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Pharmacokinetic Study of Biliary Excretion. II. Comparison of Excretion Behavior in Triphenylmethane Dyes[1,2]

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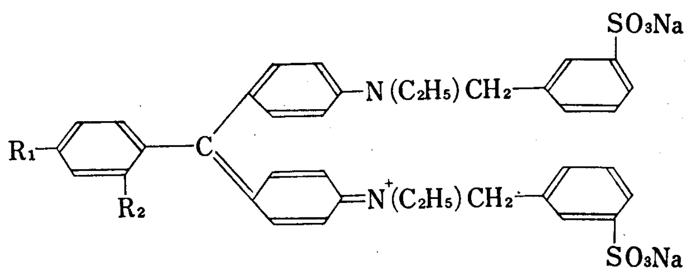
In the previous paper, the authors reported the kinetic studies on the factors affecting the biliary excretion with azo dyes and indigoid dye. The further investigation was tried with triphenylmethane dyes in rat. Six triphenylmethane dyes, *i.e.*, Brilliant Blue FCF, Light Green SF, Fast Green FCF, Guinea Green B, Acid Violet 6B and Acid Violet 5B were used.

(1) The excretion behaviors were similar to those of azo dyes which had the dose dependency. And the excretion ratios were generally larger than those of azo dyes except Light Green SF and Acid Violet 5B.

(2) It was concluded that a sulfonate group beyond other groups had a remarkable effect on the biliary excretion ratio and pattern, and that the position of a sulfonate group as well as the number was important to determine its effect.

(3) The possibility of the compartmental analysis for the biliary excretion process was suggested in the excretion pattern of Brilliant Blue FCF.

In order to investigate some factors affecting the biliary excretion, the authors intended the kinetic study using water soluble dyes, and found in the previous paper[4] that the increase of the sulfonate groups of azo dyes did not always increase the biliary excretion ratio, but in a case decreased the ratio depending on the substituted position. This was not so simple as in the past report[5] that the larger the number of the groups, was a dye the more excretable. From these results, it was concluded that the excretion pattern and the ratio were affected with the position of the sulfonate groups as well as the number of them.



Brilliant Blue FCF: $R_1=H$, $R_2=SO_3Na$
Light Green SF: $R_1=SO_3Na$, $R_2=H$
Fast Green FCF: $R_1=OH$, $R_2=SO_3Na$
Guinea Green B: $R_1=H$, $R_2=H$
Acid Violet 6B: $R_1=N(CH_3)_2$, $R_2=H$
Acid Violet 5B: $R_1=N(CH_3)_2$, $R_2=C_2H_5$

Chart 1. Chemical Structure of Triphenylmethane Dyes

And it was also reported in the previous paper that the same biliary excretion mechanism was suggested for azo dyes which were mainly excreted in bile, and Indigo Carmine which was mainly excreted in urine and scarcely in bile from the comparison between the excretion patterns of them. In the present paper, the further studies have been tries with six

1) This forms Part II of "Pharmacokinetic Study of Biliary Excretion," by T. Iga; Part I: T. Iga, S. Awazu, M. Hanano and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **18**, 2431 (1970).
2) Partial fulfillment of Doctor of Pharmaceutical Science degree requirement of Tatsuji Iga to the Graduate school, University of Tokyo.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) T. Iga, S. Awazu, M. Hanano and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **18**, 2431 (1970).
5) J.L. Radomsky and T.J. Mellinger, *J. Pharmacol. Expt. Therap.*, **136**, 259 (1962).

triphenylmethane dyes,[6] and the same effect mainly respective to the chemical structure as in the previous paper with azo dyes,[4] was also found in this series of dyes. And in addition, the interesting phenomenon in the excretion pattern of Brilliant Blue FCF was found and is to be reported here.

Experimental

Materials——Dyes used in this study were shown in Table I. All dyes were purchased from Wako pure chemical industries, LTD and Tokyo chemical industries, Co., Ltd. All other reagents were commercially available and of special grade.

Table I. Dyes used in This Study

Dye	Name	Molecular formula	Molecular weight	Absorption max. (mμ)
Triphenylmethane dyes	Brilliant Blue FCF (FD Blue No. 1)	$C_{37}H_{34}O_9N_2S_3Na_2$	792.9	618
	Light Green SF (FD Green No. 2)	$C_{37}H_{34}O_9N_2S_3Na_2$	792.9	632
	Fast Green FCF (FD Green No. 3)	$C_{37}H_{34}O_{10}N_2S_3Na_2$	809.0	638
	Guinea Green B (FD Green No. 1)	$C_{37}H_{35}O_6N_2S_2Na$	713.8	630
	Acid Violet 6B (FD Violet No. 1)	$C_{39}H_{40}O_6N_3S_2Na$	729.9	544
	Acid Violet 5B	$C_{41}H_{44}O_6N_3S_2Na$	762.0	545

Drug Administration and Samplings and Analytical Methods——The procedure was carried out in the same way as described in the previous paper.[4] The excreted dye was determined as the equivalent amount to the authentic dye from the optical density at the wave length for each dye listed in Table I using Hitachi 124 spectro-photometer.

Bile Flow——As reported previously,[4] the bile flow rate, under light ether anesthesia tended to fluctuate somewhat until 1 hr. But the effect of fluctuation on the excreted amount of a dye was found to be neglected. And the detailed data of bile flow and dye concentration in bile are available from the authors.

Binding with Plasma Protein——The equilibrium dialysis methods were used. Three ml rat plasma in Visking tube was put into a light resistant glass stoppered tube containing 30 ml isotonic pH 7.3 buffer solution of 1 μmole/ml dye concentration and dialysed at 37°. The dye concentration of the buffer solution was determined after 160 hr dialysis. The dye concentration was selected to correspond to approximately to the plasma concentration when the dye administered intravenously.

Result and Discussion

Triphenylmethane dyes have been used as the popular blue fed dyes for a relatively long time. But the metabolism and excretion of them as far as the authors understand, have not been studied enough up to this time. In this paper, six triphenylmethane dyes, *i.e.*, Brilliant Blue FCF (BB), Light Green SF (LG), Fast Green FCF (FG), Guinea Green B (GG), Acid Violet 6B (AV6B) and Acid Violet 5B (AV5B) were used. Their molecular weights are between about 700 and 800 (Table I) and all these values are larger than those of azo dyes which were used in the previous paper. As shown in Chart 1, all these dyes have the common structure and differentiated only with the number and position of sulfaonate groups and other groups substituted in one benzene ring.

6) The symbols for dyes in the present study are as follows: Brilliant Blue FCF=BB, Light Green SF=LG, Fast Green FCF=FG, Guinea Green B=GG, Acid Violet 6B=AV6B, Acid Violet 5B=AV5B.

(1) Excretion Ratio

The biliary excretion was almost completed for each dye until 4 hr and the excretion ratio was obtained from the cumulative amount until 4 hr. Typical examples were shown in Fig. 1, for the most excretable BB and the least excretable LG.

And the biliary excretion ratios of 3, 15 and 30 μmole doses for each dye were listed in Table II. The ratios were generally larger than those of azo dyes except LG and AV5B.

The following structural effects on the biliary excretion ratio were found for the number and position of sulfate groups, and were the same as in the previous paper where azo dyes were used.

Assuming GG which has no substituents in the benzene ring as the standard, the excretion ratios of BB and FG which have a sulfonate group at R_2 position were compared with that of GG. Both of the former had about 90% of the ratio and the latter had about 65% of it. This coincided well with the results of Radomsky[5] that the larger the number of sulfonate group in azo dyes, the larger was the excretion ratio. But on the other hand, LG showed only 17—25% excretion ratio which was far less tha GG, although LG has also a sulfonate group in the benzene ring as BB and FG. The only difference between the most excretable BB and the least excretable LG is that the former has a sulfonate group at R_2 position and the latter at R_1 position instead of R_2.

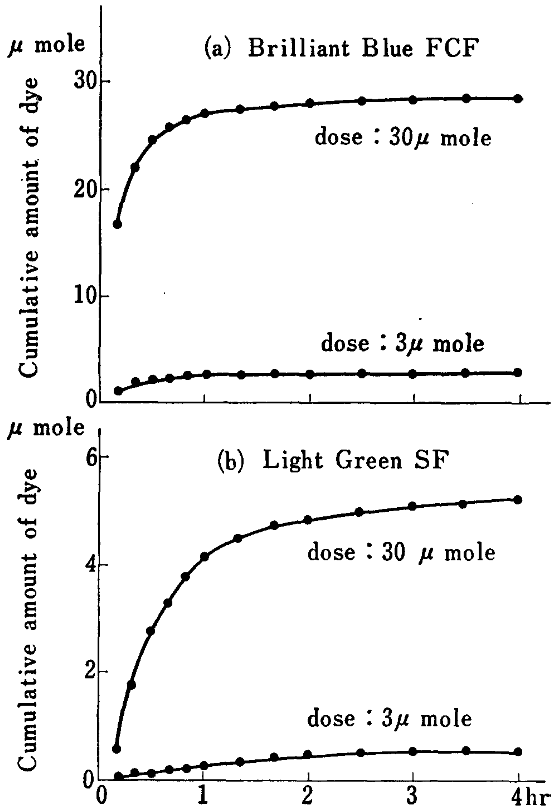


Fig. 1. (a) Cumulative Brilliant Blue (BB) Excretion Curves in Bile; (b) Cumulative Light Green SF (LG) Excretion Curves in Bile

Accordingly, it was suggested that substitution with some group at R_1 may decrease the biliary excretion ratio. But FG showed the similar excretion ratio to that of BB. As FG has one hydroxy group at R_1 of BB, it could be said that substitution with hydroxy group at R_1

TABLE II. Averaged Excretion Ratio in 4 hr (%)[a]

Dye	Dose (μmole) 3	15	30
Brilliant Blue FCF	94.2 (2)[b]	96.0 (3)[b]	91.0 (3)[b]
Light Green SF	25.2 (2)	21.0 (3)	18.7 (3)
Fast Green FCF	92.9 (3)	90.3 (2)	91.2 (3)
Guinea Green B	68.1 (2)	73.7 (3)	73.3 (3)
Acid Violet 6B[c]	65.4 (2)	59.9 (2)	60.0 (3)
Acid Violet 5B		26.1 (2)	26.2 (2)

a) $\text{ratio} = \frac{\text{excreted amount in 4 hr}}{\text{dose}} \times 100(\%)$
b) number of experiments
c) 7.5 μmole dose was administered in stead of 3 μmole.

had not remarkable effect in the excretion ratio. And moreover, substitution with dimethyl-amino group at R_1 was also found not to affect the excretion ratio as shown in the comparison with GG and AV6B where difference of the ratio was not clear. In this case AV6B has dimethyl group at R_1 of GG.

From these results, it was found that sulfonate group at R_1 has the decreasing effect specifically, and it was concluded that the effect of number of the sulfonate group is not so straightforward as Rado msky reported[5] and that the position of the sulfonate group has an important effect on the biliary excretion ratio.

As for the effect of hydroxy group, Williams[7] found the increasing effect of the group on the biliary excretion ratio in the study on hydroxy biphenyls. But in the present case the effect of hydroxy group on the ratio was hardly found, although only one compound, *i.e.*, FG was studied. But as stated in the latter section, it was found that hydroxy group had some effect on the excretion pattern. Accordingly, it may be said that hydroxy group effect is remained to be further investigation as Williams said.

Since plasma protein binding of dyes are expected to be one of the factors influencing the biliary excretion ratio, the authors tries to study on the binding of these dyes to plasma protein with equilibrium dialysis methods. The results were shown in Table III.

TABLE III. Binding Ratio with Plasma Protein after Dialysis at 37° (%)[a]

Dye	%	Dye	%
Brilliant Blue FCF	65.3	Fast Green FCF	74.6
Light Green SF	94.7	Guinea Green B	80.5

a) Visking tube contained three ml plasma and the external phase contained 30 ml isotonic pH 7.3 buffer solution. The initial dye concentration was 1 μmole/ml in the external phase.

BB had 65% ratio, but LG had almost 95% and it was suggested that the binding ratio was also one of the factors in the biliary excretion and further studies now are proceedihg in the authors' laboratory and will be reported in the latter paper.

(2) Excretion Pattern

In the previous paper, the authors reported three types of the biliary excretion patterns according to Nelson plots[8] from the cumulative excretion data. Type A was composed of two straight lines and Type B of one straight line. And Type C gave one straight line with the early lag period. These types appeared in an azo dye and riboflavin depending on dose. When a dose increased, the type changed from C to A. All of three types were not always observed for one compound in a studied dose range, but the above tendency of the type always appeared. In the present study of triphenylmethane dyes, the similar excretion patterns and dose dependency were observed. Typical examples for each dye were shown in Fig. 2—16, and types were listed in Table IV.

The standard compound of the present paper, GG showed Type B excretion pattern in all dose (3—30 μmole), but when substitutents were introduced in the benzene ring, the following effects on the excretion pattern were observed.

The sulfonate group at R_2 gave the most interesting effects, that is, BB gave remarkable curve line in the period corresponding to K_1 of high dose type (Type A), but the latter period gave straight line as shown in Fig. 2—4. Since the secondary plots of the usual method gave straight lines as also shown in Fig. 2—4, the possibility of compartmental model analy-

7) R.T. Williams, P. Millbrun and R.L. Smith, *Ann. N.Y. Acad. Sci.*, 123, 110 (1965).
8) E. Nelson, *J. Pharm. Sci.*, 50, 181 (1961).

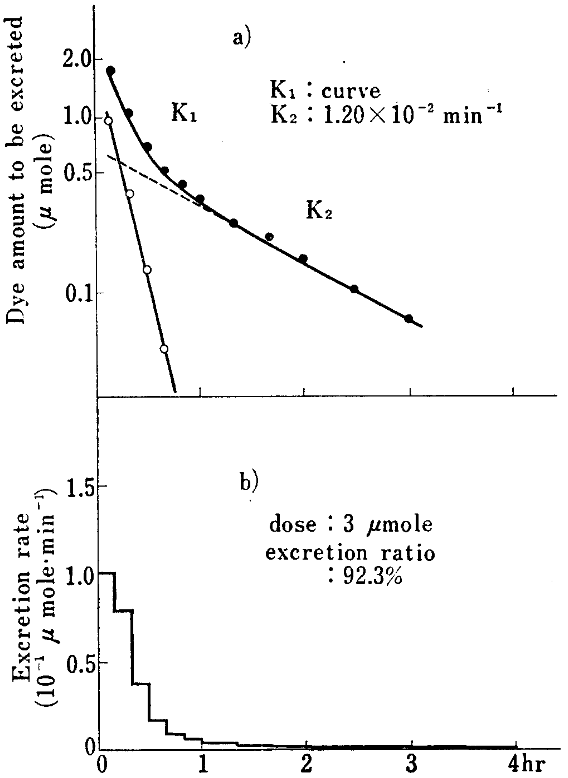


Fig. 2. a) Semilogarithmic Plots of Brilliant Blue FCF (BB) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Brilliant Blue (BB)

—●—: observed values —○—: secondary plots

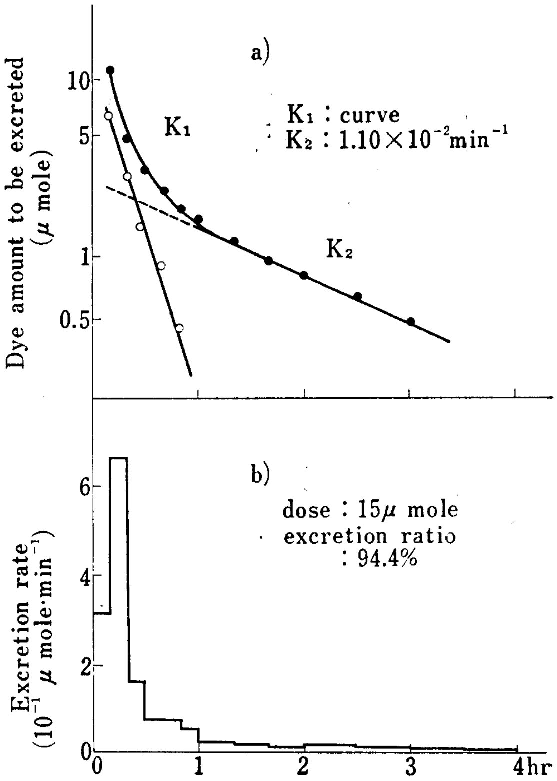


Fig. 3. a) Semilogarithmic Plots of Brilliant Blue FCF (BB) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Brilliant Blue (BB)

—●—: observed values —○—: secondary plots

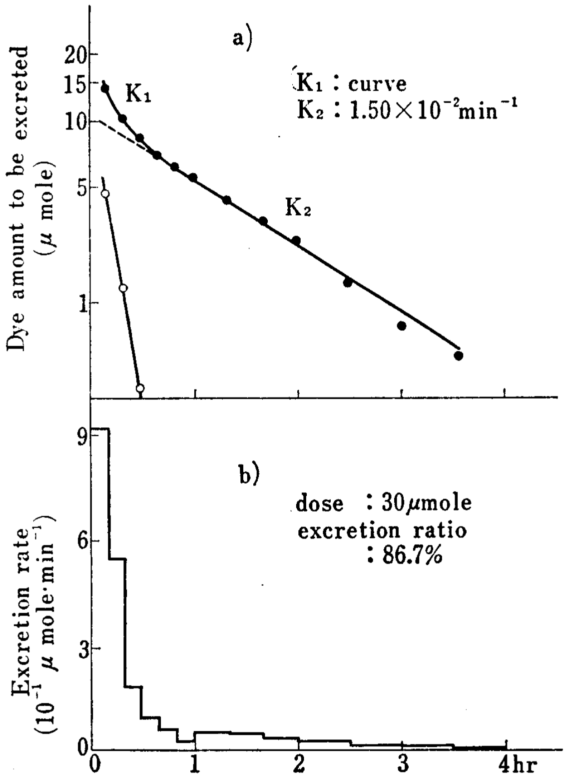


Fig. 4. a) Semilogarithmic Plots of Brilliant Blue FCF (BB) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Brilliant Blue (BB)

—●—: observed values —○—: secondary plots

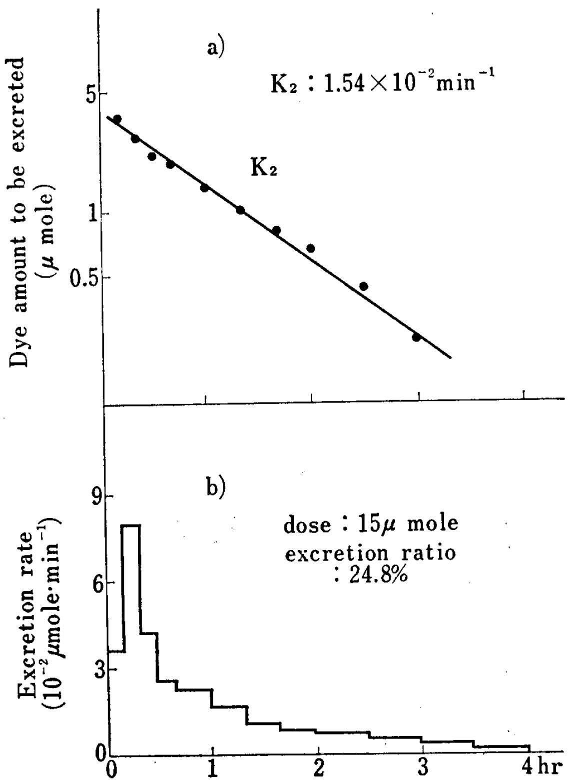


Fig. 5. a) Semilogarithmic Plots of Light Green SF (LG) in the Body to be excerted in Bile; b) Averaged Excretion Rate of Light Green SF (LG)

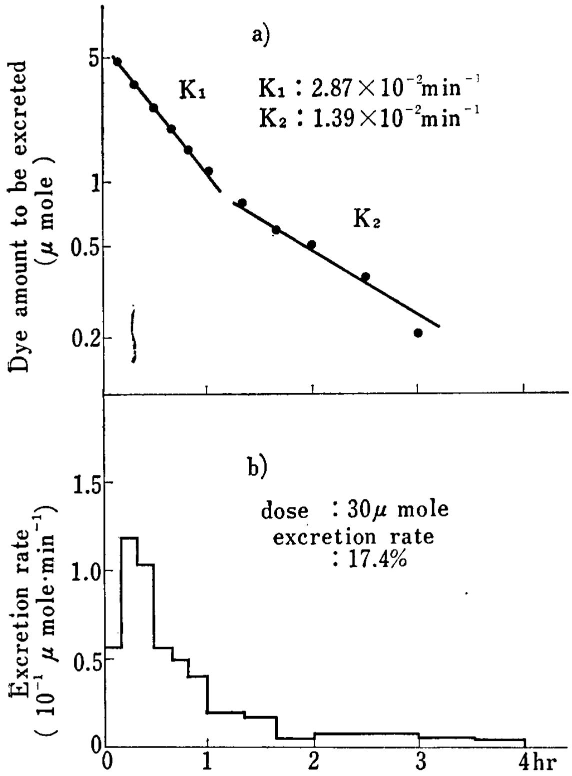


Fig. 6. a) Semilogarithmic Plots of Light Green SF (LG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Light Green SF (LG)

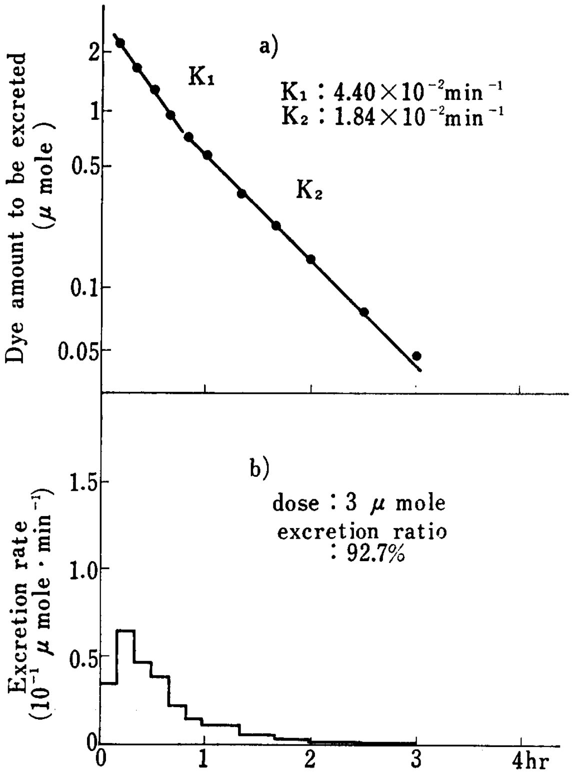


Fig. 7. a) Semilogarithmic Plots of Fast Green FCF (FG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Fast Green FCF (FG)

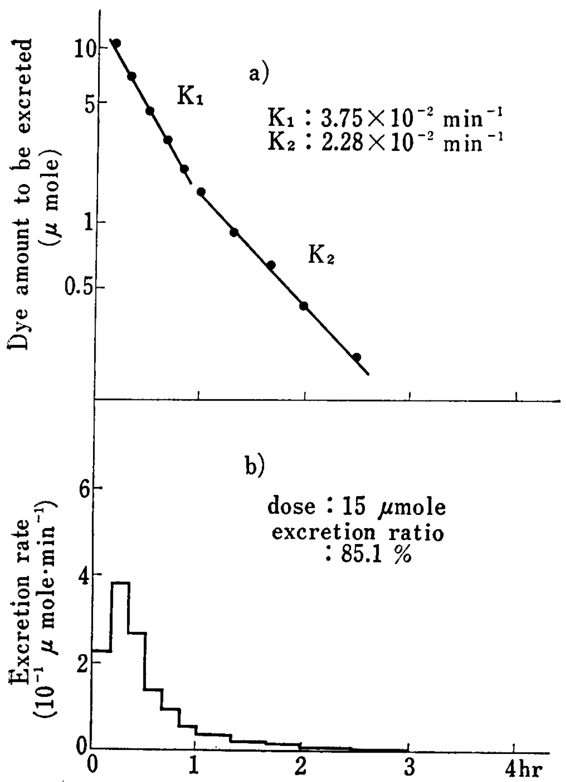


Fig. 8. a) Semilogarithmic Plots of Fast Green FCF (FG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Fast Green FCF (FG)

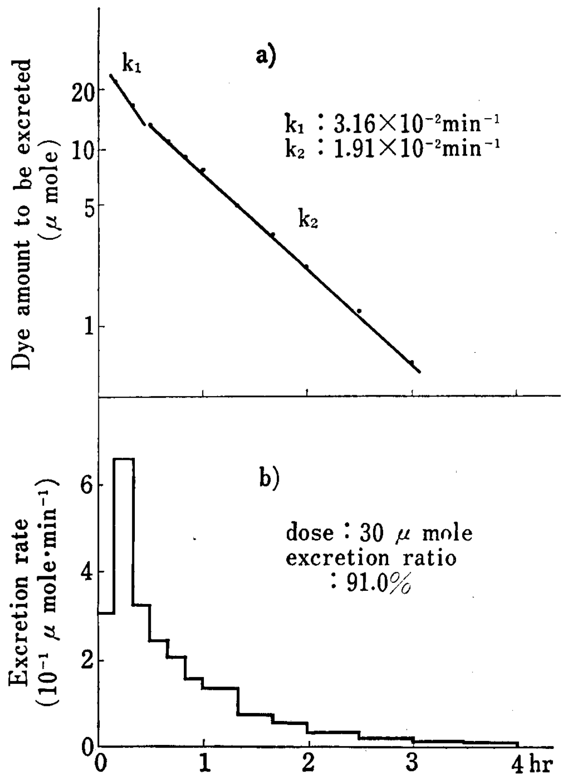


Fig. 9. a) Semilogarithmic Plots of Fast Green FCF (FG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Fast Green FCF (FG)

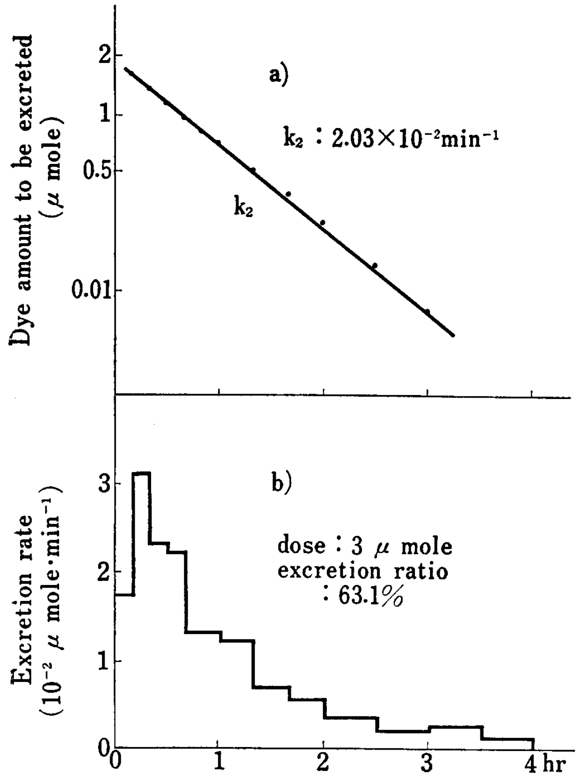


Fig. 10. a) Semilogarithmic Plots of Guinea Green B (GG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Guinea Green B (GG)

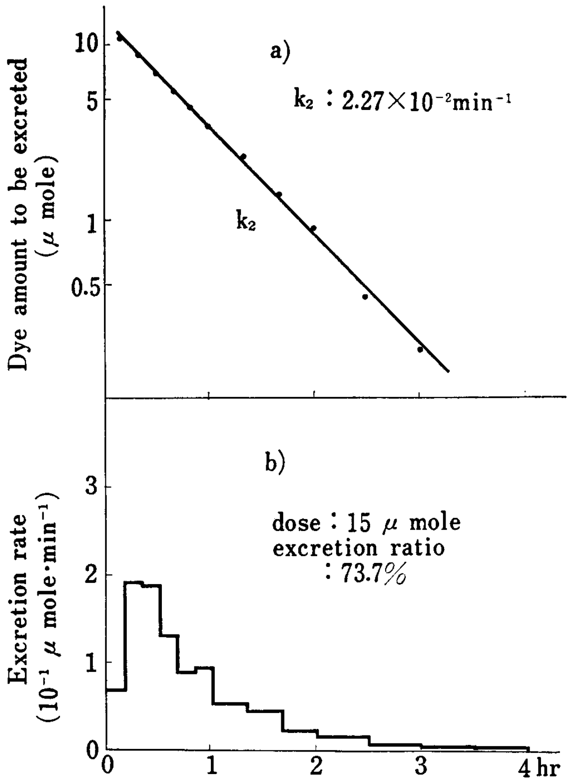


Fig. 11. a) Semilogarithmic Plots of Guinea Green B (GG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Guinea Green B (GG)

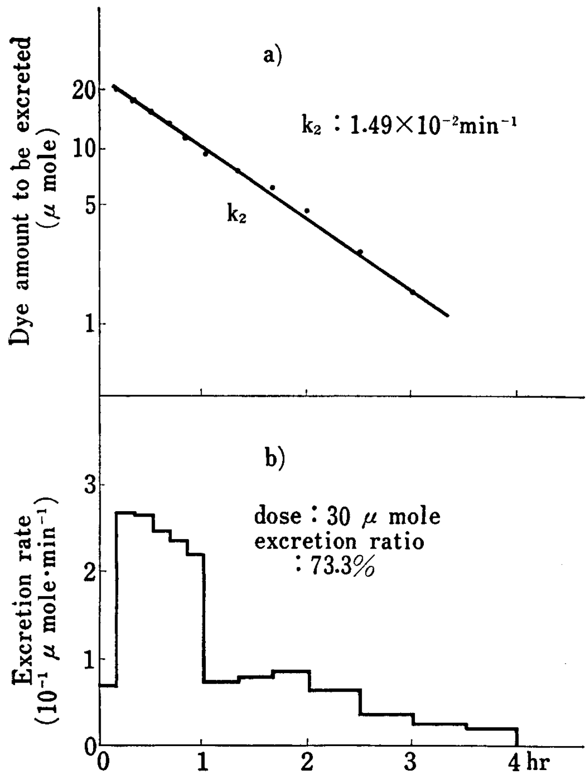


Fig. 12. a) Semilogarithmic Plots of Guinea Green B (GG) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Guinea Green B (GG)

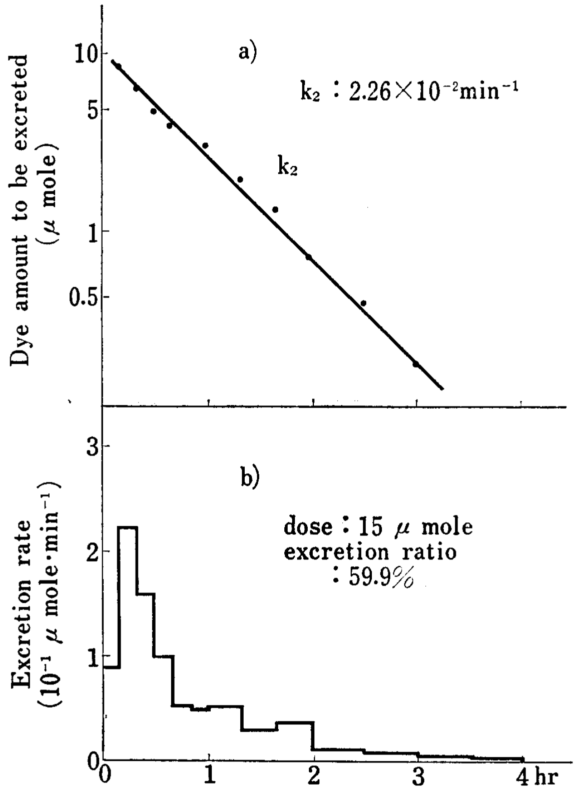


Fig. 13. a) Semilogarithmic Plots of Acid Violet 6B (AV6B) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Acid Violet 6B (AV6B)

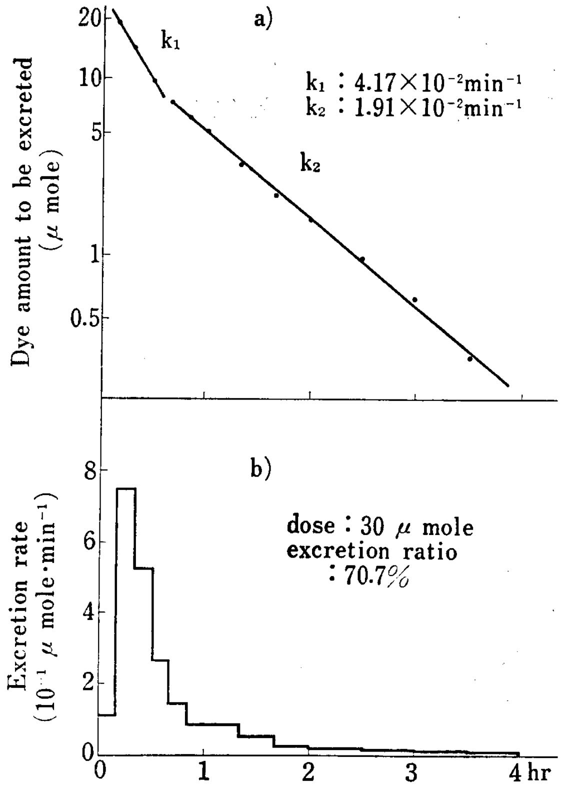


Fig. 14. a) Semilogarithmic Plots of Acid Violet 6B (AV6B) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Acid Violet 6B (AV6B)

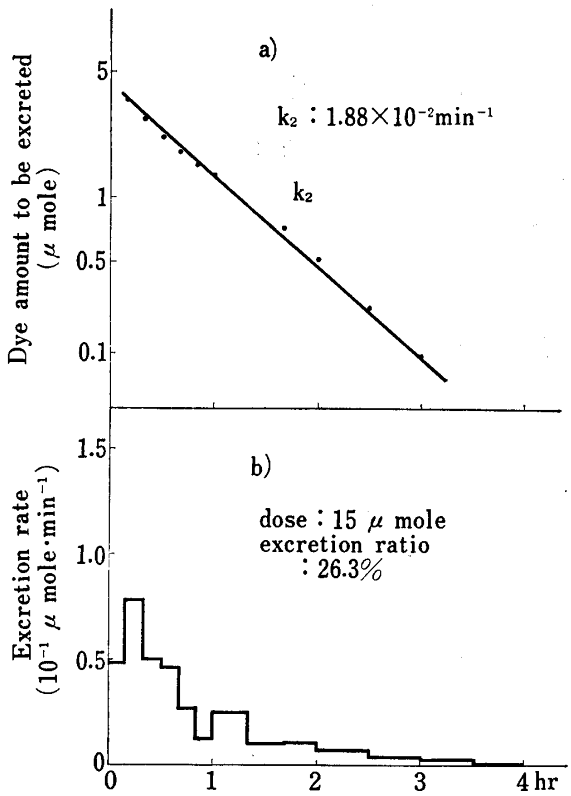


Fig. 15. a) Semilogarithmic Plots of Acid Violet 5B (AV5B) in the Body to be excreted in Bile; b) Averaged Excretion Rate of Acid Violet 5B (AV5B)

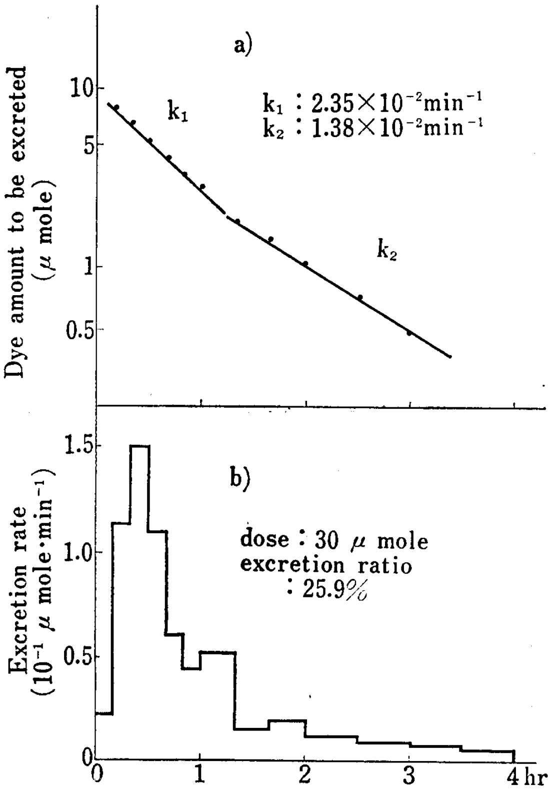


Fig. 16. a) Semilogarithmic Plots of Acid Violet 5B (AV5B) in the Body to be excreted in Bile; b) Averaged excretion Rate of Acid Violet 5B (AV5B)

Table IV. Biliary Excretion Rate Constant

Dye	Dose (μmole)	K_1 (10^{-2} min^{-1})	k_2 (10^{-2} min^{-1})	Type
Brilliant Blue FCF	30	curve	1.50	A*
	15	curve	1.10	A*
	3	curve	1.20	A*
Light Green SF	30	2.87	1.39	A
	15		1.54	B
	3		1.56	B
Fast Green FCF	30	3.16	1.91	A
	15	3.75	2.28	A
	3	4.40	1.84	A
Guinea Green B	30		1.49	B
	15		2.27	B
	3		2.03	B
Acid Violet 6B	30	4.17	1.91	A
	15		2.26	B
	7.5		2.14	B
Acid Violet 5B	30	2.35	1.38	A
	15		1.88	B

sis was suggested. The compartmental model analysis which includes blood concentration time course data is being tried in the authors' laboratory.

On the other hand LG, which has the same molecular weight and number of sulfonate groups as BB, showed different patterns depending on dose. At 3 and 15 μ mole dose, they showed Type B, and at dose of 30 μ mole showed Type A, and the ratio of k_1 to k_2 was about 2 and no difference from those of azo dyes was found. Accordingly, it was found that the sulfonate group at R_1 showed remarkable effect on the excretion ratio as described above, but had not essential effect on the excretion pattern.

FG showed Type A excretion pattern in the dose range studied here between 3 and 30 μ mole and the ratio of k_1 to k_2 were between 1.7 and 2.4. And from the comparison with BB, it was suggested that the hydroxy group at R_1 had some effect on the excretion pattern, but had little effect on the excretion ratio. And moreover AV6B which has a dimethyl amino group at R_1 of benzene ring instead of sulfonate in LG, showed dose dependency from Type B to Type A. As for AV5B which has ethyl group at R_2 in AV6B showed similar dose dependency to AV6B.

From these results, it was found that a sulfonate group beyond other groups had a remarkable effect on the biliary excretion ratio and pattern, and that the position of a sulfonate group as well as the number was important to determine its effect. And the possibility of the compartmental analysis for the biliary excretion process was suggested.