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Effect of Salicylate on the Metabolism and Urinary Excretion of Sulfonamides in the Rat[1,2]

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The effect of concomitant administration of salicylate on the acetylation and urinary excretion of sulfonamides (sulfanilamide, sulfamethoxazole, sulfadiazine, sulfisoxazole and sulfathiazole) after intravenous administration to rats was studied.

The degree of acetylation of sulfamethoxazole in rat urine was found to be significantly decreased by salicylate treatment, but that of sulfathiazole was increased. It was previously found that the eliminations of sulfamethoxazole and sulfathiazole from rat blood were markedly reduced by the concomitant administration of salicylate.

To reconcile these apparently conflicting effects of salicylate treatment on the fate of the two sulfonamides, urine samples were collected at intervals and the rate constant of urinary excretion and that of acetylation were calculated employing the elimination rate constant from rat blood. The decrease in the elimination of sulfamethoxazole on salicylate treatment was explained on the basis of a decrease in the acetylation rate constant. However, for sulfathiazole, the decrease in the elimination was a result of a decrease in the excretion rate constant due to the inhibitory effect of salicylate treatment on the excretion of unchanged sulfathiazole.

Keywords——sulfonamides; salicylate; concomitant administration; metabolism; urinary excretion; rat; excretion rate constant; acetylation rate constant

In the previous report,[1] it was shown that the effects of salicylate on the blood concentration profiles of sulfonamides in the rat could be classified into three types and that the tissue distributions of sulfonamides were increased by the concomitant administration of salicylate. The eliminations of sulfamethoxazole and sulfathiazole were reduced by salicylate treatment, increasing the duration of their presence in the blood.

In the present report, the urinary excretion and metabolism of sulfonamides, mainly sulfamethoxazole and sulfathiazole, were studied in the rat to elucidate the effect of salicylate on the time course of their blood levels.

Experimental

Materials——Commercially available sulfonamides, sulfanilamide (S), sulfamethoxazole (SMX), sulfathiazole (ST), sulfadiazine (SD), and sulfisoxazole (SIX), and sodium salicylate (SA) were used without further purification. N^4-Acetylsulfonamides were synthesized by the method of Kano *et al.*[4] for sulfamethoxazole and the method of Uno *et al.*[5] for sulfathiazole. All other reagents were of analytical reagent grade, and distilled water was used throughout.

Drug Administration——Administration of drugs was as described previously.[1] Male Sprague–Dawley rats weighing 250—350 g were used. Sulfonamides and their N^4-acetylated derivatives were given by intravenous injection into a tail vein. To study the effect of salicylate treatment, salicylate was administered subcutaneously to the rat dorsum 25 min prior to the administration of sulfonamide or its N^4-acetylate.

1) Concomitant Administration of Drugs. III. Part II: Y. Higashi, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **27**, 1299 (1979).
2) This work was presented at the 26th Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Osaka, October 1976.
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4) H. Kano and K. Ogata, *Ann. Rept. Shionogi Res. Lab.*, **7**, 301 (1957).
5) T. Uno and M. Ueda, *Yakugaku Zasshi*, **80**, 1785 (1960).

Urine Collection——Rats were kept in individual metabolic cages with free access to water. For the experiments requiring chronological collection of urine, rats were exposed briefly to ether vapor, which usually brought about urination,[6] at the appropriate times. To secure a sufficient amount of urine, 2 ml of water was administered orally by intubation 1 hr prior to the administration of sulfonamide or its N^4-acetylate, and 1 ml of water was administered orally each time after urine collection. For experiments on the urinary recovery of total sulfonamide, rats were kept in individual metabolic cages until the experiment was over.

Analytical Method——Sulfonamides were assayed by diazotization using a modification of the Bratton–Marshall method.[7] Unchanged sulfonamide in the urine sample was assayed by the diazotization method. To assay the total amount of all forms of sulfonamide in the urine, aliquots of urine were acidified with HCl and heated for 1.5 hr in a boiling water bath, then the solution was assayed by the diazotization method. In the preliminary experiment, the amount of glucuronide of sulfonamide in the rat urine was assayed by the method of Koechlin *et al.*[8] and was found to be negligible in comparison with the amounts of unchanged and N^4-acetylated sulfonamides for all the sulfonamides tested in the present study.

Glomerular Filtration Rate——Endogenous creatinine clearance was measured to estimate the glomerular filtration rate (GFR) in the rat. Rats were anesthetized with ether and forced to empty the bladder by pressing the pubic region just before the experiment. Water (2 ml per rat) was administered orally by intubation. The rats were kept in individual metabolic cages. Urine samples were collected during the 2 hr experimental period and, at the end of experiments, rats were forced to urinate by inhalation of ether and pressing the pubic region. Blood samples were also collected before and after the experiments. The concentrations of creatinine in blood and urine samples were assayed employing a clinical test kit, Creatinine-Tste Wako (Wako Pure Chemical Industries, Ltd.).

Protein Binding——Protein binding of sulfonamides to bovine serum albumin (BSA) and displacement by salicylate were studied by an equilibrium dialysis method[9] at 37° and pH 7.4.

Results

Degree of Acetylation

For the study of concomitant administration, intravenous administration of sulfonamide and subcutaneous administration of salicylate were employed. The degree of acetylation of a sulfonamide in the urine was calculated by assaying the amounts of total and unchanged sulfonamide in urine samples which were collected during 48 hr following the adminstration. By that time, the recovery of sulfonamide in all forms in the urine was assumed to be essentially complete for the sulfonamides studied. The effects of salicylate treatment on the acetylation of sulfonamides were also studied (Fig. 1). Eighty percent of sulfanilamide excreted in the urine was found to be acetylated, and salicylate treatment did not affect the degree of acetylation. In the previous study,[1] it was found that the elimination of sulfanilamide from rat blood following intravenous administration was not influenced by salicylate treatment. It is interesting to note that, in the present experiments, the process of metabolism of sulfanilamide in terms of the degree of acetylation was also found to be little influenced by salicylate treatment.

The degree of acetylation of sulfamethoxazole was significantly decreased by salicylate treatment. Similar results were obtained for sulfisoxazole and sulfadiazine. However, in the case of sulfathiazole, the degree of acetylation was markedly increased by salicylate treatment. It should be noted that, in the previous work,[1] the disposition rate constant, k_β, for sulfamethoxazole and sulfathiazole from rat blood were found to be similarly decreased by salicylate treatment. It is interesting that the effects of salicylate treatment on the fates of the two sulfonamides in the rat are quite different in terms of the rate of disposition and the degree of acetylation.

6) K. Kakemi, H. Sezaki, T. Komuro, K. Ikeda, and H. Kishi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2386 (1970).
7) M. Yamazaki, M. Aoki, A. Kamada, and N. Yata, *Yakuzaigaku*, **27**, 37 (1967).
8) B.A. Koechlin and W. Engerberg, *Antibiot. Med. Clin. Ther.*, **6**, (Suppl. 1) 22 (1959).
9) Y. Higashi, T. Nishihata, K. Kubo, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3572 (1978).

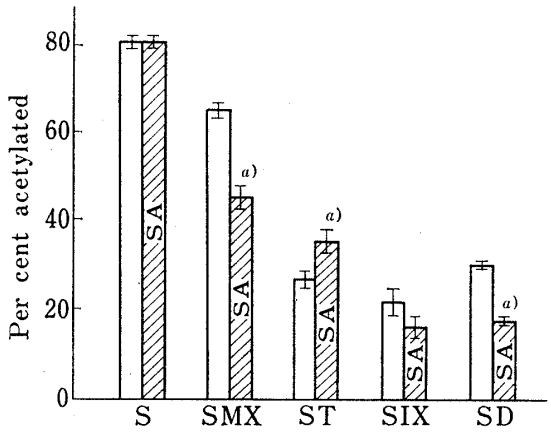

Fig. 1. Effect of Salicylate on the Acetylation of Sulfonamides in Rat Urine collected for 48 hr after Intravenous Administration of Sulfonamides

□, without salicylate.
▨, with salicylate (500 μmol/kg, *s.c.*).
Each bar represents the mean for five rats, and the vertical line indicates the standard error.
a) Significant differences at $p < 0.05$.
Doses: sulfamethoxazole, 50 μmol/kg, *i.v.*; sulfanilamide, sulfathiazole, sulfisoxazole, and sulfadiazine, 100 μmol/kg, *i.v.*

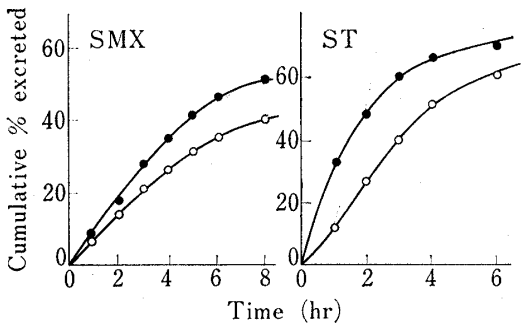

Fig. 2. Effect of Salicylate on the Excretion Profiles of Total Sulfamethoxazole and Sulfathiazole after Intravenous Administration of Sulfonamides

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Each point represents the mean for five rats.
Dose : 250 μmol/kg, *i.v.*

Urinary Excretion Profiles of Sulfonamides

To clarify the different effects of salicylate treatment on the degree of acetylation of sulfamethoxazole and sulfathiazole in the urine, urine samples were collected at 1 hr intervals for 6 to 8 hr after intravenous administration of sulfamethoxazole or sulfathiazole at a dose of 250 μmol/kg and subcutaneous administration of salicylate at a dose of 500 μmol/kg. Cumulative excretion profiles of total sulfonamides excreted in the urine were measured with and without salicylate treatment (Fig. 2). The data in the figure are expressed as the percentage of sulfonamide in all forms excreted in the urine against the dose. The salicylate treatment was found to result in a marked decrease in the cumulative urinary excretion of total sulfonamides for both sulfonamides. The cumulative excretion profiles of unchanged and N^4-acetylated sulfonamides excreted in the urine were also studied and plotted as before

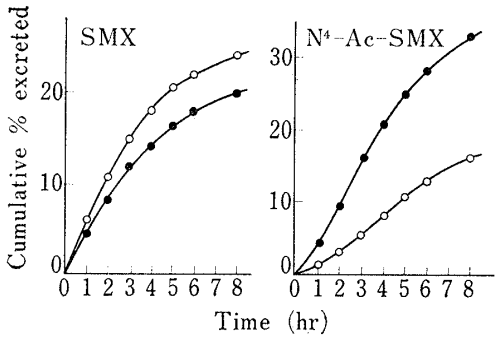

Fig. 3. Effect of Salicylate on the Excretion Profiles of Unchanged and N^4-Acetylated Sulfamethoxazole after Intravenous Administration of Sulfamethoxazole

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Each point represents the mean for five rats.
Dose : 250 μmol/kg, *i.v.*

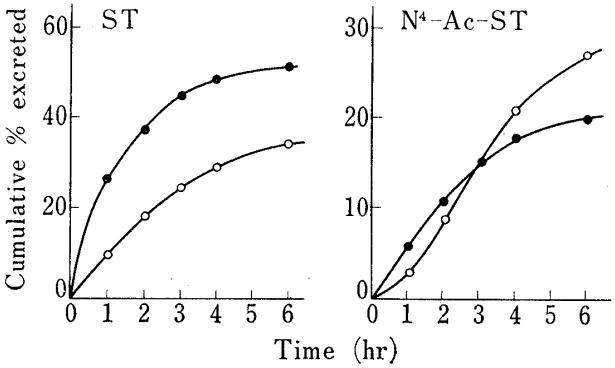

Fig. 4. Effect of Salicylate on the Excretion Profiles of Unchanged and N^4-Acetylated Sulfathiazole after Intravenous Administration of Sulfathiazole

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Each point represents the mean for five rats.
Dose: 250 μmol/kg, *i.v.*

(Fig. 3 and 4). From the results for total and N^4-acetylated sulfonamides in the urine samples, the fractions of N^4-acetylated material in each urine sample were obtained. The chronological changes in the fraction of N^4-acetylated material and the effects of salicylate treatment on the profiles are shown in Fig. 5. Salicylate treatment resulted in a marked decrease in the acetylated fraction of sulfamethoxazole, but in the case of sulfathiazole, the treatment increased the acetylated fraction.

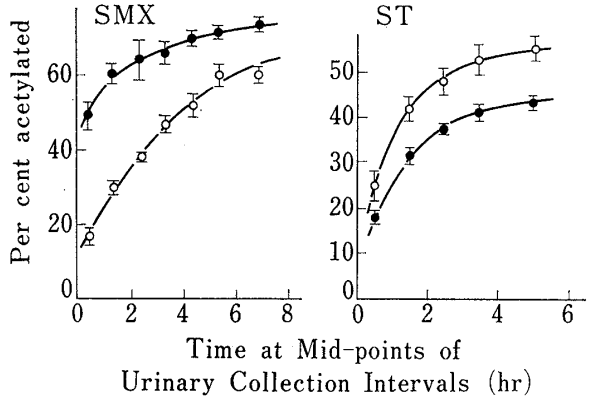

Fig. 5. Effect of Salicylate on the Acetylation Profiles of Sulfamethoxazole and Sulfathiazole in Rat Urine after Intravenous Administration of Sulfonamides

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Each point represents the mean±standard error for five rats.

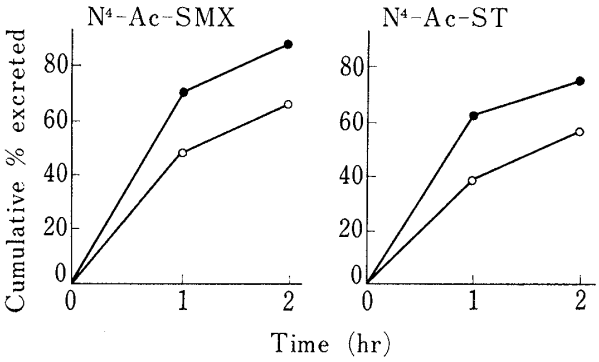

Fig. 6. Effect of Salicylate on the Excretion of N^4-Acetylated Sulfamethoxazole and Sulfathiazole after Intravenous Administration of N^4-Acetylsulfonamides

—●—, without salicylate.
—○—, with salicylate (500 μmol/kg, *s.c.*).
Each point represents the mean for four rats.

Urinary Excretion Profiles after Intravenous Administration of N^4-Acetylsylfonamides

The effects of salicylate treatment on the urinary excretion of N^4-acetylsulfonamides were studied following intravenous administration of N^4-acetylsulfamethoxazole or N^4-acetylsulfathiazole at a dose of 125 μmol/kg and subcutaneous administration of salicylate at a dose of 500 μmol/kg. Cumulative excretion profiles of N^4-acetylated material with and without the salicylate treatment are presented in Fig. 6. The urinary excretions of the two N^4-acetylated compounds were very rapid and about 80% of the dose was excreted within 2 hr after the administration. The salicylate treatment resulted in a marked decrease in the urinary excretions in both cases.

Effect of Salicylate on the Glomerular Filtration Rate (GFR)

To clarify the effects of salicylate treatment on the physiological function of rat kidney, endogenous creatinine clearance was determined as a measure of GFR. It is known[10] that

Table I. Creatinine Clearance in Rats

	Control	SA treat[a]
Serum Concn. (mg/dl)	0.743±0.023[b]	0.748±0.014
Amt. excreted (mg/kg/2 hr)	3.07 ±0.25	3.13 ±0.13
Clearance (ml/min/kg)	3.56 ±0.31	3.48 ±0.17

a) Subcutaneous administration of 500 μmol/kg of salicylate.
b) Each datum represents the mean±standard error for six rats.

10) J.D. Wallin, "Physiological Chemistry," 16th ed., ed. by H.A. Harper, V.W. Rodwell, and P.A. Mayes, Lange/Maruzen, California, 1977, p. 623.

the concentration of endogenous creatinine in blood plasma is maintained constant in the normal living body and that creatinine passes through the glomerular filter and is excreted in the urine without reabsorption. Thus, the creatinine clearance is clincally used for the estimation of GFR.

The values of creatinine clearance with and without salicylate treatment are presented in Table I. The concentration of creatinine in rat plasma and the value of creatinine clearance were not influenced by salicylate at a subcutaneous dose of 500 μmol/kg. The pH value of fresh urine was also found to be little influenced by salicylate treatment, and remained at 6.8. The urinary pH is considered to be an important factor influencing the urinary excretion of weak electrolytes[11] including sulfonamides. Thus, it may be considered that salicylate treatment does not affect the physiological function of rat kidney in terms of glomerular filtration and tubular reabsorption of sulfonamides.

Discussion

The degree of acetylation of sulfamethoxazole in rat urine was found to be significantly decreased by salicylate treatment, but that of sulfathiazole was increased by the same treatment (Fig. 1). Previously,[1] it was reported that the eliminations of sulfamethoxazole and sulfathiazole from rat blood were markedly reduced by the concomitant administration of salicylate. To clarify these conflicting effects of salicylate treatment on the fate of the two sulfonamides, urine samples were collected and the cumulative excretion of total sulfonamide was measured for each drug. The urinary excretion profiles of the two sulfonamides in terms of total excretion indicated that the treatment had an inhibitory effect on the excretion (Fig. 2). From the amounts of unchanged and N^4-acetylated fractions in urine samples which were collected chronologically, it was found that the decrease in acetylation of sulfamethoxazole was mainly attributable to a decrease in the excretion of N^4-acetylsulfamethoxazole (Fig. 3), and the increase in acetylation of sulfathiazole was attributable to a decrease in the excretion of unchanged sulfathiazole (Fig. 4). The rate constant of urinary excretion of unchanged sulfonamides, k_e, and that of acetylation, k_m, were next calculated, as follows.

Elimination of a sulfonamide from the blood stream is performed by processes of excretion into the urine and metabolism in the living body. Thus, the elimination rate constant, k_{el}, for unchanged sulfonamide from the blood stream is the sum of the excretion rate constant and the rate constant of metabolism.

$$k_{el} = k_m + k_e \qquad \text{(Eq. 1)}$$

And k_m is given by

$$k_m = \frac{\text{metabolites excreted in infinite time}}{\text{total drugs excreted in infinite time}} \cdot k_{el} \qquad \text{(Eq. 2)}$$

where k_{el} is the first-order rate constant for elimination of sulfonamide from the blood stream after a rapid intravenous injection. k_e is given by Equation 3.

$$k_e = k_{el} - k_m \qquad \text{(Eq. 3)}$$

In the present study, the value of k_β, which was obtained from the slope of semilogarithmic plots of unchanged drug concentration in the blood *versus* time at the elimination phase following intravenous injection of sulfonamide, was used as the value of k_{el}. In the preliminary experiment, little glucuronide conjugation was observed in the rat. Thus, only acetylation was considered in the process of metabolism of sulfonamides.

Employing k_β from the previous report[1] and the degree of acetylation in the urine excreted during 48 hr (Fig. 1), the values of k_e and k_m for five sulfonamides were calculated

11) A.B. Gutman, T.F. Yu, and J.H. Sirota, *J. Clin. Invest.*, **34**, 711 (1964); M. Cohen and R. Pocelinko, *J. Pharmacol. Exp. Ther.*, **185**, 703 (1973).

Table II. Excretion Rate Constants of Sulfonamides

Sulfonamides	$k_e \times 10^2$ [a] (hr^{-1})	
	Control[b]	SA treat[c]
Sulfanilamide	14.4 ±1.3	14.5 ±1.1
Sulfamethoxazole	3.74±0.20	3.55±0.31
Sulfadiazine	6.67±0.30	7.55±0.31
Sulfisoxazole	19.4 ±1.6	24.2 ±3.3
Sulfathiazole	38.5 ±2.4	26.8 ±1.6[d]

a) Mean±standard error.
b) Without salicylate.
c) With salicylate (500 μmol/kg, *s.c.*).
d) $p<0.01$.

Table III. Acetylation Rate Constants of Sulfonamides

Sulfonamides	$k_m \times 10^2$ [a] (hr^{-1})	
	Control[b]	SA treat[c]
Sulfanilamide	59.6 ±3.1	59.8 ±3.8
Sulfamethoxazole	6.98±0.27	2.93±0.27[d]
Sulfadiazine	2.72±0.20	1.77±0.13[d]
Sulfisoxazole	5.5 ±1.0	4.0 ±0.9
Sulfathiazole	14.6 ±1.3	14.9 ±1.3

a) Mean±standard error.
b) Without salicylate.
c) With salicylate (500 μmol/kg, *s.c.*).
d) $p<0.01$.

(Tables II and III). It was found that salicylate treatment did not influence k_m but decreased k_e of sulfathiazole, while for sulfamethoxazole, the treatment decreased k_m but had no effect on k_e. Thus, it may be considered that the decrease in elimination of sulfamethoxazole from the blood on salicylate treatment can be explained on the basis of a decrease in k_m. However, for sulfathiazole, the decrease in the elimination from rat blood causes a decrease in k_e on salicylate treatment. The possibility of an increase in the enzymatic activity for acetylation of sulfathiazole can be ruled out, because the treatment had little effect on k_m.

Kakemi *et al.*[6] reported that the elimination of sulfonamides from rat blood was enhanced by bucorome, a non-steroidal anti-inflammatory drug, as a result of an increase in the concentration of unbound sulfonamide due to the displacing effect of bucorome on protein-bound sulfonamides. It has been reported that the protein binding of sulfonamides is easily inhibited

Table IV. Effect of Salicylate on the Unbound Fraction of Sulfonamides at 37° and pH 7.4

Sulfonamides	Per cent unbound		Ratio[c]
	Without[a]	With SA[b]	
Sulfanilamide	95.0	98.7	1.0
Sulfamethoxazole	56.7	85.3	1.5
Sulfadiazine	85.6	93.4	1.1
Sulfisoxazole	24.8	70.3	2.8
Sulfathiazole	49.5	73.6	1.5

a) Concentration of sulfonamides: 0.2 mM.
b) Concentration of salicylate : 1.0 mM.
c) With SA/without SA.
Concentration of BSA: 1% (w/v).

by the addition of salicylate,[9] and we reinvestigated this point. The concentrations of sulfonamide, salicylate, and bovine serum albumin used were 0.2 mM, 1 mM, and 1% (w/v), respectively. The results are presented in Table IV in terms of unbound fractions of sulfonamides with and without salicylate, and the degree of displacement by salicylate is expressed in terms of the ratio of the values with and without salicylate.

It has been well established that the glomerular filtration of drug is closely correlated to the concentration of the unbound fraction of the drug in the blood. The small effect of the salicylate treatment on k_e for sulfanilamide could be partially due to its small effect on the protein binding of the drug.

The effect of the salicylate treatment in increasing the unbound fraction was largest for sulfisoxazole among the sulfonamides studied, but the increase in k_e of the drug was not significant (Table II). One reason for this was considered to be the buffering action of red blood cells in the protein binding of the drug. It was reported previously[12] that red blood cells participate in the equilibrium of unbound and bound fractions of sulfonamides in blood. Displacement of a drug from binding sites on protein may also produce a redistribution of the drug into tissues, especially in the liver. Takada *et al.*[13] reported that the elimination of sulfamethizole from dog blood was inhibited by acidic non-steroidal anti-inflammatory drugs, due to an inhibition of renal tubular secretion of sulfamethizole. Salicylate, an acidic anti-inflammatory drug, was reported to be secreted by the *p*-aminohippurate mechanism,[14] and was considered to inhibit the tubular secretion process of acidic drugs which were excreted by this means. The tubular secretion of sulfisoxazole was also reported to be an important pathway for the urinary excretion of the drug in rabbits.[15] Thus, a possible inhibitory effect of salicylate treatment on the tubular secretion of sulfisoxazole should be considered in connection with the urinary excretion of the drug in rats. The insignificant effect of salicylate treatment on k_e for sulfisoxazole was considered to be caused by compensating effects of the salicylate treatment, *i.e.*, possible inhibition of tubular secretion, and competitive displacement in protein binding resulting in an increase in unbound sulfisoxazole.

For sulfanilamide, the absence of a tubular secretion process for urinary excretion[15] was considered to be an important factor in minimizing the effect of salicylate treatment.

The existence of a tubular secretion process was reported for sulfathiazole in the dog.[16] A possible contribution of this process should be considered to be the decrease in the excretion of sulfathiazole in the rat on salicylate treatment. In general, acetylated sulfonamides are excreted mainly by tubular secretion.[16] The present results (Fig. 6) that salicylate treatment decreases the excretion of N^4-acetylated sulfamethoxazole and sulfathiazole were considered to reflect a possible inhibitory effect of salicylate treatment on their tubular secretion. Thus, the decrease in k_e of sulfathiazole may be mainly due to the inhibitory effect of salicylate on the tubular secretion.

Brody[17] found that salicylate uncoupled oxidative phosphorylation in mitochondrial preparations of rat tissues and influenced many enzymatic reactions in tissues. It was also reported that the acetylation of sulfonamides occurred enzymatically.[18] This process is thought to be mainly performed in the liver, and would be influenced by salicylate treatment. Anton *et al.*[19] reported that the acetylation of sulfonamides *in vitro* was affected by the

12) Y. Higashi, S. Uei, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **27**, 23 (1979).
13) M. Takada, S. Akuzu, A. Misawa, R. Hori, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **22**, 542 (1974); M. Takada, A. Misawa, K. Fujimoto, R. Hori, and T. Arita, *ibid.*, **22**, 551 (1974).
14) I.M. Weiner and G.H. Mudge, *Am. J. Med.*, **36**, 743 (1964).
15) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2526 (1969).
16) T. Arita, R. Hori, M. Takada, S. Akuzu, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 570 (1972).
17) T.M. Brody, *J. Pharm. Exp. Ther.*, **114**, 39 (1956).
18) T.C. Chou and F. Lipmann, *J. Biol. Chem.*, **196**, 89 (1952); W.W. Weber and S.N. Cohen, *Mol. Pharmacol.*, **3**, 266 (1967); M.S. Steinberg, S.N. Cohen, and W.W. Weber, *Biochim. Biophys. Acta*, **235**, 89 (1971).
19) A.H. Anton and J.J. Boyle, *Can. J. Physiol. Pharmacol.*, **42**, 809 (1964).

addition of plasma albumin and concluded that the unbound fraction of sulfonamide is involved in the acetylation process. In the present work, the effects of the treatment on k_m were quite different with different sulfonamides. It is considered that the overall effect of enzymatic inhibition and the increase in the unbound fraction on the acetylation of sulfonamides are responsible for the differences. The k_m values of sulfamethoxazole and sulfadiazine were significantly reduced by salicylate treatment. Sulfinpyrazone was reported to inhibit the acetylation of sulfonamide *in vitro*.[19] So far, the acetylation of sulfonamides *in vitro* has not been studied, and such a study may be useful to clarify the present results.