

Effect of Salicylate on the Distribution of Sulfonamides to Red Blood Cells in Rat¹⁾

YUTAKA HIGASHI,^{2a)} SACHIKO UEI, NOBORU YATA, and AKIRA KAMADA²⁾

Faculty of Pharmaceutical Sciences, Osaka University²⁾

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Effects of salicylate on the distribution of five sulfonamides to red blood cells in rat were studied *in vitro* and *in vivo*. The distribution of sulfonamides to red blood cells in whole blood *in vitro* increased with an increase in their concentration in blood. But, in cell suspension without plasma, the distribution of the drugs to red blood cells was not influenced by their concentration. The distribution of sulfonamides except for sulfanilamide to red blood cells was enhanced by the addition of salicylate in whole blood. But, in cell suspension, the effect of salicylate was not observed. Addition of rat plasma to the cell suspension reduced the distribution of sulfonamides to red blood cells. It was considered that the unbound fraction of sulfonamides was responsible for their distribution to red blood cells. Thus, it may be concluded that salicylate inhibits the binding of sulfonamides to plasma protein, resulting in an increase in their unbound fraction and an enhancement of the distribution of sulfonamides to red blood cells.

Keywords—sulfonamides; salicylate; distribution to red blood cells; drug interaction; concomitant administration; protein-binding

In the previous report,³⁾ it was shown that sulfonamides bound to bovine serum albumin were easily displaced by the addition of salicylate and the amount of sulfonamide displaced by the addition of salicylate was linearly correlated with their intrinsic binding ability to the protein which was measured without salicylate. And sulfonamides were considered to be competitively displaced at the same binding site on the protein.

Anton⁴⁾ have demonstrated that the antimicrobial activity of sulfonamides is markedly reduced by the addition of protein to the test solution being subjected to a decrease in the unbound fraction of the drugs in solution. Pharmacologic effect of a highly bound drug can be increased when the drug is displaced from its binding site by another drug resulting in an increase in the unbound fraction.⁵⁾

Many drugs⁶⁾ were reported to penetrate into red blood cells as well as to bind to the plasma protein. But little was studied on the effect of uptake of drugs into red blood cells on their pharmacokinetics.^{7,8)}

Presently, the effect of salicylate on the distribution of sulfonamides to red blood cells was studied *in vitro* and *in vivo*.

- 1) Concomitant Administration of Drugs. I. A part of this work was presented at the 26th Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, October 1976.
- 2) Location: 133-1, Yamadakami, Suita-shi, Osaka; a) Present address: Department of Pharmacy, Osaka University Hospital, 1-50 Fukushima-1-chome, Fukushima-ku, Osaka-shi, Osaka.
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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 of Drugs to Red Blood Cells in Whole Blood *in Vitro*—Male rats of Sprague-Dawley strain weighing 250–350 g were used. Blood was collected through cardiac puncture using EDTA to prevent clotting, and used immediately. In the preliminary experiments, the distribution equilibrium of sulfonamides between plasma and red blood cells was found to be rapidly established within 10 min at 37° in a mixture of one portion of aqueous drug solution and 9 portions of whole blood. In the present study, to avoid the effect of dilution, sulfonamide and salicylate were dissolved in whole blood by the following method. Sulfonamide and salicylate were dissolved in ethanol. Aliquots of the ethanol solution of sulfonamide and salicylate were taken into glass-stoppered bottles, and evaporated to dryness under reduced pressure. Two ml of whole blood was placed into each bottle. Time allowed to establish the distribution equilibrium was fixed at 1 hr including the dissolution period of drugs. After the bottles were kept for 1 hr at 37° under intermittent shaking, the value of hematocrit, H_t , and concentration of sulfonamide in whole blood, C_b , were measured. Then, the blood was centrifuged at 2500 rpm for 10 min, and the concentration of sulfonamides in plasma, C_p , was measured. The distribution of sulfonamide to red blood cells was obtained employing Eq. 1.

$$\text{per cent distributed} = \frac{C_b - C_p(1 - H_t)}{C_b} \times 100 \quad (\text{Eq. 1})$$

Distribution of Drugs to Red Blood Cells *in Vivo*—Male rats were kept in individual cages with free access to food and water. Drugs were dissolved in distilled water with the aid of sodium hydroxide. Sulfamethoxazole was administered into the tail vein at a dose of 250 $\mu\text{mol/kg}$. Salicylate was subcutaneously administered to the dorsum at a dose of 500 $\mu\text{mol/kg}$ 25 min before the administration of sulfamethoxazole. Blood samples were chronologically collected by cardiac puncture, and C_b , C_p and H_t were measured.

Distribution of Drugs to Red Blood Cells in the Cell Suspension—Rat red blood cells were separated from plasma by centrifugation for 10 min at 2500 rpm, the plasma and buffy layer were discarded, and the packed red blood cells were washed three times with saline. The isotonic suspending medium was composed of one part of an isotonic phosphate buffer of pH 7.4, three parts of saline and 100 mg% of glucose. The washed red blood cells were suspended in the suspending medium which contained sulfonamide (0.05–0.2 mM) and salicylate (0–2.0 mM). The value of hematocrit of the suspension was adjusted to 0.45 which was the normal value of rat blood.⁹⁾ The suspension was incubated for 30 min at 37° in a water-bath under intermittent shaking. The distribution of drug to red blood cells was calculated employing Eq. 1.

Assay Method—A modified Bratton-Marshall method¹⁰⁾ was employed for the assay of sulfonamides.

Results

Distribution of Sulfonamides to Red Blood Cells in the Whole Blood *in Vitro*

Distribution of sulfamethoxazole to red blood cells in the rat blood was studied *in vitro* in the presence of salicylate at various concentrations (Fig. 1). The distribution of sulfamethoxazole to red blood cells markedly increased with an increase in its concentration in whole blood and the distribution was greatly enhanced by the addition of 2.0 mM of salicylate (Fig. 1 (A)). The effect of salicylate concentration on the distribution of sulfamethoxazole to red blood cells is presented in Fig. 1 (B) at various concentrations of sulfamethoxazole. Similar results were obtained for the distribution of sulfathiazole to red blood cells in the whole blood of rat (Fig. 2).

Ratio of the amount of sulfonamide distributed to red blood cells in the presence of salicylate to that without salicylate is presented against the concentration of salicylate in blood (Fig. 3). The effect of salicylate on the relative distribution of sulfamethoxazole or sulfathiazole to red blood cells increased with increase in the concentration of salicylate in blood. And the relative distribution was also found to decrease with an increase in the total concentration of sulfonamide in blood.

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The results of the effect of salicylate (2.0 mM) on the distribution of five sulfonamides (0.1 mM) to red blood cells are presented in Table I. It was noted that the effect of salicylate on the distribution of sulfanilamide to red blood cells was not observed, but other sulfonamides were markedly influenced by the presence of salicylate.

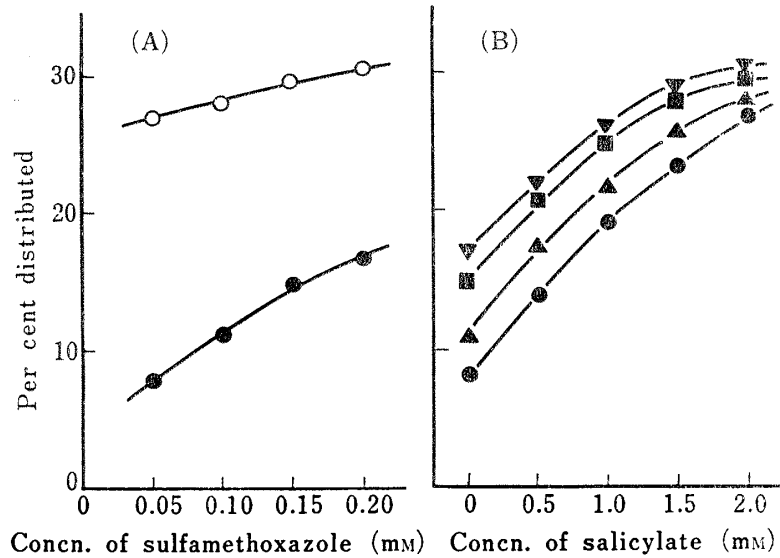


Fig. 1. Distribution of Sulfamethoxazole to Red Blood Cells in Rat Whole Blood *in Vitro*

(A) Concentration dependency of sulfamethoxazole.
 —●—, without salicylate.
 —○—, with 2 mM salicylate.
 (B) Effect of salicylate.
 Concentration of sulfamethoxazole.
 —●—, 0.05 mM; —▲—, 0.10 mM;
 —■—, 0.15 mM; —▼—, 0.20 mM.

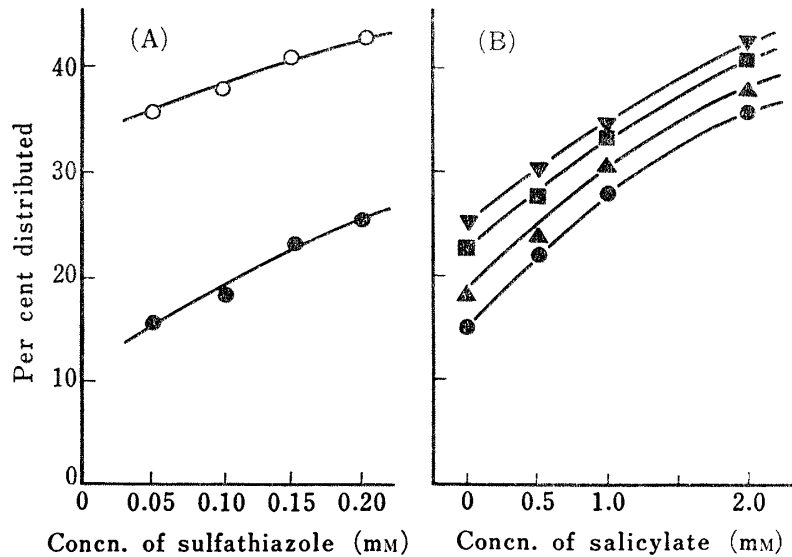


Fig. 2. Distribution of Sulfathiazole to Red Blood Cells in Rat Whole Blood *in Vitro*

(A) Concentration dependency of sulfathiazole.
 —●—, without salicylate.
 —○—, with 2 mM salicylate.
 (B) Effect of salicylate.
 Concentration of sulfathiazole.
 —●—, 0.05 mM; —▲—, 0.10 mM;
 —■—, 0.15 mM; —▼—, 0.20 mM.

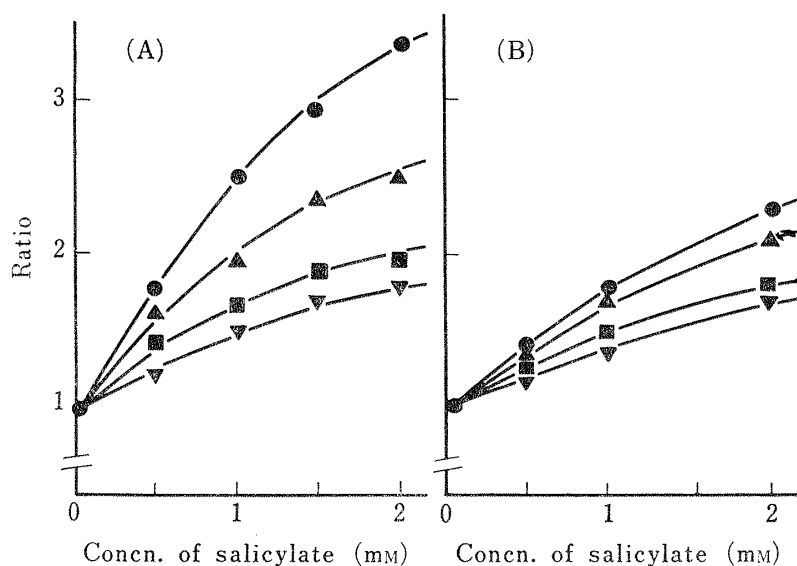


Fig. 3. Relative Effect of Salicylate on the Distribution of (A) Sulfamethoxazole, (B) Sulfathiazole to Red Blood Cells in Rat Whole Blood *in Vitro*

Concentration of sulfonamide in whole blood.
 ●—, 0.05 mM; ▲—, 0.10 mM;
 ■—, 0.15 mM; ▼—, 0.20 mM.

TABLE I. Distribution of Sulfonamides to Red Blood Cells in Rat Whole Blood *in Vitro*

Sulfonamides	Per cent distributed		Ratio ^{b)}
	without SA	with SA ^{a)}	
Sulfanilamide	77.4	75.1	0.97
Sulfamethoxazole	11.1	27.9	2.51
Sulfathiazole	18.2	38.2	2.09
Sulfadiazine	17.9	26.5	1.48
Sulfisoxazole	21.4	48.0	2.24

a) Concentration of salicylate: 2 mM.

b) With SA/without SA.

Distribution of Sulfonamides to Red Blood Cells in the Cell Suspension

The effect of salicylate on the distribution of sulfonamides to red blood cells was studied employing the cell suspension without plasma. The concentration of sulfamethoxazole distributed to red blood cells without salicylate linearly increased with an increase in the concentration of sulfamethoxazole in cell suspension (Fig. 4 (A)). Per cent distribution of sulfamethoxazole to red blood cells in the cell suspension which contained 0.1 mM sulfamethoxazole was found to be little influenced by the presence of salicylate at the concentration range of 0—2.0 mM and remained constant at around 50% (Fig. 4 (B)). The results for the distribution of five sulfonamides at the concentration of 0.1 mM with (2.0 mM) or without salicylate are presented in Table II. Salicylate did not influence on the distribution of sulfonamides to red blood cells in the cell suspension.

Effect of Plasma on the Distribution of Sulfamethoxazole to Red Blood Cells in the Cell Suspension

To study the effect of plasma on the distribution of sulfamethoxazole to red blood cells in rat blood, cell suspensions were prepared by suspending red blood cells in the suspending

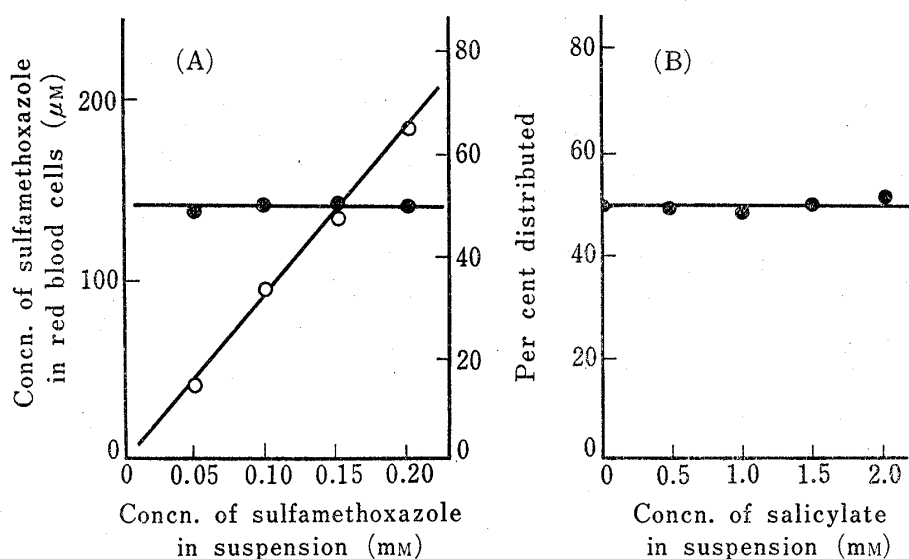


Fig. 4. Distribution of Sulfamethoxazole to Red Blood Cells in Rat Cell Suspension

(A) Concentration dependency of sulfamethoxazole.
 (B) Effect of salicylate.
 —●—, per cent distributed.
 —○—, concentration in red blood cells.

TABLE II. Distribution of Sulfonamides to Red Blood Cells in Rat Cell Suspension

Sulfonamides	Per cent distributed		Ratio ^{b)}
	without SA	with SA ^{a)}	
Sulfanilamide	66.7	66.4	0.99
Sulfamethoxazole	50.3	49.5	0.98
Sulfathiazole	65.2	65.3	1.00
Sulfadiazine	46.8	47.0	1.00
Sulfisoxazole	53.3	51.7	0.96

a) Concentration of salicylate: 2 mM.
 b) With SA/without SA.

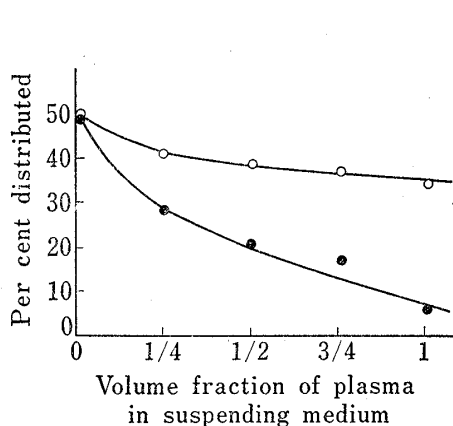


Fig. 5. Effect of Plasma on the Distribution of Sulfamethoxazole to Red Blood Cells in Cell Suspension

Concentration of sulfamethoxazole in suspension: 0.1 mM.
 —●—, without salicylate.
 —○—, with 2 mM salicylate.

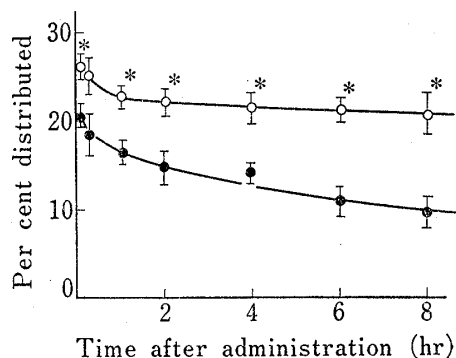


Fig. 6. Distribution of Sulfamethoxazole to Red Blood Cells after Intravenous Administration to Rat

Dose of sulfamethoxazole: 250 μmol/kg.
 —●—, without salicylate treatment;
 —○—, with salicylate treatment (500 μmol/kg, s.c.).
 Each point represents the mean standard errors for four to six rats.
 Asterisk indicates significant difference at $p < 0.01$.

medium containing various amounts of rat plasma. The total concentration of sulfamethoxazole was adjusted to 0.1 mM in the cell suspensions with (2.0 mM) or without salicylate. The distribution of sulfamethoxazole to red blood cells was definitely decreased with an increase in the fraction of plasma in the suspending medium regardless of the presence or absence of salicylate (Fig. 5). It was noted that the inhibitory effect of plasma on the sulfamethoxazole distribution to red blood cells was markedly reduced by the addition of salicylate.

Distribution of Sulfamethoxazole to Red Blood Cells *in Vivo*

The effects of salicylate on the distribution of sulfamethoxazole to red blood cells in rat blood *in vivo* were studied following a subcutaneous administration of salicylate and then an intravenous administration of sulfamethoxazole to rat (Fig. 6). The fraction of sulfamethoxazole distributed to red blood cells chronologically decreased in the control experiments of a single administration of sulfamethoxazole. But, the concomitant administration of salicylate increased the fraction of sulfamethoxazole distributed to red blood cells and the large distribution value were maintained for the experimental period studied.

Discussion

It has been well established that the passage of organic anions into human red blood cells suspended in Tyrode's solution advances following a process of simple diffusion and is roughly correlated to the lipid-to-water partition coefficient of the anions.¹¹⁾ Yamazaki *et al.*¹²⁾ reported that the distribution of sulfonamides to human red blood cells suspended in a phosphate buffer proceeded following Freundlich's adsorption isotherms. The results of the present study employing rat blood revealed that the fraction of sulfamethoxazole or sulfathiazole distributed to red blood cells increased with an increase in the concentration of drugs, suggesting a process of concentration dependent permeation through red blood cell membrane (Fig. 1—3). But the concentration dependent distribution to red blood cells was not observed for the studies employing the suspension of red blood cells without plasma (Fig. 4). Thus, it may be considered that the distribution of sulfonamides to red blood cells proceeds through the processes of adsorption to cell membrane following a passive diffusion permeation. And the presence of plasma in the cell suspension as well as in whole blood should be responsible for the concentration dependent distribution of sulfonamides to red blood cells.

Many works have revealed that acidic non-steroidal anti-inflammatory drugs including salicylate stabilize red blood cell membrane *in vitro* and protect red blood cells from heat-induced¹³⁾ or hypotonic haemolysis¹⁴⁾ *in vitro*. And the stabilizing effects of the anti-inflammatory drugs on the red blood cell membrane are due to their stabilizing effects on certain proteins in the cell membrane.¹⁵⁾ These works strongly suggest that salicylate can modify the red blood cell membrane *in vivo* as well as *in vitro*. But the results presented in Fig. 4 (B) and Table II revealed that, in cell suspension, salicylate did not influence on the distribution of sulfonamides to red blood cells, suggesting little effect of salicylate on the cell membrane with respect to the distribution of drugs to red blood cells.

To explain the difference of the effects of salicylate on the distribution of sulfonamides to red blood cells in whole blood from those in cell suspension, the fraction of plasma in the cell suspension was changed (Fig. 5). The distribution of sulfamethoxazole to red blood cells was markedly decreased by the addition of plasma to cell suspension. It may be explained

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on the basis of a decrease in the unbound concentration of sulfamethoxazole. These results strongly support that the distribution of sulfamethoxazole to red blood cells is preferentially controlled by the unbound concentration of the drug in whole blood as well as in cell suspension.

In the previous report,³⁾ it was reported that the unbound concentrations of sulfonamides in the bovine serum albumin solution were markedly increased by the addition of salicylate being subjected to the competitive binding of the drugs at the same binding site on the protein. Thus, the displacement of sulfamethoxazole from the binding site of protein by the addition of salicylate results in an increase in the unbound concentration of sulfamethoxazole in the protein solution. The influence of salicylate on the distribution of sulfamethoxazole to red blood cells in whole blood was remarkable, but, in the cell suspension without plasma, the addition of salicylate influenced little on the distribution of the drug. These results were explained on the basis of the change of unbound fraction of sulfamethoxazole in plasma by the addition of salicylate, resulting in the change of distribution of unbound drug between red blood cells and plasma.

Kitazawa *et al.*⁸⁾ reported that the distribution ratios of metocloramide and sulfadimethoxine between erythrocyte and plasma increased with an increase in the dose of drugs intravenously administered to rat vein. And they considered that the relative fraction of drugs to protein decreased with an increase in the concentration of drugs in blood, and the unbound fraction of drugs, which were responsible for their distribution to erythrocyte, increased with an increase in their blood concentration.

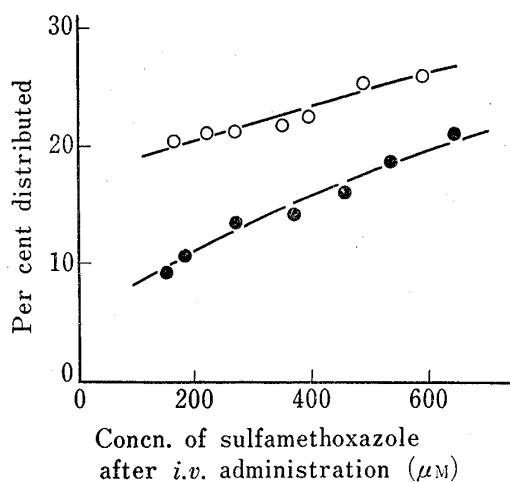


Fig. 7. Distribution of Sulfamethoxazole to Red Blood Cells after Intravenous Administration to Rat

Dose of sulfamethoxazole: 250 $\mu\text{mol}/\text{kg}$.
 —●—, without salicylate treatment;
 —○—, with salicylate treatment (500 $\mu\text{mol}/\text{kg}$, s.c.).
 Each point represents the mean for four to six rats.

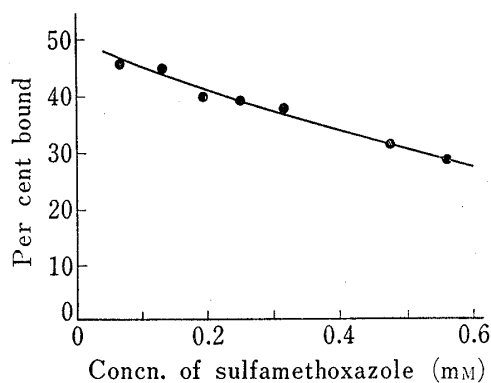


Fig. 8. Binding of Sulfamethoxazole to BSA at pH 7.4 and 37°

Concentration of BSA: 1% (w/v).

In the present *in vitro* and *in vivo* studies, similar results were obtained that the fraction of sulfamethoxazole distributed to red blood cells against its concentration in blood *in vitro* (Fig. 1) and *in vivo* (Fig. 7) increased with an increase in its concentration. The fraction of sulfamethoxazole bound to bovine serum albumin was found to decrease with an increase in the concentration of sulfamethoxazole in the protein solution (Fig. 8). Thus, the increased distributions of sulfamethoxazole and sulfathiazole to red blood cells with an increase in their concentrations in blood (Fig. 1 and 2) are subjected to the increased fractions of unbound drugs in blood.

The distribution of sulfonamides to red blood cells in blood was markedly increased by the presence of salicylate due to the increased concentration of unbound sulfonamides by the

displacing mechanism of salicylate at the same binding site of protein. The inconsiderable influence of salicylate on the distribution of sulfanilamide to red blood cells in blood is considered to be subjected to the small amount of the drug bound to protein and also the slight change in the unbound fraction of the drug regardless of the presence or absence of salicylate.