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Pharmacokinetic Studies of Biliary Excretion. V. The Relationship between the Biliary Excretion Behavior and the Elimination from Plasma of Xanthene Dyes and Bromsulphthalein in Rat[1,2]

TATSUJI IGA, SHOJI AWAZU and HISASHI NOGAMI

Faculty of Pharmaceutical Sciences, University of Tokyo[3]

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The relationship between the biliary excretion behavior and the elimination from plasma was studied using five xanthene dyes, *i.e.* Phloxine B (PB), Rose Bengal (RB), Erythrosine B (EB), Eosine Y (EY) and Fluorescein sodium (FS), and Bromsulphthalein (BSP) *in vivo* and *in vitro* in rat.

1) It was of interest that the plasma elimination patterns of xanthene dyes showed two types, the elimination following the one and two compartments models. PB and RB were the former group, and EB, EY and FS were the latter group.

2) The plasma elimination of xanthene dyes except for FS which has no halogen substituent, were relatively rapid ($T_{1/2}$=3—12 min). But the elimination of FS was very slow ($T_{1/2}$=60 min), and it was suggested that the effect of the halogen substituent was shown in the plasma elimination as well as in the biliary excretion.

3) The binding ratio with rat plasma of xanthene dyes showed similar ratios (55—70%) with each other except for FS. The ratio of FS was very low (15%) in spite of it's slow plasma elimination.

4) The plasma elimination of BSP was very rapid ($T_{1/2}$=3.3 min) and followed the two compartment model, but the binding ratio with plasma protein was rather high (about 50%) in spite of its rapid plasma elimination and concentrical biliary excretion.

5) It was also found that the bile acid gave some influence on the binding with BSA.

In the present serial paper,[1] the authors intended the kinetic study of the relationship between the biliary excretion behavior and the elimination from plasma using azo and triphenylmethane dyes *in vivo* and *in vitro* in rat. In azo dyes, Amaranth (AM) and New Coccine (NC) were studied, and it was found that the remarkable difference in the biliary excretion of these two dyes did not depend on the difference of the plasma elimination or binding to the plasma proteins, and was suggested that there were some other factors such as uptaking into liver cells, metabolism, urinaly excretion and so on. On the other hand, in triphenylmethane dyes, Brilliant Blue FCF (BB) and Light Green SF (LG) were studied, and it was also found that the remarkable difference in the biliary excretion behavior of these two dyes, mainly depended upon the difference of the affinity to the plasma proteins, since the binding ratio of LG was two times larger than that of BB.

In the present paper, the further studies have been tried with five xanthene dyes[4a] and Bromsulphthalein (BSP) *in vivo* and *in vitro* in rat. It was found that these dyes, except for Phloxine B (PB) and Rose Bengal (RB), showed the plasma elimination following the two exponential as well as azo and triphenylmethane dyes, but both PB and RB showed typical first order elimination, which was the first examples among the dyes investigated in the present series.

1) Part IV: T. Iga, S. Awazu, M. Hanano and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **19**, 2609 (1971).
2) Partial fulfilment of Doctor of Pharmaceutical Science degree requirement of Tatsuji Iga to the Graduate School, University of Tokyo.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) *a*) Phloxine B (PB), Rose Bengal (RB), Eosine Y (EY), Erythrosine B (EB) and Fluorescein sodium (FS); *b*) T.G. Richards, V.R. Tindall and A. Young, *Clin. Sci.*, **18**, 499 (1959).

Experimental

Materials——Dyes used in this study were shown in Table I. All dyes except for Bromsulphthalein were purchased from Wako Pure Chemical Industries, Ltd., and Tokyo Chemical Industires, Co., Ltd. Bromsulphthalein was used as the injective solution (Daiichi Chemical Industires, Ltd.). The bovine serum albumin was also purchased from Wako Pure Chemical Industries (powder fraction V). The bile salts was purchased from Difco Laboratories in USA (mixture type). And 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)
Xanthene dyes	Phloxine B (FD Red No. 104)	$C_{20}H_2O_5Br_4Cl_4Na_2$	829.71	538
	Rose Bengal (FD Red No. 105)	$C_{20}H_2O_5I_4Cl_4Na_2$	1018.58	547
	Erythrosine B (FD Red No. 3)	$C_{20}H_6O_5I_4Na_2$	879.92	526
	Eosine Y (FD Red No. 103)	$C_{20}H_6O_5Br_4Na_2$	691.88	517
	Fluorescein sodium	$C_{20}H_{10}O_5Na_2$	376.27	494
Sulfophthalein dye	Bromsulphthalein	$C_{20}H_8O_{10}S_2Br_4Na_2$	838.05	580

Drug Administration and Samplings——The procedure was carried out in the same way as described in the previous paper.[1]

Binding with Plasma Protein and Bovine Serum Albumin (BSA)——The procedure was carried out in the same way as described in the previous paper.[1] In the study of the effect of the bile salts on the binding, isotonic pH 7.3 buffer solution containing 1,2 and 4% of bile salts respectively, were used.

Analytical Methods——The procedure was also carried out in the same way as described in the previous paper[4] except for the elimination from blood study of BSP.

Bromsulphthalein (BSP)——One tenth ml of plasma samples were diluted 30 or 50 times with 1/2N NH_4OH and photometrically measured at the two wave lengths, 580 mμ and 425 mμ using Hitachi 124 spectro-photometer. The calculation was followed with the Richards'[4b] regression formula, viz: 1.02× (reading at 580 mμ) − 0.29× (reading at 425 mμ) = True reading of BSP at 580 mμ.

Result and Discussion

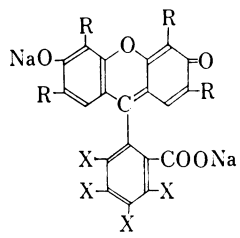
I. Elimination from Plasma

It was reported in the present serial paper[1] that the behavior of the elimination from plasma had great importance in the biliary excretion systems and has been studied by many workers in the clinical or physiological fields.

In this present paper, the authors intended to try the kinetic study of the elimination from plasma using five xanthene dyes, *i.e.* Phloxine B (PB), Rose Bengal (RB), Erythrosine B (EB), Eosine Y (EY) and Fluorescein sodium (FS), and Bromsulphthalein (BSP). The chemical structures of these dyes were shown in Chart 1. And the relationship between the plasma-elimination pattern and the biliary excretion behavior reported in the previous paper,[5] was discussed in the same dose (30 μ mole).

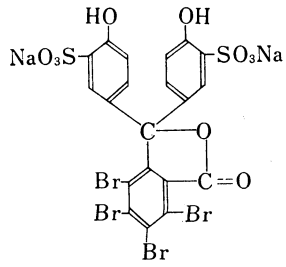
Xanthene Dyes——In the previous paper,[5] the effect of the halogen substituents on the biliary excretion were studied using the same five xanthene dyes. It was found that the excretion behavior was similar to those of azo dyes and triphenylmethane dyes which had some sulfonate substituents. As for the biliary excretion ratios, these dyes of this series showed common larger excretion ratios than 75% except for FS which had no halogene groups, and furthermore, it was characteristic of xanthene dyes that in the excretion pattern, except for PB which had both -Br and -Cl, all other dyes did not show high dose type (Type A)[1] in the same dose range as azo and triphenylmethane dyes (3—30 μ mole doses) and in addition,

5) T. Iga, S. Awazu and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **19**, 297 (1971).



Xanthene dyes

	R	X
Phloxine B (PB)	Br	Cl
Rose Bengal (RB)	I	Cl
Erythrosine B (EB)	I	H
Eosine Y (EY)	Br	H
Fluorescein sodium (FS)	H	H



Sulfophthalein dye

Bromsulphthalein (BSP)

Chart 1. Chemical Structure of Xanthene Dyes and Bromsulphthalein

RB and EB showed low dose type with the early lag period (Type C)[1] in 15 and 3 μ mole doses and this type was never seen in both azo and triphenylmethane dyes.

In this paper, the relationship between the characteristic behavior in the biliary excretion of xanthene dyes and the plasma elimination pattern, were studied.

1. Phloxine B and Rose Bengal: It was reported in the previous paper[5] that PB showed high excretion ratio (about 90%) and typical high dose type (Type A) in 30 μ mole, dose, and that this depended mainly upon both -Br and -Cl. On the other hand, RB which has -I and -Cl showed only low dose type in the same dose range and in 15 and 3 μ mole doses, showed Type C, and it was suggested that -I gave these delay of excretion. The biliary excretion patterns of these dyes in 30 μ mole dose, were shown in Fig. 1.

The plasma elimination curves of these dyes were shown in Fig. 2.

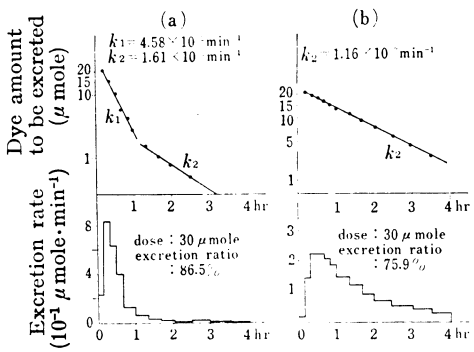


Fig. 1. Semilogarithmic Plots of Dyes in the Body to be Excreted in Bile and Averaged Excretion Rate of Dyes

(a) Phloxine B (PB), (b) Rose Bengal (RB)
(data from the previous paper[6])

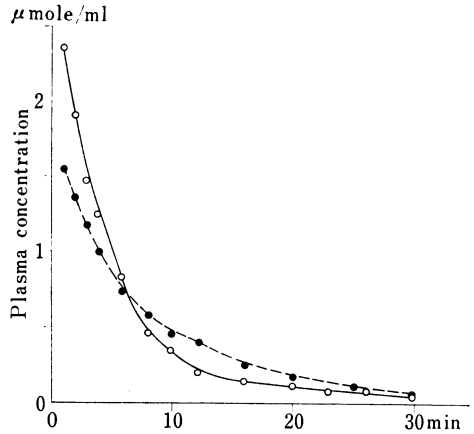


Fig. 2. Plasma Elimination Curves of Phloxine B (PB) and Rose Bengal (RB)

dose: 30 μ mole
—○—: Phloxine B (PB)
---●---: Rose Bengal (RB)

It is of very interest that the plasma elimination of these two dyes did not follow the two compartment open model, but the tybical first order as shown in the semilogarithmic plots of the plasma concentration of these dyes (Fig. 3).

It was found that PB disappeared from plasma very rapidly and its elimination rate constant and half-life were 2.16×10^{-1} min^{-1} and 3.2 min, respectively. On the other hand, as for RB, the disappearance was also rapid but the elimination rate constant was two times smaller than that of PB. These values were 1.10×10^{-1} min^{-1} and 6.3 min. In spite of the rapid elimination, RB did not show the rapid biliary excretion shown in PB termed as Type A, this was probably due to the larger uptake into liver cells as Glaser reported.[6]

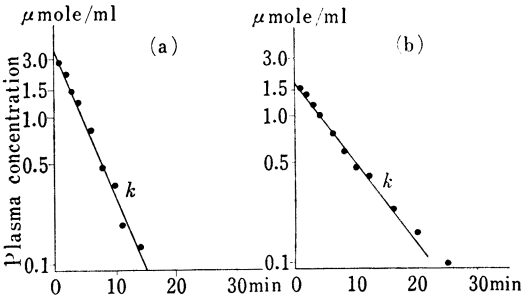


Fig. 3. Semilogarithmic Plots of Plasma Concentration of (a) Phloxine B (PB), (b) Rose Bengal (RB)

(a) $K=2.16\times10^{-1}$ min, $T_{1/2}=3.2$ min
(b) $K=1.10\times10^{-1}$ min, $T_{1/2}=6.3$ min

Furthermore, as to the disappearance of RB from blood or plasma, it has been studied in the medical fields up to this time, since the I^{131}-RB had been often used for the target of liver function test for a longtime as well as BSP and Azorubin S (AS). Delprat[7] reported that the elimination of RB was influenced by the liver injury. Taplin,[8] *et al.* studied the medical use of I^{131}-RB in rabbit and men. And Glaser,[6] *et al.* also studied the mechanism of removal of I^{131}-RB from plasma in rat using the technique of autoradiography, and reported the distribution of radioactivity among plasma, liver and gastrointestinal tract based upon radioassay data. From these results, the liver concentration of I^{131}-RB reached maximum at about 20 min, and the plasma concentration decreased below one half of initial concentration at about 7 min and this data coincided well with our data ($T_{1/2}=6.3$ min).

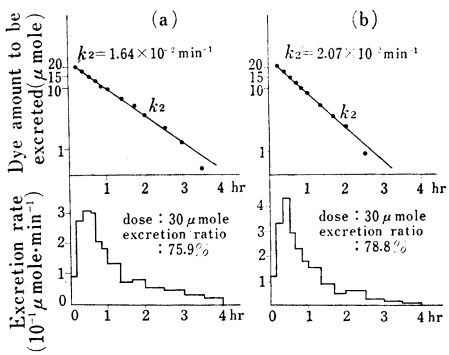


Fig. 4. Semilogarithmic Plots of Dyes in the Body to be Excreted in Bile and Averaged Excretion Rate of Dyes

(a) Erythrosine B (EB), (b) Eosine Y (EY)
(data from the previous paper[6])

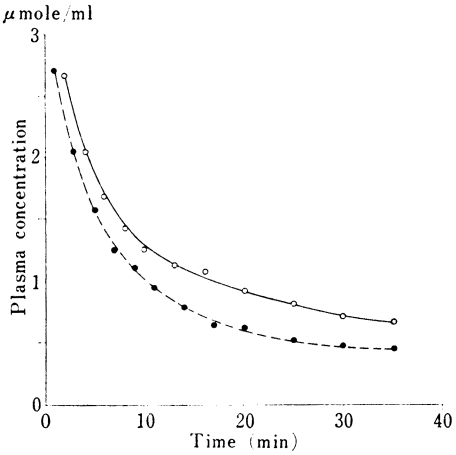


Fig. 5. Plasma Elimination Curves of Erythrosine B (EB) and Eosine Y (EY)

dose: 30 μmole
○ : observed values of Erythrosine B
● : observed values of Eosine Y
——: calculated curve of Erythrosine B
------: calculated curve of Eosine Y

6) W. Glaser, W.D. Gibbs and G.A. Andrews, *J. Lab. Clin. Med.*, **54**, 556 (1959).
7) G.D. Delprat, Jr., *Arch. intern. Med.*, **32**, 401 (1923).
8) G.V. Taplin, O.M. Meredith and H. Kade, *J. Lab. Clin. Med.*, **45**, 665 (1955).

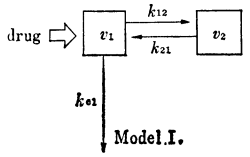
2. Erythrosine B and Eosine Y: As reported previously,[5] EY which had -Br showed Type B in all dose range studied, but EB which had -I instead of -Br showed Type C in 15 and 3 μmole dose, although no significant difference was found in the excretion ratio between them. And this was probably due to -I as well as in RB. The excretion patterns in 30 μ mole dose of these dyes were shown in Fig. 4.

The plasma elimination curves of these dyes were shown in Fig. 5. It was found that the elimination followed the two compartment open system, and the pharmacokinetic constants were calculated with the iterative least square method programed as the routine in our laboratory[9] The results of the calculation were shown in Table II.

Table II. Pharmacokinetic Constants for the Two Compartment Model[a]

Dye	k_{12}[a,b]	k_{21}[a,b]	k_{el}[a,b]	$T_{1/2}$[c]	V_1[a,d]	V_2[a,d]
Erythrosine B (EB)	0.143	0.139	0.054	12.8	7.9	8.1
Eosine Y (EY)	0.098	0.064	0.056	12.4	9.5	14.6
Fluorescein sodium (FS)	0.343	0.343	0.012	57.8	13.7	13.7
Bromsulphthalein (BSP)	0.267	0.222	0.213	3.3	15.9	19.1

a) Calculation was carried out with the least square iteration method programed in our laboratory (Model I).[10]
b) min^{-1}
c) $T_{1/2}$ (min)$=0.693/k_e$
d) ml



The elimination rate constant of EB was 0.54×10^{-1} min^{-1} and that of EY was 0.56×10^{-1} min^{-1}, and these two values were nearly equal with each other.

3. Fluorescein sodium: Fluorescein sodium (FS) which had no halogen substituent, was studied for the comparison. As reported in the previous paper,[5] the biliary excretion ratio in 4 hr, about 45%, was much smaller than those of other xanthene dyes which had 4 or 8 halogen substituents, above 74%, and the excretion pattern showed only low dose type (Type B) in all dose ranges studied (3—30 μ mole). The plasma elimination curve of FS was shown in Fig. 6.

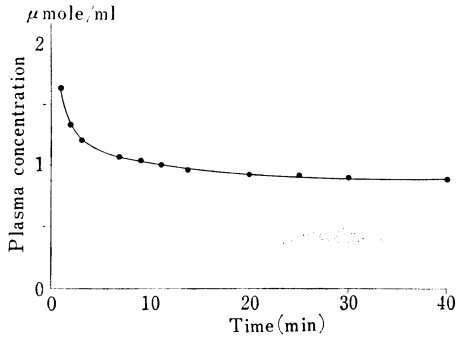


Fig. 6. Plasma Concentration Time Course of Flourescein Sodium (FS)
dose: 30 μmole
● : observed values
——: calculated curve

It was found that the elimination was very slow and the dye concentration was kept to high level for relatively long time. But the elimination followed the two compartment open model and the pharmacokinetic constants calculated with the same program,[9] were listed in Table II. The elimination rate constant (k_{el}) was 0.12×10^{-1} min^{-1}, and its half life was 57.8 min and this value was still greater than that of Light Green SF (LG), 40 min, which was the largest among azo and triphenylmethane dyes in this series and was ascribed to the strong binding with the plasma protein.[5] As for the binding with plasma protein and BSA, it is discussed in the latter section.

9) H. Nogami, M. Hanano, S. Awazu and H.H. Moon, *Chem. Pharm. Bull.* (Tokyo), **17**, 2097 (1969).

It was suggested that the effect of the halogen substituent was shown in the plasma elimination as well as in the biliary excretion.

Bromsulphthalein——Since Bromsulphthalein (BSP) is the most useful drug for the hepatic function test, there have been many reports up to this time as discussed in the previous paper.[5] Particularly, in the clinical function test, the plasma elimination has much studied by many workers, since the plasma residual concentration is used for the target. Ingelfinger, *et al.*[10] reported the disappearance from the blood after a single intravenous injection in both healthy men and patients. Mendeloff, *et al.*[11] reported the factors affecting the disapperance from the blood after a single intravenous injection in men and suggested the "saturation" of plasma disappearance ratio (PRD). Brauer, *et al.*[12] studied the removal of BSP from blood plasma with the perfused liver method in rat. Furthermore, Combes, *et al.*[13] studied the mechanism of BSP removal from the blood. As for the mathematical treatment of the plasma disappearance, Richards, *et al.*[14,15] reported that the disappearance followed the two exponential curve and showed the calculation time course curves of blood, liver and bile concentation.

In this series of study, BSP was used for the comparison with other dyes, *i.e.* azo, triphenylmethane and xanthene dyes, since BSP has both sulfonate and halogenate substituents and furthermore showed the most concentrical biliary excretion behavior even in a low dose range (0.6—6 μmole). The biliary excretion pattern at 6 μmole dose was shown in Fig. 7.

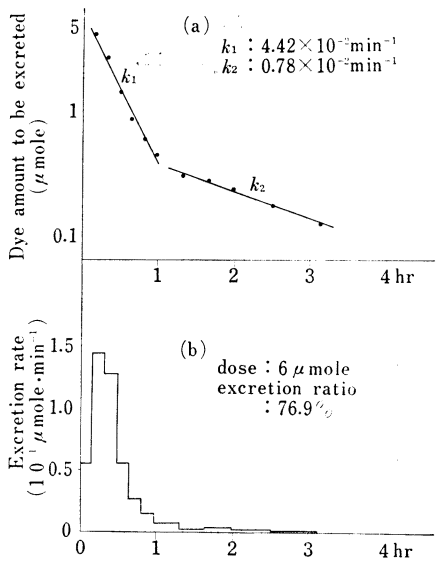


Fig. 7. (a) Semilogarithmic Plots of Bromsulphthalein (BSP) in the Body to be Excreted in Bile. (b) Averaged Excretion Rate of Bromsulphthalein (BSP)

(data from previous paper[5])

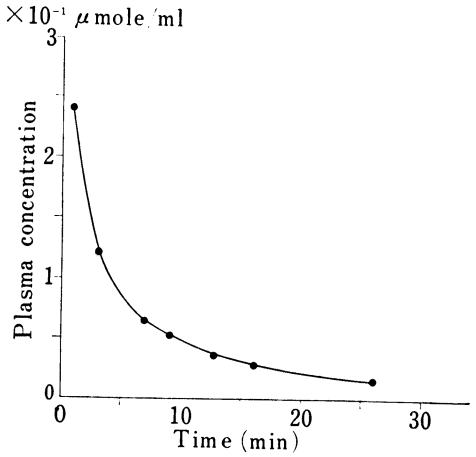


Fig. 8. Plasma Concentration Time Course of Bromsulphthalein (BSP)

dose: 6 μmole
● : observed values
——: calculated curve

10) F.J. Ingelfinger, S.E. Bradley, A.I. Mendeloff and P. Kramer, *Gastroenterology*, **11**, 646 (1948).
11) A.I. Mendeloff, P. Kramer, F.J. Ingelfinger and S.E. Bradley, *Gastroenterology*, **12**, 222 (1949).
12) R.W. Brauer and R.L. Pessotti, *J. Pharmacol. Exptl. Therap.*, **97**, 358 (1949).
13) B. Combes, H.O. Wheeler, A.W. Childs and S.E. Bradley, *Trans. Assn. Amer. Physicians*, **69**, 276 (1956).
14) T.G. Richards, V.R. Tindall and A. Young, *J. Physiol.*, **138**, 38p (1957).
15) T.G. Richards, V.R. Tindall and A. Young, *Clin. Sci.*, **18**, 499 (1959).

It was found that the plasma elimination after 6 μmole dose administered, followed to the two compartment open model as Richards, *et al.*[14] reported, and the results after calculated with the iterative least square method program,[9] were ploted in Fig. 8 and the pharmacokinetic constants were listed in Table II. The value of k_{12} was 2.67×10^{-1} min^{-1} and the plasma elimination rate constant (k_{el}) was 2.13×10^{-1} min^{-1}. These values were the same degree to those of Brilliant Blue FCF (BB)[5] which showed also the concentrical biliary excretion. The half-life of plasma elimination was 3.3 min and this value was nearly equal to that of BB (2.8 min).

It is said that the biliary excretion of BSP saturates in high dose and this depends on the capacity of uptaking into liver or secretion into bile flow. Since the dose range was relatively low (0.6—6 μmole) in this series, no saturation was observed in the plasma elimination nor in the biliary excretion.

Table III. The Relationship between the Binding Ratio and the Biliary Excretion Ratio

Dye	Binding Ratio (%)[a,b]		Excretion ratio in 4 hr (%)[c,d]
	BSA	Rat plasma protein	
Phloxine B (PB)	59.3	64.1	92.9
Rose Bengal (RB)	57.8	67.4	78.8
Erythrosine B (EB)	58.8	68.4	75.0
Eosine Y (EY)	49.5	56.7	76.2
Fluorescein sodium (FS)	13.5	15.7	45.0
Bromsulphthalein (BSP)	26.7	50.3	85.9

a) Visking tube contained three ml BSA (Bovine Serum Albumin) solution (Concn. 10^{-4}M/liter) or rat plasma (mixture of 15 animals) and the external phase contained 30 ml isotonic pH 7.3 buffer solution. The initial dye concentration was 1μmole/ml in the external phase. The results were obteined after dialysis for 120 hr at 4°.
b) The value was the average of 3 experiments.
c) data from the previous paper[6]
d) The value was the average of 8—9 animals.

II. Binding with plasma Protein and Bovine Serum Albumin

The influence of the binding with rat plasma protein and bovine serum albumin (BSA) on the biliary excretion, and the effect of the bile acid on the binding, were studied *in vitro* using equilibrium dialysis method. The results after dialysis at 4° for 120 hr were listed in Table III.

It was found that PB, RB and EB showed similar binding ratio with each other in both rat plasma and BSA, but EY had about 10% smaller ratios than those of the fomers. On the other hand, FS which had no halogen substituent, showed only about 15% in rat plasma and this value was the lowest one in this series of dyes. It is of interest that the plasma concentration of FS was kept to high level for relatively long time as shown in Fig. 6 in spite of

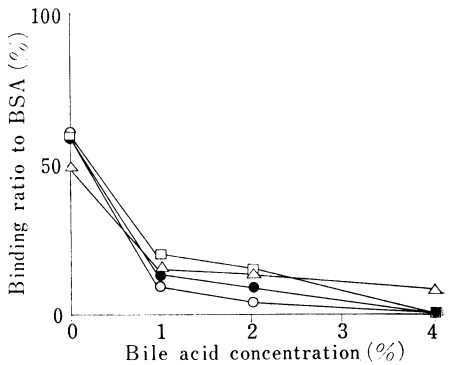


Fig. 9. The Infulence of the Bile Acid on the Binding with the Bovine Serum Albumin (BSA)

—●—: Phloxine B (PB)
—○—: Rose Bengal (RB)
—□—: Erythrosine B (EB)
—△—: Eosine Y (EY)

its low binding ratio with plasma protein. It was suggested that there were some other factors such as the interaction with blood cells, saturation in the uptaking into liver or excretion into urine, since FS was more excretable into urine than into bile as Webb, *et al.*[16] reported.

As for BSP, it was also of interest that the binding ratio with plasma protein was about 50% in spite of its concentrical biliary excretion and rapid plasma disappearance.

In the study of the effect of the bile acid, it was found that with the increasing of the bile acid concentration, the binding ratio was decreased as shown in Fig. 9. It was suggested that *in vivo*, the bile acid gave some influence on the transfar process in liver, and a future detail study will be necessary.

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.

16) J.N. Webb, M. Fonda and E.A. Brouwer, *J. Pharmacol.* 137, 141 (1962).