC-Na aqueous suspension and 13.2 mg/ml for control. These doses were determined each time by the method described previously.<sup>21)</sup>

**Experimental Procedures**—i) **Rat:** Male albino rats of Donryu (280—320 g) were used, which were fasted overnight before oral administration. Collection of urine, bile and plasma was already mentioned in detail.<sup>21)</sup> And as for sampling after 24 hr, foods as well as water could be taken *ad libitum*. The bile effect on absorption of I were investigated with the following procedure. The one rat was thrice cannulated, one cannula in the bladder and two cannulae in the bile duct. One of the two cannulae in the upper part of the bile duct was used to collect bile. The other cannula was used to infuse fresh bile collected from another rat at the constant rate of 0.8 ml/hr with the automatic infusion pump (Natsume Seisakusho Ltd., KN-I type) into the duodenum. After operation, the rat was placed in a restraining cage during recovery and experimental periods. Then, after oral administration, urine and bile samples were collected. And in order to exclude the bile effect, saline solution instead of bile was infused at the identical rate.

ii) **Man:** In order to compare with the case of rat as for the CMC-Na effect, 600 mg of I (usual dose in a day, Japanese Pharmacopoeia VIII) was pulverized in a mortar with a pestle in the presence or absence of 120 mg of CMC-Na and taken orally by five healthy male adult volunteers. Urine samples were collected at an appropriate time interval during 24 hr. Food regulation for volunteers was same as described previously.<sup>21)</sup> Two months after one type of preparations had been taken, the other type was taken by each volunteer as the cross-over test. The order of the test was randomized.

**Determination**—The method used was the separate determination of conjugated and unmetabolized I. All procedures were also reported previously in detail.<sup>21)</sup>

### Results and Discussion

#### Dose and Dosage Form Effects on Absorption of I in Rat from Urinary Excretion

a) **Effect of CMC-Na**—Fig. 1 shows the cumulative per cent of the dose excreted in urine after the oral administration of I for the low dose in aqueous suspension. Results were

16) A. Knight and J. Miller, *Ann. Int. Med.*, **30**, 1180 (1949).

17) K. Liewendahl, *Acta Endocrin. Suppl.*, **133**, 5 (1968).

18) W.T. Haskins and G.W. Luttermoser, *J. Pharmacol. Exptl. Therap.*, **109**, 201 (1953).

19) P. Ritter and M. Jermann, *Arzneimittel.-Forsch.*, **16**, 1647 (1966).

20) L. Bergger and O. Hansson, *Clin. Pharmacol. Therap.*, **9**, 67 (1968).

21) M. Hayashi, T. Fuwa, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **24**, 2589 (1976).

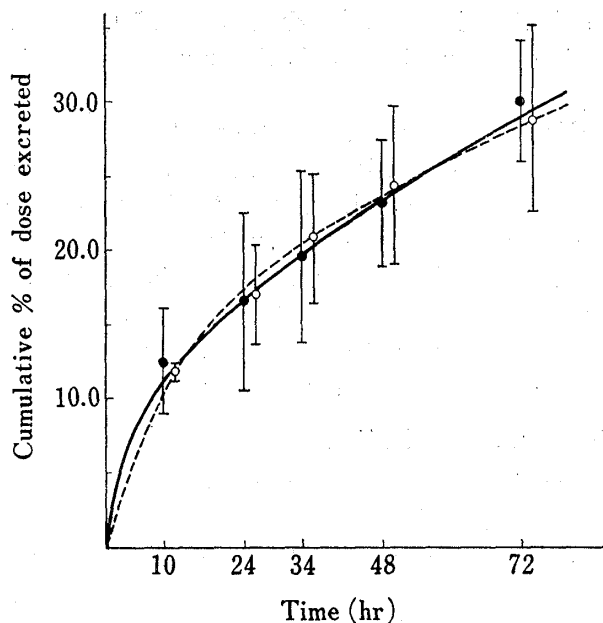


Fig. 1. Cumulative Curves of Urinary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin (Low Dose) Aqueous Suspension in Rat

●—: with CMC-Na  
 dose: 4.31 mg  
 ---○---: without CMC-Na (control)  
 dose: 3.98 mg

Circles and vertical bars indicated the mean values and  $\pm$  standard deviations ( $\pm$  S.D.) of cumulative per cent of the dose excreted as glucuronides and sulfates in 3 rats, respectively.

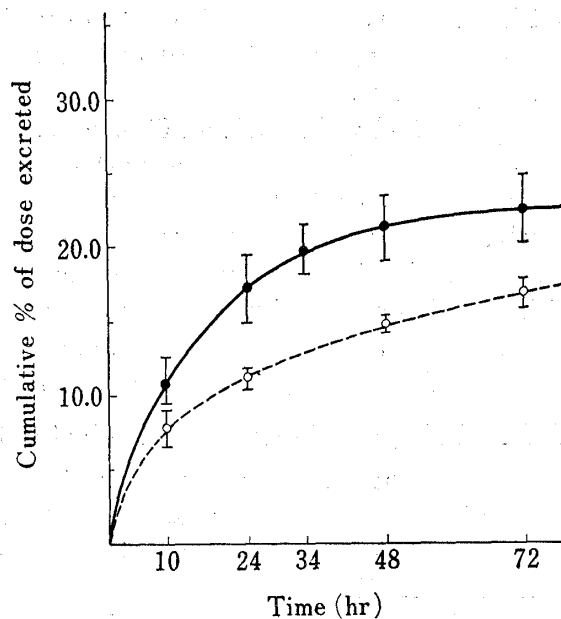


Fig. 2. Cumulative Curves of Urinary Excretion Ratio after Oral Administration of Iodochlorhydroxyquin (High Dose) Aqueous Suspension in Rat

●—: with CMC-Na  
 dose: 15.3 mg  
 ---○---: without CMC-Na (control)  
 dose: 13.2 mg

Circles and vertical bars indicated the mean values and  $\pm$  standard deviations ( $\pm$  S.D.) of cumulative per cent of the dose excreted as glucuronides and sulfates in 3 rats, respectively.

expressed as the mean value  $\pm$  standard deviation (S.D.) of three runs. Although the suspensibility of I just before the administration was observed remarkably different for both suspensions visually, excretion patterns were not so different each other.

The result for the high dose (about three and half times dose) was shown in Fig. 2. The difference of excretion patterns between both dosage forms was observed clearly.

TABLE I. Cumulative Excretion Ratio to Dose until 72 hr after Oral Administration of Iodochlorhydroxyquin Aqueous Suspension in Rat

	Low dose		High dose	
	With CMC-Na <sup>a)</sup> (%)	Without CMC-Na <sup>b)</sup> (control) (%)	With CMC-Na <sup>c)</sup> (%)	Without CMC-Na <sup>d)</sup> (control) (%)
G	1.0 $\pm$ 0.6	2.9 $\pm$ 0.8	2.8 $\pm$ 1.1	2.6 $\pm$ 1.6
S	29.1 $\pm$ 6.7	26.0 $\pm$ 6.6	19.7 $\pm$ 1.9	14.2 $\pm$ 0.9
T	30.1 $\pm$ 4.1	28.9 $\pm$ 6.3	22.5 $\pm$ 2.3	16.8 $\pm$ 1.0

G: glucuronide S: sulfate T: total (G+S)  
<sup>a)</sup> dose: 4.31 mg <sup>b)</sup> dose: 3.98 mg <sup>c)</sup> dose: 15.3 mg <sup>d)</sup> dose: 13.2 mg  
 Results are expressed as the mean value  $\pm$  S.D. of 3 rats.

The results above mentioned were summarized together in Table I. And these data until 72 hr were discussed in this report. Consequently, I-S was excreted much more than I-G as shown previously.<sup>21)</sup> In this case of the low dose, the cumulative per cent to the dose excreted as I-G and I-S until 72 hr was 30.1 $\pm$ 4.1% for CMC-Na suspension and was 28.9 $\pm$ 6.3% for control, respectively. And this difference was not of 5% level significance.

But in the case of the high dose, the excretion as I-G and I-S to the dose was  $22.5 \pm 2.3\%$  for CMC-Na suspension and  $16.8 \pm 1.0\%$  for control, respectively. This difference was of 5% level significance.

**b) Statistical Analysis for CMC-Na and Dose Effect**—Since it was observed that CMC-Na and dose affected the excretion pattern and ratio of I as seen in Fig. 1, 2 and Table I, the cumulative per cent of the dose excreted until 72 hr was examined statistically to show it clearly. Factors analyzed were the dose (low and high) and dosage form (with and without CMC-Na). Each set of experiments contained three iterative runs, and the variance analysis with two elements design was carried out. Consequently, only the difference between doses was of 1% level significance. Although the difference between dosage forms was not significant, it seemed likely that the effect of CMC-Na at the high dose was larger than at the low dose.

### CMC-Na Effect on Absorption of I in Rat from Plasma Concentration

Plasma concentration curves until 24 hr after the oral administration of the high dose in overnight fasted rats were shown in Fig. 3. In Fig. 3-a, b, three examples after the oral administration of aqueous suspension with and without CMC-Na (control), respectively, were described. As the scattering on the plasma concentration of each rat at each time was very large, and therefore, it was difficult to discuss the mean value  $\pm$ S.D. of three rats, in this report the CMC-Na effect on absorption of I was discussed from the total plasma concentration (free+glucuronide+sulfate). Comparing the mean value in the area under concentration until 24 hr of three rats in each dosage form, the ratio of CMC-Na aqueous suspension to control was  $1.73(\pm 0.21)/1.00(\pm 0.11)=1.73$ .

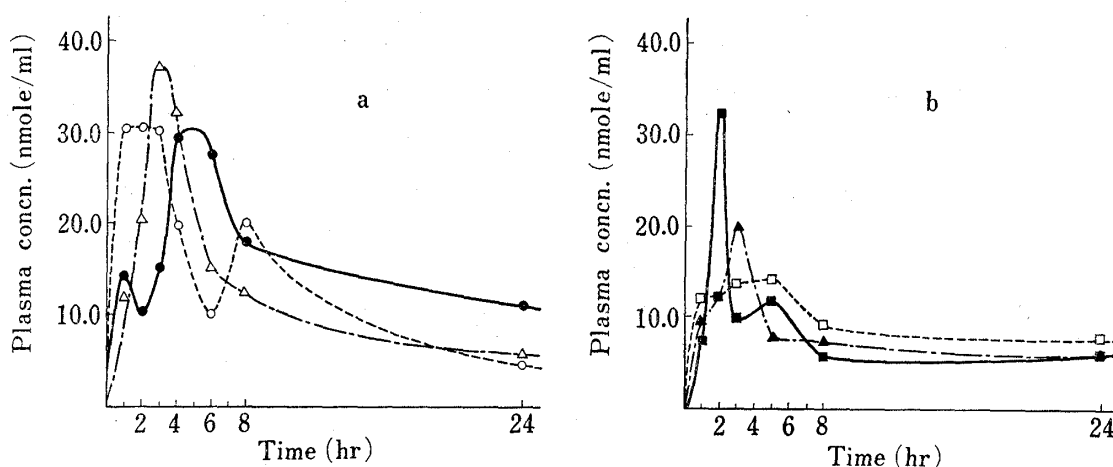


Fig. 3a,b. Plasma Concentration after Oral Administration of Iodochlorhydroxyquin Aqueous Suspension with and without CMC-Na in Rat

a: CMC-Na aqueous suspension *p.o.*, dose=14.7 mg

b: aqueous suspension without CMC-Na (control) *p.o.*, dose=14.4 mg

In both cases, results are expressed as the total value of free, glucuronide and sulfate in 3 rats, respectively.

Each symbol represents data obtained from separate rats.

On the other hand, comparing the cumulative per cent to the dose excreted in urine until 24 hr at the high dose above mentioned (in Fig. 2), the ratio was  $17.2(\pm 2.3)/11.1(\pm 0.7)=1.55$ . Consequently, the availability until 24 hr after oral administration in the rat at the high dose was almost equally enhanced about 1.5 to 1.7 times by CMC-Na from the plasma concentration and urinary excretion. However, at the low dose, the CMC-Na effect in the plasma concentration was not clear similarly with the urinary excretion. Thus, from the plasma concentration and urinary excretion data, the CMC-Na effect on the availability of I was clearly observed for the high dose, but the effect was vague for the low dose. And the comparative rapid absorption in the case of the aqueous suspension without CMC-Na (control) was also observed in the plasma concentration. The above-mentioned was probably explained with the specula-

tion that there were sufficient endogeneous or biological suspending agents, probably bile components, to suspend a small amount of I, however that these agents were not sufficient for the high dose and added CMC-Na was helpful.

### CMC-Na Effect on Absorption of I in Man from Urinary Excretion

The cumulative per cent of the dose excreted in urine until 24 hr was shown for each volunteer in Table II. I-G was excreted mainly and the excretion of I-S and unmetabolized I was of trace (less than 1%). And so total urinary excretion ratio was able to be regarded as that of I-G. The excretion ratio was rather scattered among volunteers. CMC-Na

TABLE II. Effect of CM-CNa on Cumulative Urinary Excretion of Iodochlorhydroxyquin Metabolites (Glucuronide+Sulfate) until 24 hr after Oral Administration in Man

Subject	Sex	Age in years	Body Wt. (kg)	Cumulative % of dose excreted	
				Dose: Iodochlorhydroxyquin 600 mg + CMC-Na 120 mg (%)	Dose: Iodochlorhydroxyquin 600 mg (%)
(H)	M	26	67	31.1	16.1
(K)	M	25	55	15.0	7.3
(F)	M	33	75	8.6	6.9
(A)	M	39	72	10.7	12.2
(J)	M	27	67	16.3	18.2

increased the ratio about two times for volunteer H and K, but it hardly affected the ratio for volunteer F, A, and J. Accordingly, it can be likely said that it is difficult to conclude whether CMC-Na affected the absorption of I in man, or whether there were two types of man, sensitive and insensitive for CMC-Na, from their urine data for such a limited number of volunteers and a limited dose. At present, a study with a large number of subjects or the higher dose is difficult because of the I toxicity of inducing SMON.

### Absorption Region

The gastric absorption of I in pylorus-ligated fasted rats was studied. From the plasma concentration after oral administration of I, the gastric absorption was found to be very small or of trace. Considering these data, it was shown that I was hardly absorbed in the stomach but probably absorbed mainly in the intestine.

### Bile and CMC-Na Effects on Intestinal Absorption from Biliary and Urinary Excretion

In this report, it was already speculated that the endogeneous suspending agents, probably bile components, had an important effect on the absorption of I, and therefore in this section, the bile effect on the absorption of I in the intestine, which was regarded as the main absorption region of I, was chiefly investigated.

As shown in the experimental part and Table III, the following four experimental systems in rats were designed.

TABLE III. Experimental System for Investigating Bile and CMC-Na Effects on Intestinal Absorption of Iodochlorhydroxyquin

	Infused solution	Oral dosage form
A	bile	CMC-Na aqueous suspension
B	bile	aqueous suspension
C	saline solution	CMC-Na aqueous suspension
D	saline solution	aqueous suspension

Results for these cases (A, B, C, D) were shown respectively in Fig. 4. In all cases, the excreted amount of I-G in bile was much greater than I-S or I-G in urine after oral administration, but I-S in bile was negligibly small as shown previously.<sup>21)</sup> In urine, I-S was greater than I-G as in the previous report,<sup>21)</sup> and unmetabolized I excretion was of trace in both bile and urine. Furthermore, the total biliary and urinary excretion in A with both bile and

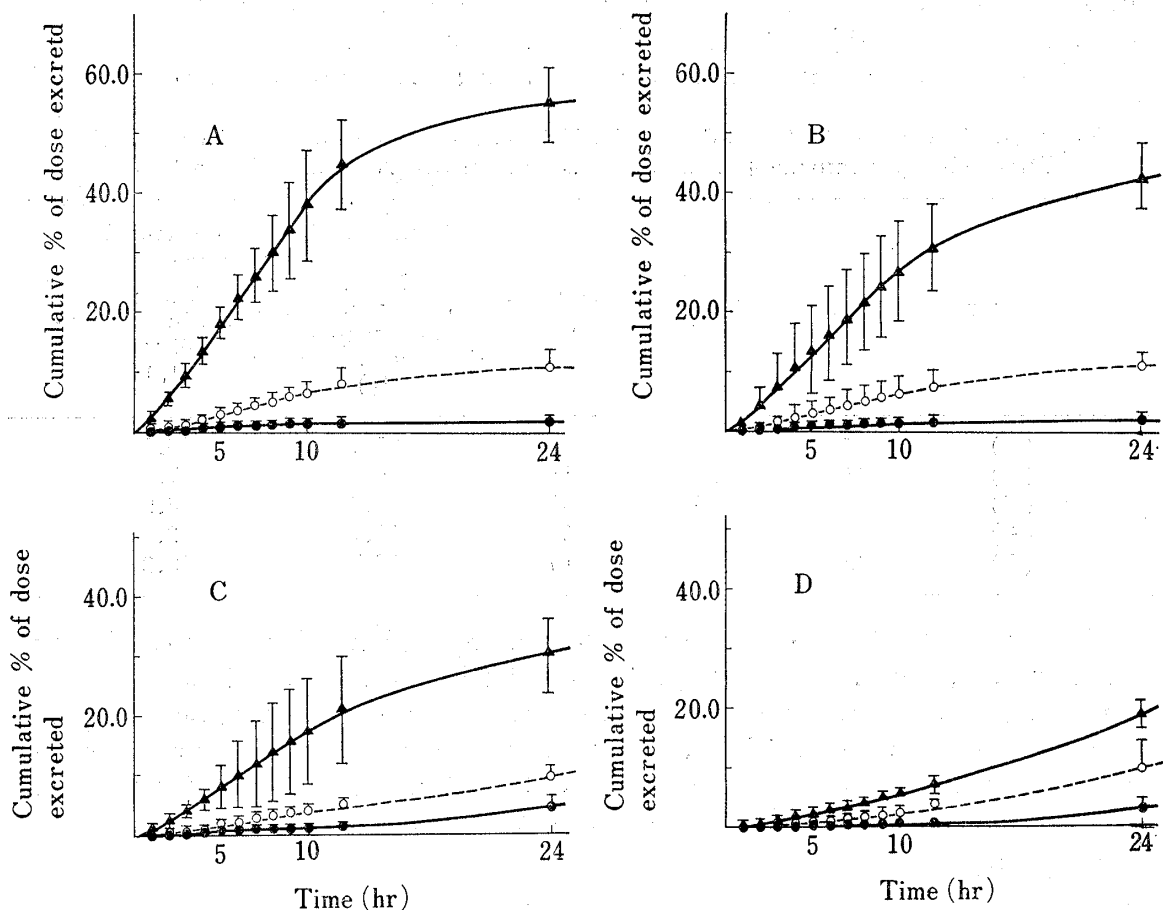


Fig. 4. Cumulative Urinary and Biliary Excretion Ratio of Iodochlorhydroxyquin Glucuronide and Sulfate during Bile or Saline Solution Infusion into the Duodenum after Oral Administration of aqueous Suspension with or without CMC-Na in Rat

- A: with CMC-Na, dose=15.5 mg, bile infusion  
 B: without CMC-Na, dose=14.0 mg, bile infusion  
 C: with CMC-Na, dose=15.0 mg, saline solution infusion  
 D: without CMC-Na, dose=13.2 mg, saline solution infusion

—●—: glucuronide in urine

---○---: sulfate in urine

—▲—: glucuronide in bile

Results expressed the mean value and S.D. in each direction by vertical bars of 3 rats

CMC-Na to be suspending agents was much greater than D without them. D also showed slight delay for excretion. On the other hand, B and C without either bile or CMC-Na, showed the intermediate types of A and D. Moreover, although the excretion still continued after 24 hr in all cases, the excretion ratio until 24 hr was discussed here, because the control of these experimental conditions for a long time was much more difficult than the experiment described previously. The results above mentioned were listed together in Table IV. Comparing the total excretion ratio to the dose of biliary and urinary excretion in 24 hr, the order of the experimental system  $A > B > C > D$  was observed. And it seemed that their differences were derived mainly from the biliary excretion of I-G since the total urinary excretion ratio was hardly different in all systems.

TABLE IV. Total Urinary and Biliary Excretion Ratio of Iodochlorhydroxyquin Glucuronide and Sulfate until 24 hr during Bile or Saline Solution Infusion into the Duodenum after Oral Administration of aqueous Suspension with or without CMC-Na in Rat

Infused solution	Oral dosage form	Urine		Bile Glucuronide (%)	Urine + Bile Total (%)
		Glucuronide (%)	Sulfate (%)		
A	bile CMC-Na aq. suspension	2.0±0.8	10.9±2.1	55.0±6.2	68.0± 8.0
B	bile aq. suspension	2.1±1.4	11.3±2.3	43.2±5.6	56.6± 9.0
C	saline solution CMC-Na aq. suspension	4.3±2.1	9.4±2.1	29.7±6.2	43.3±10.3
D	saline solution aq. suspension	2.4±2.2	9.4±5.1	18.7±2.4	30.5± 5.6

total: glucuronide+sulfate in bile and urine  
 dose: 15.5, 14.0, 15.0, 13.2 mg in experimental system A, B, C, and D, respectively  
 Results are expressed as the mean value S.D. of 3 rats.

The cumulative per cent of the dose excreted in urine and bile until 24 hr, however, was examined statistically. Factors analyzed were the infused solution (bile or saline solution) and the oral dosage form (aqueous suspension with or without CMC-Na). Each set of experiments contained three iterative runs, and the variance with two elements design was carried out in the same way as the previous statistical analysis for the CMC-Na and dose effect. Consequently, only the factor for the infused solution was of 1% level significance. On the other hand, the difference between dosage forms was not significant.

#### Conclusion on Bile and CMC-Na Effects

The conclusions on the experimental system A to D were as follows. (1) It was shown that the excretion ratio until 24 hr was I-G in bile > I-S in urine > I-G in urine (Fig. 4). (2) So far as the mean value of the total biliary and urinary excretion ratio showed, the order of the excretion was A > B > C > D and it was observed that bile had a larger effect on the intestinal absorption of I than CMC-Na. And therefore, bile was admitted to be a powerful endogeneous suspending agent (Table IV). (3) As a result of the statistical analysis for the CMC-Na and bile effect on the total urinary and biliary excretion ratio, only the bile effect was significant.

Of course, these experimental conditions were entirely different from the case of the normal rat concerning that bile was collected with bile fistula and the same volume as collected was made up by infusing another rat bile into the duodenum. Namely, the role of the enterohepatic circulation of bile acids and I could not be understood in these experiments. However, from the fact that the CMC-Na effect on the urinary excretion was not shown statistically in these experimental systems differently from the previous result in the rats without bile fistula for the same dose in this paper but that the effect on the biliary excretion was of 10% level significance, the role of enterohepatic circulation seemed very important.

Moreover, although saline solution infusion to exclude the bile effect had possibilities of changing the intestinal motion and absorption of water, these points were yet unknown at present.

Actual effects of bile could be regarded as dispersion and solubilization of I with bile acids. Since bile contains lecithin as well as various bile acids, the relationship between intestinal absorption and solubilization of I by means of both bile acids and lecithin is under investigation at present.