[Chem. Pharm. Bull. 27(6)1299—1306(1979)]

UDC 615.281.033.076.9 : 615.31.015.2

Effect of Salicylate on the Blood Concentration Profile and Distribution of Sulfonamides in Rat[1,2]

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(Received August 25, 1978)

The effect of concomitant administration of salicylate on the pharmacokinetics of five sulfonamides in rats was studied. Changes in the blood concentration profiles of sulfonamides at the elimination phase due to salicylate treatment were classified into three types: Type I– little change was observed (sulfanilamide); Type II– the blood concentration was decreased, but the disposition rate constant, k_β, was hardly influenced (sulfadiazine and sulfisoxazole); Type III– the value of k_β decreased (sulfamethoxazole and sulfathiazole). An intravenous administration of salicylate after administration of sulfamethoxazole brought about a sudden decrease in the blood concentration of sulfamethoxazole. The effects of salicylate treatment on the distribution of sulfamethoxazole and sulfadiazine were studied employing a two-compartment open model. Their distribution to the tissue compartment of the model was increased by the salicylate treatment. The effect of salicylate was interpreted on the basis of a redistributional interaction of sulfonamides with salicylate.

Keywords——sulfonamides; salicylate; concomitant administration; pharmacokinetics; tissue distribution; rat; blood concentration; liver concentration

Drugs distributed in the blood are usually bound to some extent to plasma proteins. The extent of protein binding influences the pharmacokinetics of drugs in a living body as well as their pharmacologic and therapeutic efficacies, since only the unbound fraction is generally believed to be effective as regards distribution to the active site. Many drugs bound to plasma protein are easily displaced by non-steroidal anti-inflammatory drugs, including salicylate.[4–6] Many studies[7–9] have demonstrated that concomitant administration of anti-inflammatory drugs modifies the distribution and elimination of other drugs. This has been discussed on the basis of displacement of the bound drugs from plasma protein by the anti-inflammatory drugs.

In the previous reports, sulfonamides were found to be displaced[6] from bovine serum albumin by the addition of salicylate and their distribution to rat red blood cells[1] improved in the presence of salicylate. In the present work, the effects of salicylate on the blood concentration and tissue distribution of sulfonamides were studied following intravenous administration of sulfonamides to rats with concomitant administration of salicylate.

1) Concomitant Administration of Drugs II. Part I: Y. Higashi, S. Uei, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **27**, 23 (1979).
2) A Part of this work was presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, Apr. 1975.
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4) H.M. Solomon, J.J. Schroge, and D. Williams, *Biochem. Pharmacol.*, **17**, 143 (1968).
5) J.H. Perrin and D.A. Nelson, *J. Pharm. Pharmacol.*, **25**, 125 (1973).
6) Y. Higashi, T. Nishihata, K. Kubo, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3572 (1978).
7) E.G. McQueen and W.H. Wardell, *Br. J. Pharmacol.*, **43**, 312 (1971).
8) B.H. Thomas, B.B. Coldwell, H.S. Buttar, and W. Zeitz, *Chem. J. Physiol. Pharmacol.*, **51**, 205 (1973).
9) Y. Imamura, K. Shigemori, and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **23**, 1377 (1975).

Experimental

Materials——Commercially available sulfonamides and sodium salicylate were used without further purification. Other reagents were of analytical reagent grade, and distilled water was used throughout.

Distribution Study in the Rat——Male rats (Sprague–Dawley strain, 250—300 g body weight) were kept in individual cages with free access to food and water. They were lightly anesthetized with ether for about 1 min when a drug was to be administered or a blood sample taken. Anton[10] reported that this amount of ether had no effect on the distribution of sulfonamide in rats. Similar results were obtained in the present study. Thus, the effect of ether on the distribution of sulfonamides was neglected. Drugs were dissolved with the aid of NaOH. The solution of sulfonamide was administered into the tail vein. Blood samples were taken by cardiac puncture, using ethylene diamine tetraacetic acid (EDTA) to prevent clotting. For the study of concomitant administration of salicylate, it was subcutaneously administered to the dorsum of the rats. The blood concentration of salicylate, which was measured by a gas chromatographic method,[11] reached a maximum at 30 to 40 min after administration, and its elimination profile from blood was similar to that after intravenous administration. Thus, in the present experiments, salicylate was subcutaneously administered 25 min prior to the administration of sulfonamide. Preliminary experiments revealed that the subcutaneous administration of saline did not affect the blood concentration of sulfonamides. Thus, the effect of salicylate on the fate of sulfonamide in the rat was considered to be a result of interaction between the two drugs. Control experiments were performed by single administration of sulfonamide without salicylate. Salicylate had a marked effect on the blood concentration of sulfamethoxazole. Preliminary experiments indicated that a difference of administration route of salicylate (subcutaneous or intravenous injection) had little effect on the salicylate-induced change in the blood concentration of sulfonamide. Thus, subcutaneous injection of salicylate was employed in the present experiments.

Assay of Drugs——For the assay of unchanged sulfonamides in whole blood, 0.5 ml of blood sample was hemolyzed with 5 ml of distilled water, and mixed with 3 ml of 10% aqueous trichloroacetic acid solution to remove protein. The mixture was filtered and the filtrate was assayed employing a modification of the Bratton–Marshall method.[12]

For the assay of sulfamethoxazole in liver, rats were decapitated at a designated time after dosing, and the livers were immediately dissected and frozen until analyzed. Five grams of liver was weighed, homogenized in 50 ml of water, and then centrifuged for 30 min at $10000 \times g$ under cooling. The supernatant was collected and deproteinized with trichloroacetic acid. After standing for 15 min followed by filtration, the filtrate was treated with Tsuda's reagent according to the Bratton–Marshall method,[12] and then it was extracted with iso-amyl alcohol. The organic layer was measured colorimetrically at 545 nm.

Results and Discussion

Blood Concentration Profiles

To study the effects of salicylate on the blood concentration of sulfonamides, the animals were first given salicylate by subcutaneous injection at a dose of 500 μmol/kg. Twenty-five minutes were then allowed for the drug to distribute throughout the body. Sulfonamide was then given by intravenous injection and blood samples were taken at appropriate intervals thereafter to follow the time course of the drug concentration in the blood. The profiles of concentrations of five sulfonamides are presented in Fig. 1 a—e. The blood concentrations are expressed as micromoles of unchanged sulfonamide per liter of blood. The profiles obtained from the control rats given sulfonamide alone were compared with the profiles obtained when salicylate was administered concomitantly. Linear correlations were obtained for semilogarithmic plots of the blood-concentration profiles of the five sulfonamides studied. These results were regarded as representing the elimination profile at the β-phase of a two-compartment model. The disposition rate constant,[13] k_β, was obtained from the slope, and

10) A.H. Anton, *J. Pharmacol. Exp. Ther.*, **134**, 291 (1961).
11) B.H. Thomas, G. Solomonraj, and B.B. Coldwell, *J. Pharm. Pharmacol.*, **25**, 201 (1973).
12) M. Yamazaki, M. Aoki, A. Kamada, and N. Yata, *Yakuzaigaku*, **27**, 37 (1967).
13) M. Gibaldi and D. Perrier, "Drugs and the Pharmaceutical Sciences Vol. I: Pharmacokinetics," ed. by J. Swarbrick, Marcel Dekker, Inc., New York, 1975, p. 56.

the extrapolated volume of distribution,[14] Vd_{ext}, calculated by dividing the dose by the intercept, employing the results in Fig. 1 (Tables I and II).

The concomitant administration of salicylate was found to have little effect on the two pharmacokinetic parameters of sulfanilamide. Similar results were reported by Imamura *et al.*[9] employing rabbits. Takada *et al.* also reported that the blood concentration profiles of sulfanilamide in the dog were not influenced by the coadministration of bucolome,[15] oxyphenbutazone or sulfinpyrazone.[16] In the present study, the values of k_β for sulfadiazine and sulfisoxazole were not influenced by the concomitant administration of salicylate, but the values of Vd_{ext} were found to be significantly decreased by salicylate treatment. Imamura *et al.*[17] reported that a concomitant intravenous administration of sulfadimethoxine and salicylate to rabbits resulted in a decrease in the blood concentration of sulfonamide at the β-phase, but the elimination rate constant of the drug was not influenced by the salicylate treatment.

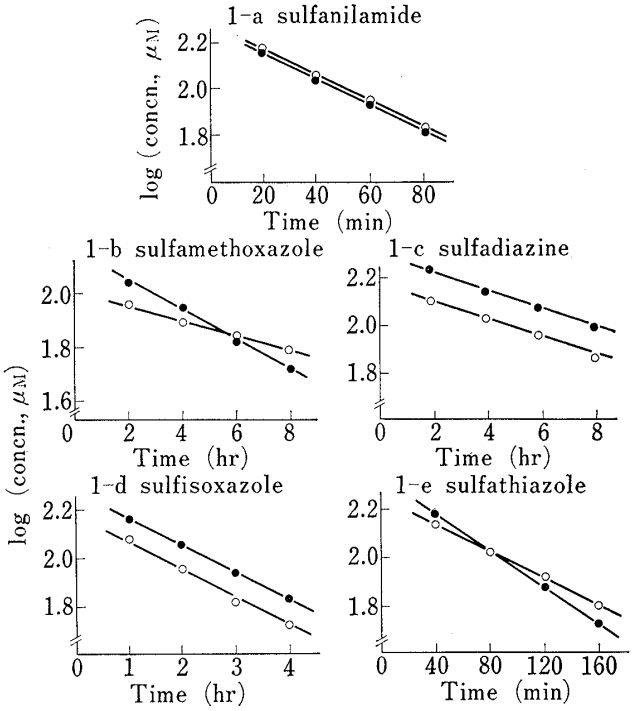

Fig. 1a—e. Effect of Salicylate on the Blood Concentrations of Sulfonamides after Intravenous Administration

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Doses of sulfonamides;
sulfamethoxazole: 50 μmol/kg, *i.v.*
sulfanilamide, sulfadiazine, sulfsoxazole and sulfathiazole: 100 μmol/kg, *i.v.*
Each point represents the mean for four to six rats.

Table I. Effect of Salicylate on the Disposition Rate Constants at the Elimination Phase after Intravenous Administration of Sulfonamides

Sulfonamides	$k_\beta \times 10^2$ [a] (hr^{-1})	
	Control[b]	SA treatment[c]
Sulfanilamide	74.0 ±4.7	74.3 ±4.3
Sulfamethoxazole	10.72±0.34	6.48±0.50[d]
Sulfadiazine	9.39±0.35	9.32±0.36
Sulfisoxazole	24.9 ±1.7	28.2 ±3.8
Sulfathiazole	53.1 ±3.0	41.7 ±1.8[d]

a) Mean±standard error.
b) Single administration of sulfonamides.
c) With salicylate (500 μmol/kg, *s.c.*).
d) $p<0.01$.

14) J.G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Ill., 1975, p. 83.
15) M. Takada, S. Akuzu, A. Misawa, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 542 (1974).
16) M. Takada, A. Misawa, K. Fujimoto, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 551 (1974).
17) Y. Imamura, K. Shigemori, and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2324 (1974).

Table II. Effect of Salicylate on the Extrapolated Volume of Distribution after Intravenous Administration of Sulfonamides

Sulfonamides	Vd_{ext}[a] (ml/kg)	
	Control[b]	SA treatment[c]
Sulfanilamide	538± 4	531±36
Sulfamethoxazole	377±30	499±28[d]
Sulfadiazine	505±10	673± 6[e]
Sulfisoxazole	545±36	647±25[d]
Sulfathiazole	462±32	545±13[d]

a) Mean±standard error.
b) Single administration of sulfonamides.
c) With salicylate (500 μmol/kg, *s.c.*).
d) $p<0.05$.
e) $p<0.01$.

The disposition rate constants of sulfamethoxazole and sulfathiazole were found to be significantly decreased by salicylate treatment. It was interesting to note that their blood concentrations at the initial stage of the experiments were lower than those of the control experiments, but their blood levels were maintained higher in the later period of the experiments (Fig. 1 b and e). However, increases in the elimination rate of sulfonamides, reported in a coadministration study of bucolome by Kakemi *et al.*,[18] were not observed in the present study.

Thus, we consider that the effects of salicylate on the blood concentration profiles of sulfonamides can be classified as follows:

Type I: Little influence is observed on salicylate treatment (sulfanilamide).

Type II: The blood level of sulfonamide is decreased by salicylate treatment, but the value of k_β is not greatly influenced (sulfadiazine, sulfisoxazole).

Type III: The value of k_β is decreased by salicylate treatment (sulfamethoxazole, sulfathiazole).

It may be supposed that the disposition of sulfonamides of Type III is inhibited by the coadministration of salicylate, resulting in a possible increase in the duration of their antibacterial activity in the living body.

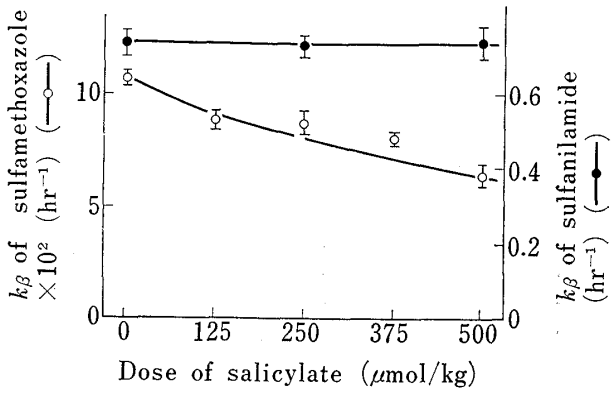

Fig. 2. Effect of Salicylate on the Disposition Rate Constants of Sulfanilamide and Sulfamethoxazole

—●—, sulfanilamide (100 μmol/kg, *i.v.*).
—○—, sulfamethoxazole (50 μmol/kg, *i.v.*).
Each point represents the mean±standard error for three to six rats.

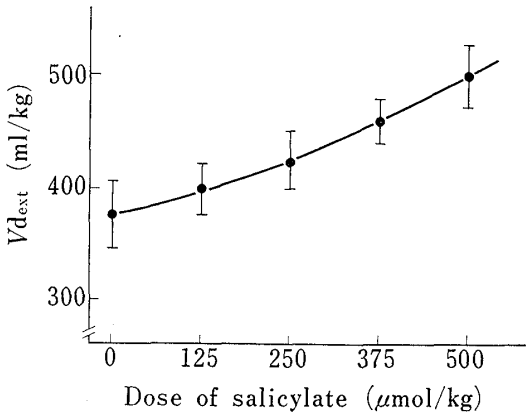

Fig. 3. Effect of Salicylate on the Extrapolated Volume of Distribution of Sulfamethoxazole

Dose of sulfamethoxazole: 50 μmol/kg, *i.v.*
Each point represents the mean±standard error for four to six rats.

18) K. Kakemi, H. Sezaki, T. Komuro, K. Ikeda, and H. Kishi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2386 (1970).

To study the effects of dose of salicylate on the fate of sulfonamide in the rat, the values of k_β for sulfanilamide and sulfamethoxazole with concomitant administration of various doses of salicylate (0—500 μmol/kg) were measured (Fig. 2). The elimination rate constant of sulfanilamide was not influenced by salicylate treatment, but the rate constant of sulfamethoxazole decreased markedly with increase in the dose of salicylate. The extrapolated volume of distribution of sulfamethoxazole, $V_{d_{ext}}$, was found to increase with increase in the dose of salicylate (Fig. 3).

To confirm these observations, the disposition rate constant of sulfamethoxazole was measured at various doses of sulfamethoxazole (50—250 μmol/kg) with concomitant administration of salicylate at a dose of 500 μmol/kg (Fig. 4). In the control experiments without salicylate, the value of k_β for sulfamethoxazole was found to remain constant regardless of the dose, suggesting simple elimination processes for the drug. However, with the concomitant administration of salicylate, the value of k_β was found to increase with increase in the dose of sulfamethoxazole, suggesting that the relative dose of the two drugs is one of the factors regulating the degree of drug interaction.

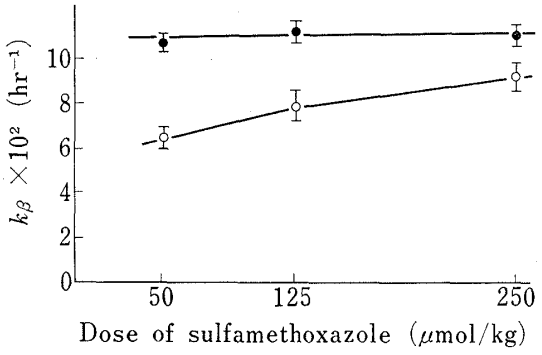

Fig. 4. Dose Dependency on the Disposition Rate Constant of Sulfamethoxazole with or without Salicylate

—●—, without salicylate.
—○—, with salicylate (500μ mol/kg, *s.c.*).
Each point represents the mean±standard error for four to six rats.

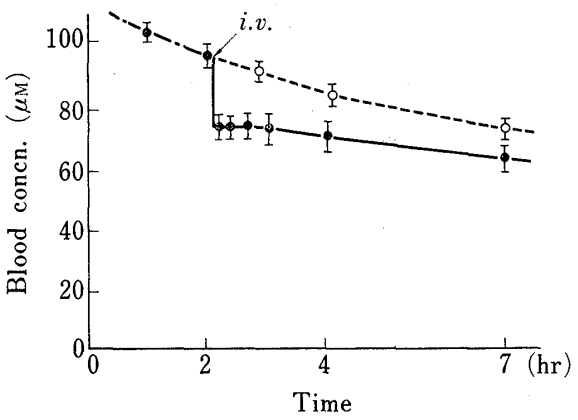

Fig. 5. Effect of Intravenous Administration of Salicylate on the Blood Concentration of Sulfamethoxazole

—•—•—, sulfamethoxazole (50 μmol/kg, *i.v.*).
The arrow indicates the intravenous administration of salicylate or saline.
—●—, salicylate (500 μmol/kg, *i.v.*).
—○—, saline (0.5ml/kg, *i.v.*).
Each point represents the mean±standard error for three to eight rats.

Time Course of the Blood Concentration of Sulfamethoxazole after Intravenous Administration of Salicylate

In these experiments, the animals were first given sulfamethoxazole intravenously at a dose of 50 μmol/kg. Two hr were then allowed for the drug to distribute throughout the body, and to permit its subsequent elimination phase to become established. Salicylate (500 μmol/kg) was then given by rapid intravenous injection and blood samples were taken at appropriate intervals thereafter to follow the time course of the blood concentration of the drug (Fig. 5). The effect of salicylate was rapid and profound. Within 30 seconds after the injection of salicylate, there was a profound fall in the blood concentration of sulfamethoxazole. The fall was complete within 5 min and thereafter the blood concentration of sulfamethoxazole remained relatively constant for 5 hr. Control experiments showed a negligible change in the elimination profiles of sulfamethoxazole from the blood. McQueen *et al.*[7] reported a similar result, *i.e.*, that the blood concentration of sulfadoxine rapidly decreased on intravenous administration of phenylbutazone. They concluded that the effect of phenylbutazone on the blood concentration of sulfadoxine was solely due to its redistribu-

tion by competitive inhibition of its binding to plasma protein. Similarly, in the present study, it may be concluded that a significant increase in Vd_{ext} and a rapid decrease in the blood concentration of sulfamethoxazole on salicylate treatment are due to an increase in the unbound fraction of sulfamethoxazole caused by salicylate, resulting in the redistribution of sulfamethoxazole in rats.

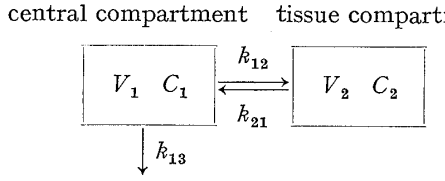

Fig. 6. Schematic Representation of the Body as a Two-Compartment Open Model

k_{12} is the transfer rate constant from the central compartment to the tissue compartment, k_{21} is the transfer rate constant from the tissue compartment to the central compartment, and k_{13} is the elimination rate constant of the drug. V_1 and V_2 are the volumes of the central and tissue compartments, respectively.

Effect of Salicylate on the Distribution of Sulfonamides Analyzed in Terms of a Two-Compartment Open Model

It has been well established that the fate of a drug administered to a living body can be described employing compartment models. To clarify the effect of salicylate on the tissue distribution of sulfonamides, two sulfonamides with different types of blood concentration profiles, sulfadiazine (Type II) and sulfamethoxazole (Type III), were studied employing a two-compartment open model (Fig. 6). The blood concentrations of sulfonamides after intravenous administration with or without salicylate were determined at the distribution and elimination phases (Fig. 7 and 8). The blood con-

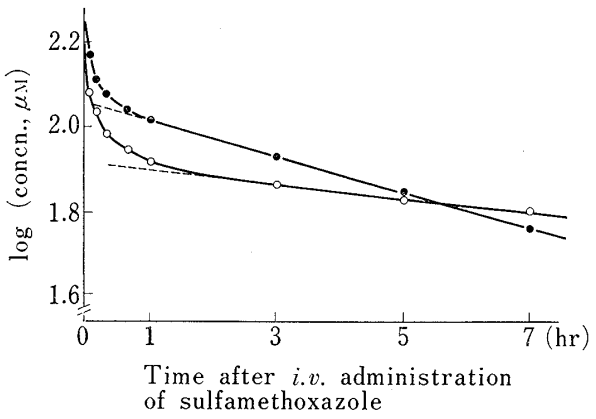

Fig. 7. Effect of Salicylate on the Blood Concentration of Sulfamethoxazole

—●—, withoutsalicylate.
—○—, with salicylate (500 μmol/kg, s.c.).
Dose of sulfamethoxazole: 50 μmol/kg, i.v.
Each point represents the mean for four to six rats.

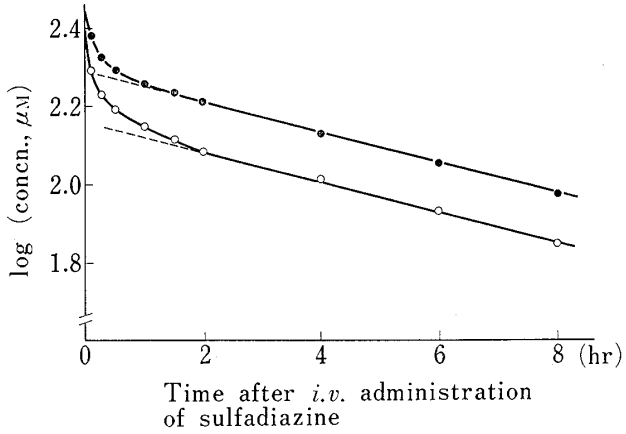

Fig. 8. Effect of Salicylate on the Blood Concentration of Sulfadiazine

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, s.c.).
Dose of sulfadiazine: 100 μmol/kg, i.v.
Each point represents the mean for five to six rats.

Table III. Pharmacokinetic Parameters of Sulfamethoxazole

	Control[a]	SA treatment[b]
k_{13} (min^{-1})	2.35×10^{-3}	1.08×10^{-3}
k_{12} (min^{-1})	2.48×10^{-2}	1.54×10^{-2}
k_{21} (min^{-1})	5.89×10^{-2}	3.20×10^{-2}
V_1 (ml/kg)	302	395
V_2 (ml/kg)	127	189

a) Single administration of sulfamethoxazole.
b) With salicylate (500 μmol/kg, s.c.).

centration of a drug, C_1, at time t after intravenous administration is expressed by Equation 1.

$$C_1 = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \qquad \text{(Eq. 1)}$$

The values of A, B, α and β can be estimated by a graphical analysis of semilogarithmic plots of the blood concentration profiles of the drug. Employing these values, the values of k_{12}, k_{21}, k_{13}, V_1 and V_2 in the model were routinely calculated following the method of Riegelman *et al.*[19] (Tables III and IV).

Table IV. Pharmacokinetic Parameters of Sulfadiazine

	Control[a]	SA treatment[b]
k_{13} (min^{-1})	1.91×10^{-3}	2.03×10^{-3}
k_{12} (min^{-1})	8.15×10^{-3}	7.73×10^{-3}
k_{21} (min^{-1})	3.88×10^{-2}	2.63×10^{-2}
V_1 (ml/kg)	410	505
V_2 (ml/kg)	86	148

a) Single administration of sulfadiazine.
b) With salicylate (500 μmol/kg, *s.c.*).

The value of k_{13} for sulfamethoxazole, but not for sulfadiazine, was found to be decreased by salicylate treatment. The values of k_{12} and k_{21} for both drugs were found to be decreased by salicylate treatment.

Next, the chronological changes in the fraction of drug distributed into the tissue compartment against the dose were calculated employing Equation 2.

$$\frac{X_2}{X_0} = \frac{k_{12}}{\beta - \alpha} e^{-\alpha t} + \frac{k_{12}}{\alpha - \beta} e^{-\beta t} \qquad \text{(Eq. 2)}$$

where X_0 is the dose, and X_2 is the amount of drug distributed into the tissue compartment at time t. The results for two drugs with and without salicylate treatment are presented in Fig. 9. It was noted that the time required to establish the maximum value of the fraction, X_2/X_0, distributed in the tissue compartment was delayed by salicylate treatment,

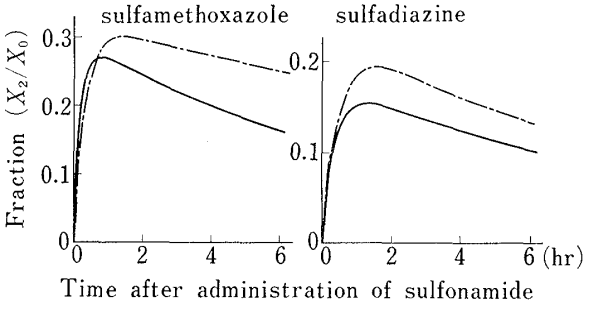

Fig. 9. Amounts of Sulfonamides in the Tissue Compartment calculated from the Pharmacokinetic Parameters

———, without salicylate.
———, with salicylate.

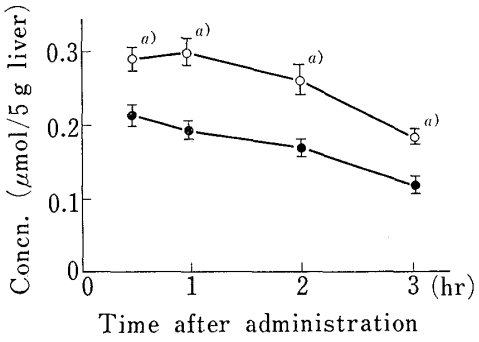

Fig. 10. Effect of Salicylate on the Concentration of Sulfamethoxazole in Rat Liver

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Dose of sulfamethoxazole: 50 μmol/kg, *i.v.*
Each point represents the mean ± standard error for four to six rats.
a) Significant differences at $p < 0.05$.

19) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 312 (1971).

and the fraction distributed into the tissue compartment was increased by the treatment. Thus, it may be considered that the effect of concomitant administration of salicylate on the distribution of sulfonamides into tissues is most marked for drugs which belong to Types II and III.

Distribution of Sulfamethoxazole to Rat Liver

The concentration of sulfamethoxazole was measured at designated periods after intravenous administration of sulfamethoxazole at a dose of 250 μmol/kg with or without salicylate treatment (Fig. 10). It was noted that the concentration of the drug distributed to the liver was significantly increased by the concomitant administration of salicylate. Thomas *et al.*[8] reported that the blood concentration of ^{14}C-bishydroxycoumarin in rats was decreased by salicylate treatment. They suggested that the displacement of bound drug from the plasma protein was the reason for the rapid uptake into liver. Thus, it may be concluded that the increased distribution of sulfamethoxazole into liver on salicylate treatment is partly responsible for an increase in the fraction of the drug distributed into the tissue compartment upon salicylate treatment.

The present results were interpreted on the basis of a redistributional drug interaction suggested by McQueen *et al.*,[7] *i.e.*, by supposing that salicylate caused a rapid change in the concentration of unbound sulfamethoxazole in the blood due to competitive binding of the drugs at the same binding sites on proteins. Thus, salicylate treatment increased the initial rate of elimination for sulfonamides of Types II and III. The increase in the unbound concentration was also responsible for the enhanced distribution of the drug into tissues. As was described in the previous report,[1] an increase in the distribution of sulfonamides into red blood cells may be responsible for an increase in the volume of distribution. Further study should be undertaken to elucidate the redistributional mechanism of sulfonamides upon salicylate treatment.