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Biliary Excretion Behavior Difference of Quaternary Ammonium Compounds between Portal Vein and Femoral Vein Infusion in Rat

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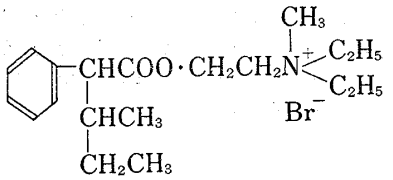
Dependence of biliary excretion behavior of a quaternary ammonium compound on administration route was reported. When valethamate bromide or scopolamine-N-butyl bromide was infused through portal vein at a constant rate, it produced a higher biliary excretion rate and total excreted amount than when they were infused through femoral vein at the same rate. This phenomenon was discussed to estimate the availability of an absorbed drug from a small intestine with biliary excretion data when the excretion data of the intravenously administered drug was taken as the standard which corresponded to the complete absorption. That is, if the dependence is overlooked, the estimated availability from biliary excretion data would be overestimated, because a drug through portal vein was more excretable than that through femoral vein.

During the study of the absorption behavior of valethamate bromide as a specimen of quaternary ammonium compounds, the following apparent unreasonable results were observed. As reported in the later section, after intraduodenal administration of valethamate bromide in rat, about 6.5% of the administered dose was excreted in bile in 8 hours. To calculate the absorption ratio from the biliary excretion data, the excretion ratio after single intravenous administration was studied and the ratio was found about 3.2%. If it is assumed simply that the intravenous administration corresponds to the complete absorption, the absorption ratio from a small intestine of valethamate bromide would be much greater than 100%. This is entirely unreasonable.

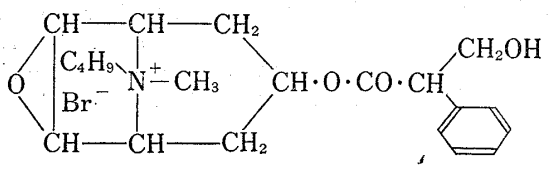
The present paper is to elucidate the unreasonable findings as above and to report that biliary excretion rate was dependent on the route of administration, *i.e.*, that the rate depended on whether a drug reached liver before or after it was distributed and eliminated from other tissue organs.

And the route dependence of the biliary excretion was ascertained with another quaternary ammonium compound, scopolamine-N-butyl bromide (SBB), and it is also to be reported.

These ammonium compounds were shown in Chart 1.



valethamate bromide



scopolamine-N-butyl bromide (SBB)

Chart 1. Chemical Structures of Valethamate Bromide and Scopolamine-N-Butyl Bromide (SBB)

Experimental

Drug Administration and Samplings——Male albino rats (Donryu) weighing 250—280 g were used. In the study of intraduodenal administration, rats were fasted for one day before the operation. Bile fistula

1) Location: *Hongo, Bunkyo-ku, Tokyo.*

was operated for the excretion of the drug in bile. The drug was administered by three different routes. In the study of intravenous administration, the drug in 0.1 ml sol. was administered through a femoral vein for 3—5 min. For the infusion study, 0.295 ml of drug saline solution was infused for one hour with Natsume Model 1H automatic micro infusion pump into portal or femoral vein, respectively. And in the study of intraduodenal administration, 1 ml of drug saline solution (concn. 10 mg/ml) was injected into the upper section of the duodenum. Bile samples were taken at given times under light ether anesthesia except for the study of intraduodenal administration where the anesthesia was used only for the operation.

Analytical Methods——Dye complex formation method was used.

1) Valethamate bromide: Bile samples of 0.3 ml was put into a 10 ml glass stoppered test tube containing 1 ml of Orange II solution (concn. 1 m mole/liter), and 6 ml of $CHCl_3$ was added. After 20 min shaking, it was centrifuged for 15 min at 3200 rpm and the upper layer (aqueous layer) was removed. To remove the residual small contents of water, the organic layer (chloroform layer) was kept at $-15°$——$-10°$ for 30 min and 4 ml of chloroform layer was put into another test tube with whole pipet. The optical density of extracted colored complex in $CHCl_3$ was determined at 485 mμ using Hitachi 124 spectro-photometer. The optical density of blank bile was less than 0.020, therefore, was neglected in this method.

To know if the hydrolytic products were detected as well in this procedure, valethamate bromide was hydrolysed in an alkaline solution. After the hydrolysis in 2N NaOH solution at 37° for 15 min, no detectable optical density was observed in this procedure.

2) Scopolamine-N-butyl bromide (SBB): SBB was determined according to Schill.[2] To apply the small sample volume of the present study and to hydrolyze SBB completely (see text), the volumes of sample, aq. phase and organic solvents, and the alkalinity of aq. phase were modified as the following.

Bile samples of 0.3 ml was put into a 10 ml test tube containing 0.2 ml of 5N NaOH and 4 ml of $CHCl_3$ saturated with dipicrylamine was added. After the similar procedure was carried out as in valethamate bromide, the opitical density was measured at 415 mμ. To test the specificity of this method (see text), SBB was determined with Orange II as in valethamate bromide.

Stability of Valethamate Bromide in Blood: To 0.2 ml of whole blood or plasma was added 0.1 ml of valethamate bromide solution (concn. 3 mg/ml) and after the solution was incubated at 37° for given time, the reaction was stopped with the addition of 1 ml of 5% CCl_3COOH. The residual amount was determined as the above.

Result and Discussion

I. Specificity for the Analitical Method

1) Valethamate Bromide——Since no information for the metabolism of valethamate bromide was available, the specificity of the method was examined with thin layer chromatography. As shown in Table I, the chromatographic behavior of the extracted complex in chloroform from bile sample was the same as that of the complex extracted from the control solution where valethamate bromide was dissolved in bile. An exception was observed in ethanol system. It was found that ethanol separated the complex into dye and the spot

TABEE I. *Rf* Values of the Dye-Valethamate Bromide Complex on Thin-Layer Chromatogram in Various Solvents

Solvent system	Bile sample	Control[a]	Valethamate[b] bromide
Acetone	0.46[c]	0.47[c]	0.00
Ethanol	0.72[d] (dye)	0.72[d] (bye)	0.00
	0.00[d] (valethamate bromide)	0.00[d] (valethamate bromide)	0.00
Dioxane	0.21[c]	0.21[c]	0.00
Acetone: ethyl acetate=4: 1	0.69[c]	0.69[c]	0.00
Dimethyl suloxide: chloroform: ethyl acetate=1 : 2 : 1	0.53[c]	0.53[c]	0.16

a) Control sample where valethamate bromide was dissolved in bile.
b) spotted directly without complex formation
c) complex
d) Complex was separated into dye and valethamate bromide which was detected with I_2 vapor.

2) G. Schill, *Anal. Chim. Acta.*, **21**, 341 (1959).

Table II. Secondary *Rf* Values after developed with Ethanol

Solvent system	Bile sample	Control[a]	Valethamate[b] bromide
$CHCl_3$: CH_3OH: NH_4OH=4 : 4 : 1	0.21	0.21	0.21
n-Butanol: formic acid: H_2O=4 : 1 : 2	0.64	0.64	0.66

a) Control sample where valethamate bromide was dissolved in bile.
b) spotted directly without complex formation

which remained in the origin. The spot was further developed with other two solvent systems, chloroform: methanol: ammonia water (28%)=4: 4: 1 and *n*-butanol: formic acid: water= 4: 1: 2. The *Rf* values of the spots of the sample complex showed the same values of the spots of the control complex as shown in Table II. Accordingly, the analytical method was proved to determine valethamate bromide itself.

2) Scopolamine-N-Butyl Bromide (SBB)——Since the dipicrylamine complex was extracted from a concentrated alkaline solution as above, it was considered to be necessary to know whether the complex was with SBB itself or with the basic moiety of hydrolytic products. After the hydrolysis of SBB in an alkaline solution, one part was neutralized and was extracted with Orange II which formed a complex with SBB, to $CHCl_3$, and the second part was extracted to $CHCl_3$ with dipicrylamine. The former gave very little complex, but on the other, the latter gave the same amount of the complex as that of SBB before hydrolyzed. Accordingly, it was considered that SBB was hydrolyzed during the determination procedure and the basic portion was extracted with dipicrylamine. It was considered, therefore, that SBB itself and its metabolites which were modified in the acidic moiety such as glucuronide were determined with the present method. As for bile sample, the complex with Orange II was scarcely observed, but the complex with dipicrylamine was determinable, therefore, it was considered that the excretes in bile were its metabolites mostly and little of SBB, almost of which was assumed glucuronide from the results of methylscopolammonium methylsulfate, analogous compound of SBB.[3]

II. Valethamate Bromide

As a probe for the small intestinal absorption of valethamate bromide, the biliary excretion was studied after intraduodenal administration, since recently the biliary excretion of drug has been interested in the fields of metabolism and pharmacokinetics. The average excretion rates after 10 mg/head administration were shown in Fig. 1, and the total excreted amount for 8 hour was 653 μg, that is, the excretion ratio was 6.5% of the administered dose. Although the fluctuations in excretion rates were observed, the excretion rate maximum was found clearly in one hour, and this meant that the absorption maximum occured in this period. This is in good coincidence with Levine[4] who reported that a quaternary ammonium compound was well absorbed in a upper segment of a small intestine. Since this absorption study was carried out under the condition without supply of raw bile into intraduodenum and without ligation of flexura duodenojejunalis, the fluctuations might be caused. Saying nothing about the mechanism or process of absorption in detail, for the present, the absorption ratio was tried to be calculated from the excretion data as the fundamental information for the absorption.

To calculate the absorption ratio, the complete absorbable formulation or other dosage route which could be simulated to produce the complete absorption was necessary. To do this, intravenous administration was taken in the present investigation.

3) K. Sano and H. Takomizu, The Abstracts of the 91 th Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, 402 (1971).
4) R.R. Levine and E.W. Pelikan, *J. Pharmacol. Exptl. Therap.*, **131**, 319 (1961).

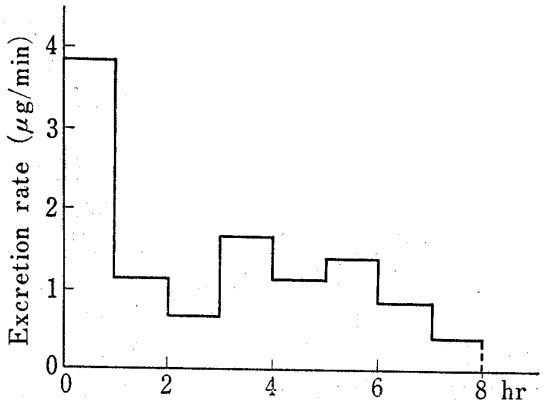


Fig. 1. Biliary Excretion Rate of Valethamate Bromide after Intraduodenal Administration, $n=6$

dose: 10 mg
excreted amount in 8 hr: 653 μg
excretion ratio in 8 hr: 6.5%

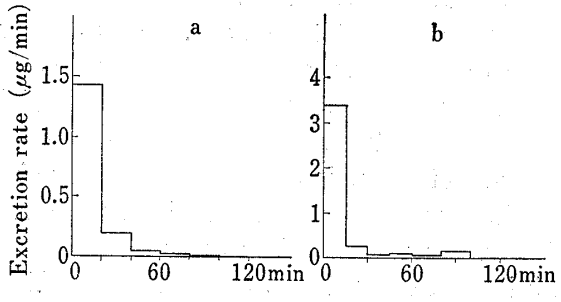


Fig. 2. Biliary Excretion Rate of Valethamate Bromide after Intravenous Injection

a) $n=4$,
dose: 4 mg/kg
excreted amount: 33.2 μg
excretion ratio: 3.3%

b) $n=3$
dose: 8 mg/kg
excreted amount: 62.7 μg
excretion ratio: 3.1%

The biliary excretion rates of valethamate bromide after 4 mg/kg and 8 mg/kg intravenous injection for 3—5 min were shown in Fig. 2. As seen easily, the excretion in bile occurred rapidly and about 85% of the total excreted amount was found within 15—20 min. But the excretion ratios until 2 hours where the excretion almost ceased were 3.3% and 3.1% for the dose of 4 mg/kg and 8 mg/kg, respectively. These ratios were different from the ratio of the intraduodenal administration. If the biliary excretion ratio was assumed to be equal between intravenous and intraduodenal administration, the excreted amount of 0.653 mg after intraduodenal absorption would mean that the absorbed amount was 20.4 mg, deviding 0.653 mg by 0.032. This value was much greater than the administered dose of 10 mg. This was entirely unreasonable.

To explain such an apparent unreasonable results, there were considered two probable mechanisms. The first one was that when valethamate bromide was administered intravenously it was rapidly hydrolyses with esterase which existed in blood before it reached liver. The second one was that when valethamate bromide was absorbed from a small intestine, it reached liver through portal vein and was excreted into bile before it was distributed with circulation, while the intravenously administered drug was eliminated from tissue organs before it reached liver.

To examine the first mechanism, the stability of valethamate bromide in whole blood and plasma was studied and it was found that the ester bond was not hydrolyzed at least until 90 min at 37°, and at 120 min slight but not significant decomposition was observed. Accordingly the first mechanism was ruled out.

As for the second mechanism, the comparative study was carried out in the two different administration routes, *i.e.*, one was the portal vein infusion which corresponded to the com-

Table III. Comparison of Biliary Excretion Ratio for Portal and Femoral Vein Infusion

Drug	Dose (mg)	Portal vein		Femoral vein	
		Excreted amount (μg)	Excretion ratio (%)	Excreted amount (μg)	Excretion ratio (%)
Valethamate Bromide	5.463	536.7[a] ±42.5[b]	9.82±0.78[b]	299.1[a] ±28.2[b]	5.48±0.52[b]
Scopolamine-N-Butyl Bromide (SBB)	1.65	510.3[c] ±53.7[b]	31.4±3.5[b]	352.9[c] ±21.4[b]	21.4±1.2[b]

a) amount excreted until three hours, $n=5$
b) standard error
c) amount excreted until four hours, $n=6$
infusion speed for valethamate bromide: 5.463 mg/0.295 ml/hr, for scopolamine N-butyl bromide (SBB): 1.65 mg/0.295 ml/hr

plete absorption and the other was the femoral vein infusion which had the distribution process before the pass of the liver. The infusion through these two different routes was carried out at the same speed (5.463 mg/0.295 ml/hr) for one hour and the results were shown in Fig. 3 for the excretion rates and in Table III for the excretion ratios.

These results showed the significant difference in t-test ($p<0.05$, $n=5$) in the biliary excretion ratios between portal vein and femoral vein infusion. The excretion rates were larger in portal vein infusion than in femoral vein infusion. And the ratio of the excretion ratio of the former infusion to the ratio of the latter was 1.8. If the ratio of 1.8 was assumed to be hold between the absorbed drug from small intestine and the instantaneously intravenously administered drug, the ratio of the excretion ratio of the absorbed drug (6.5%) to the ratio of the intravenously administered drug (3.2%) was corrected by the factor 1.8 and was 1.1. This meant that the absorption ratio of valethamate bromide was 100%. Such a high absorption ratio seemed too high for a quaternary ammonium compound,[4-6] however, valethamate bromide might have an exceptional absorbability. But in order to discuss the absorbability of it further, the relation of the excretion ratio to the infusion speed and dose was to be studied. From these results, although the true absorbability remained to be solved, the apparent unreasonable results that the absorbed amount looked to exceed the dose was taken to be elucidated mainly with the second mechanism where the drug was considered to be excreted in bile before it was distributed and eliminated from other tissue organs.

III. Scopolamine-N-Butyl Bromide (SBB)

In order to ascertain if such a biliary excretion behavior was observed in a different compound, the similar comparative study was carried out for SBB which had a different

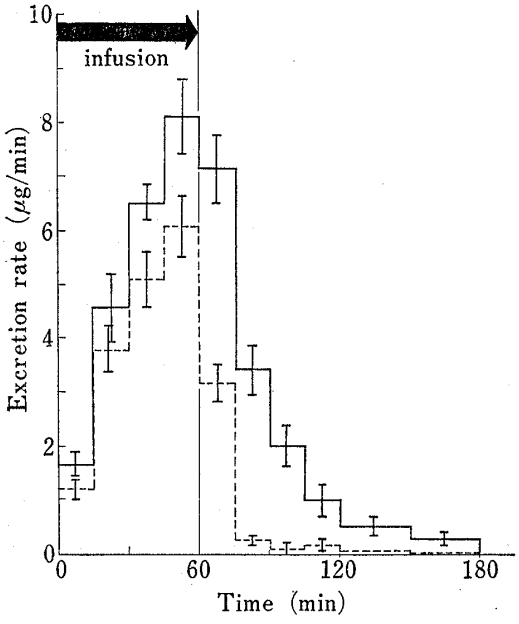


Fig. 3. Biliary Excretion Rate of Valethamate Bromide after Portal and Femoral Vein Infusion

$n=5$
—— : portal vein infusion
------ : femoral vein infusion
I : standard error
infusion speed: 5.463 mg/ 0.295 ml/hr

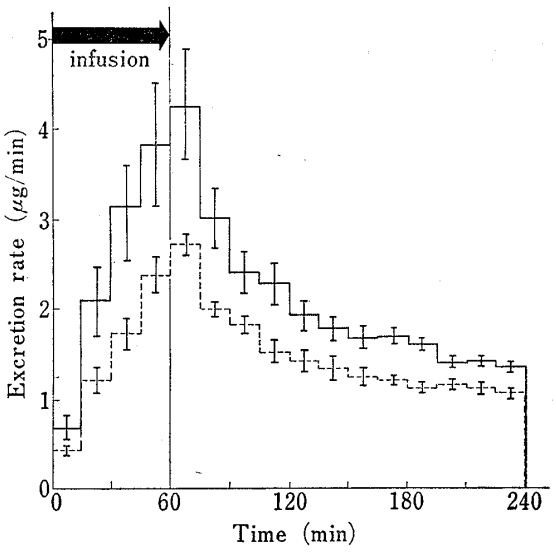


Fig. 4. Biliary Excretion Rate of Scopolamine-N-Butyl Bromide (SBB) after Portal and Femoral Vein Infusion

$n=6$
—— : portal vein infusion
------ : femoral vein infusion
I : standard error
infusion speed: 1.65 mg/0.295 ml/hr

4) L.S. Schanker, B.B. Brodie and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
5) R.R. Levine and B.B. Clark, *J. Pharmacol. Exptl. Therap.*, **114**, 63 (1955).
6) R.R. Levine, *J. Pharmacol. Exptl. Therap.*, **129**, 296 (1960).

biliary excretion behavior from that of valethamate bromide in the experimental doses as reported in the later section. The similar comparative study as in valethamate bromide between portal vein and femoral vein infusion was carried out at the infusion speed of 1.65 mg/ 0.295 ml/hr for the convenience of the determination and for the tolerance of the toxicity in rat. The results after one hour infusion were shown in Fig. 4 for the biliary excretion rates and in Table III for the biliary excretion ratio. The excretion rate was larger in the portal vein infusion than in the femoral vein infusion. And the excretion ratios for 4 hours of portal vein and femoral vein infusion were 31.4±3.5% and 21.4±1.2%, respectively, and the difference was significant with t-test ($p<0.05$, $n=6$).

From these results, it was found that the biliary excretion ratio of SBB depended on the administration route as well as in valethamate bromide, although SBB showed the following different excretion behavior from valethamate bromide. Valethamate bromide had low excretion ratio and the excretion maximum appeared in the last period of infusion, and after infusion was stopped, the excretion ceased rapidly (Fig. 3). On the other hand, SBB was excreted mostly as metabolites, and the excretion continued for a long period after infusion was stopped.

After the completion of the present study, the paper of Tajana[7] appeared. They reported only that the amount of moxicoume (4-methyl-5,7-bis(2-N-morpholinoethoxy)coumarin) excreted in bile after intravenous administration was similar to the amount excreted after intraduodenal administration of the same dose in rat. But looking up their data from the standpoint of the present study, the difference could be observed between the cumulative excreted data of 4.24±0.22[8] mg/kg ($n=10$) after intraduodenal administration and of 2.85±0.20[9] mg/kg ($n=8$) after intravenous administration. Therefore, their results were consistent with the present study although they did not take the difference into account. Accordingly, the route dependence of biliary excretion seemed to be observed for various drugs.

The findings of the route dependent biliary excretion behavior reported in the present study is of great importance both for the practical and mechanistic points. If the dependence is overlooked, the calculated availability or other pharmacokinetic evaluations would be erroneous, and the pharmacological effect would be different from the expected. And although the importance of the first pass of liver or route dependence has been reported for metabolism and blood concentration,[10–13] it has never been mentioned for biliary excretion in the scope of the authors' literature survey.

The discussion whether the route dependence is essential or rather apparent and it is due to high dose effect which comes from the concentrical administration through portal vein is the further problem to be studied.

Acknowledgement This work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education to which the authors are grateful.

7) A. Tajana, E. Massarani, D. Nardi, E. Xamin and I. Setnikar, *Arzneimittel-Forschung*, **22**, 539 (1972).
8) Data from Table I of ref. 6.
9) Data from Table II of ref. 6.
10) P.A. Harris and S. Riegelman, *J. Pharm. Sci.*, **58**, 71 (1969).
11) C.T. Dellery, D.S. Davies and M.E. Conolly, *Ann. N.Y. Acad. Sci.*, **179**, 108 (1971).
12) M. Gibaldi and S. Feldman, *Europ. J. Pharmacol.*, **19**, 323 (1972).
13) T. Suzuki, Y. Saitoh, S. Isozaki and R. Ishida, *Chem. Pharm. Bull* (Tokyo), **20**, 273 (1972).