

**Effects of Simultaneous Administration of Drugs on Absorption and Excretion.****V.<sup>1)</sup> Effect of Phenylbutazone on Antibacterial Activity and Distribution of Sulfadimethoxine in Rabbits**

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The antibacterial activities of sulfadimethoxine in rabbit plasma were significantly increased by concomitant injection of phenylbutazone.

On the other hand, the levels of unchanged sulfadimethoxine determined by chemical assay in rabbit plasma were significantly reduced by concomitant injection of phenylbutazone.

These results suggest that phenylbutazone increases the transfer of sulfadimethoxine from plasma to tissues in rabbits.

Studies on the displacement of one drug by another from protein binding sites have become one of the important problem in areas of biopharmaceutics.<sup>3)</sup>

In the previous reports of this series,<sup>1,4)</sup> we have shown that salicylic acid, which can strongly displace sulfadimethoxine (SDM) from protein binding sites of SDM with bovine serum albumin, affects on the antibacterial activity and distribution of SDM in rabbits.

In the present report, to confirm further the influence of the displacing agents on the binding of SDM with rabbit plasma albumin, the effect of phenylbutazone (PBZ) on the antibacterial activity and distribution of SDM in rabbits was investigated by the same method described in the previous paper.<sup>1)</sup> Furthermore, the effect of salicylic acid and PBZ on the distribution of sulfanilamide in rabbits was studied.

**Experimental**

**Materials**—SDM (Daiichi Seiyaku Co., Ltd.), sulfanilamide (Katayama Chemical Co., Ltd.), PBZ (Fujisawa Pharmaceutical Industry Co., Ltd.), sodium salicylate (Nakarai Chemical Co., Ltd.).

**In Vivo Experimental Methods**—(a) Animals: Male rabbits weighing 3.0–3.2 kg were fasted about 24 hours prior to the experiments. However, drinking water was allowed *ad libitum*.

(b) Administration Method of Drugs: Sulfonamides (25 mg/kg body weight as sodium salt) with PBZ (50 mg/kg body weight as sodium salt) or salicylic acid (100 mg/kg body weight as sodium salt) were administered intravenously to rabbits.

(c) Collections of Urine, Blood and Plasma: The collections of urine, blood and plasma were carried out by the same method described in our previous paper.<sup>1)</sup>

**Determination Procedure of Sulfonamides by Chemical Assay**—Sulfonamides were determined by the method of Bratton and Marshall.<sup>5)</sup> The detailed procedure was described in the previous report.<sup>1)</sup>

**Determination Procedure of SDM by Bioassay**—The determination of antibacterial activities of SDM in rabbit plasma were carried out according to the method describing in our previous papers.<sup>4,6)</sup>

1) Part IV: Y. Imamura, K. Shigemori, and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2324 (1974).

2) Location: 5-1 Oe-honmachi, Kumamoto, 862, Japan.

3) B.K. Martin, *Nature*, **207**, 274 (1965); C.M. Kunin, *Clin. Pharmacol. Therap.*, **7**, 166 (1965); P.M. Aggeler, R.A. O'Reilly, L. Leong, and P.E. Kowitz, *New. Engl. J. Med.*, **276**, 496 (1967); M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968); S. Goto, *Yakuzaigaku*, **32**, 165 (1972).

4) Y. Imamura and H. Ichibagase, *Yakugaku Zasshi*, **93**, 1206 (1973).

5) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 539 (1939).

6) M. Shibata, K. Shigemori, and Y. Imamura, *Chemotherapy*, **22**, 1424 (1974).

## Result and Discussion

The antibacterial activities of SDM in rabbit plasma following intravenous injection of SDM with or without PBZ were determined according to the method described in the previous papers.<sup>4,6)</sup> As shown in Fig. 1, the antibacterial activities of SDM in rabbit plasma were significantly increased by concomitant administration of PBZ. In the previous paper,<sup>4)</sup> we revealed that the antibacterial activities *in vitro* of SDM decreased in the presence of bovine serum albumin were significantly recovered by addition of PBZ, and that the recovery effect was attributed to the displacement of SDM by PBZ from protein binding sites of SDM with bovine serum albumin. In addition, the antibacterial activities of PBZ in rabbit plasma following single administration of PBZ were not detected. Consequently, it is considered that the binding *in vivo* of SDM with rabbit plasma albumin also can be displaced by PBZ.

Plasma levels of unchanged SDM determined by chemical assay, on the other hand, were apparently decreased by concomitant administration of PBZ (Fig. 2). To clarify this decreasing effect of PBZ on plasma levels of unchanged SDM in rabbits, the two compartment open model was applied to this system with the same method described in the preceding papers.<sup>1,7)</sup> The results were shown in Fig. 3 and Table I. As can be seen from Table I, the value of the rate constant ( $k_{12}$ ) controlling the distribution of SDM from central compartment to peripheral compartment was evidently increased by concomitant administration of PBZ. Whereas the increase in the value of the rate constant ( $k_{21}$ ) controlling the distribution of SDM from peripheral compartment to central compartment was small.

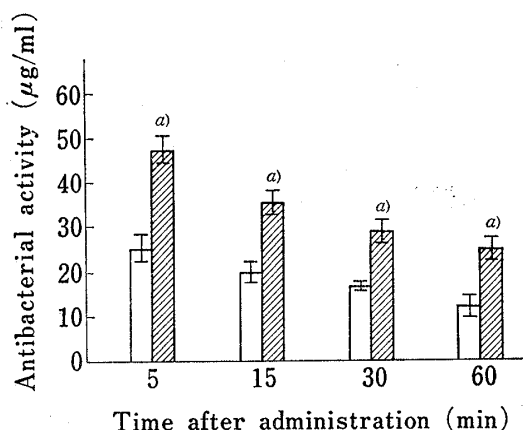


Fig. 1. Antibacterial Activities of Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with Phenylbutazone

Each value is expressed as mean  $\pm$  S.D. of 4 experiments.

a) Statistically significant difference was tested by the Student's t test ( $p < 0.01$ ).

□ : SDM alone (25 mg/kg body weight)  
 ▨ : SDM + phenylbutazone (50 mg/kg body weight)

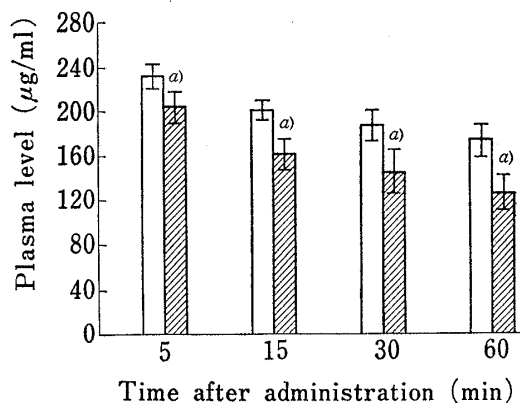


Fig. 2. Plasma Levels of Unchanged Sulfadimethoxine determined by Chemical Assay in Rabbits following Intravenous Administration of Sulfadimethoxine with Phenylbutazone

Each value is expressed as mean  $\pm$  S.D. of 4 experiments.

a) Statistically significant difference was tested by the Student's t test ( $p < 0.05$ ).

□ : SDM alone (25 mg/kg body weight)  
 ▨ : SDM + phenylbutazone (50 mg/kg body weight)

Furthermore, in order to elucidate whether this effect of PBZ on the value of  $k_{12}$  of SDM in rabbits may be caused by the displacement of SDM by PBZ from its protein binding sites, the effect of PBZ and salicylic acid on sulfanilamide with very low binding to plasma albumin was analyzed by the same method. These results were shown in Fig. 4 and Table II. As shown in Table II, the values of the distribution rate constants,  $k_{12}$  and  $k_{21}$ , for sulfanilamide in rabbits were not increased by PBZ or by salicylic acid.

7) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, 57, 117 (1968).

From these results described above, it may be concluded that the decrease in plasma levels of unchanged SDM in rabbits after concomitant administration of PBZ is attributed to the redistribution into tissues of free SDM displaced from its protein binding sites by PBZ.

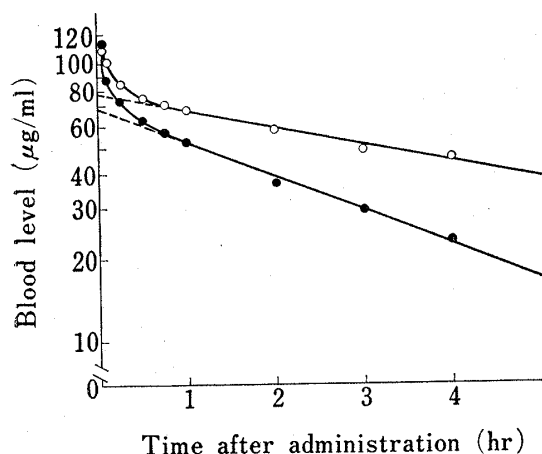


Fig. 3. Blood Level Curve for Unchanged Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with Phenylbutazone

Each value is expressed as mean of 4 experiments.

- : SDM alone (25 mg/kg body weight)  
—●— : SDM + phenylbutazone (50 mg/kg body weight)

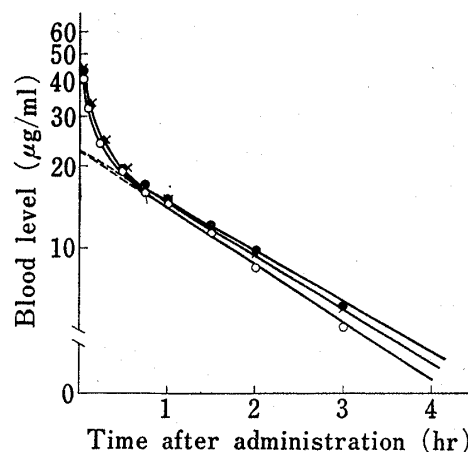


Fig. 4. Blood Level Curves for Unchanged Sulfanilamide in Rabbits following Intravenous Administration of Sulfanilamide(SA) with Phenylbutazone or Salicylic Acid

Each value is expressed as mean of 4 experiments.

- : SA alone (25 mg/kg body weight)  
—●— : SA + phenylbutazone (50 mg/kg body weight)  
—x— : SA + salicylic acid (100 mg/kg body weight)

TABLE I. Values of Individual Rate Constants for Unchanged Sulfadimethoxine in Rabbits

	$k_{12}$ (hr <sup>-1</sup> )	$k_{21}$ (hr <sup>-1</sup> )	$k_{e1}$ (hr <sup>-1</sup> )
Sulfadimethoxine alone	1.02	3.01	0.18
With phenylbutazone	2.05	3.31	0.46

These values were calculated from the data given in Fig. 3.  
Two compartment open model of the following type was applied.

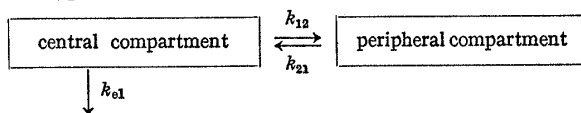


TABLE II. Values of Individual Rate Constant for Unchanged Sulfanilamide in Rabbits

	$k_{12}$ (hr <sup>-1</sup> )	$k_{21}$ (hr <sup>-1</sup> )	$k_{e1}$ (hr <sup>-1</sup> )
Sulfanilamide alone	3.01	4.29	0.90
With phenylbutazone	2.93	4.43	0.80
With salicylic acid	2.84	4.10	0.80

These values were calculated from the data given in Fig. 4.  
Refer to Table I for two compartment open model.

Recently, Anton, *et al.*<sup>8)</sup> demonstrated that the distribution of highly bound sulfamethoxy-pyridazine in rats was significantly increased by sulfinpyrazone (one of PBZ derivatives), and this effect was due to the binding displacement, since the displacing agent had little effect on the distribution of sulfanilamide. This studies by Anton, *et al.* offer additional evidence in support of our consideration. Moreover, it is assumed that the enhancement in the transfer of SDM from plasma to gastrointestinal tract, namely the enhancement in an exsorption, may result in the reduced plasma levels of unchanged SDM. Studies on the exsorption are now under way and the details will be reported in near future.

The value of the elimination rate constant ( $k_{el}$ ) for SDM, which is the sum of the rate constants for the simultaneous processes of metabolism and excretion, was increased by con-

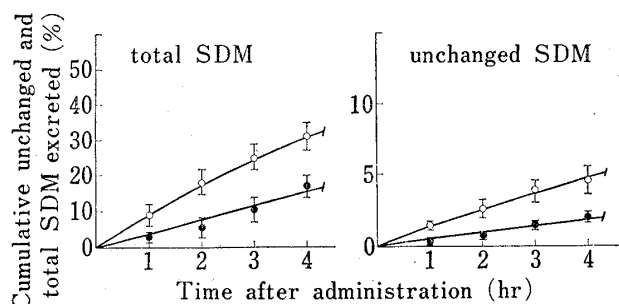


Fig. 5. Urinary Excretion Curves for Unchanged and Total Sulfadimethoxine in Rabbits following Intravenous Administration of Sulfadimethoxine with Phenylbutazone

Each value is expressed as mean  $\pm$  S.D. of 4 experiments.

—○— : SDM alone (25 mg/kg body weight)

—●— : SDM + phenylbutazone (50 mg/kg body weight)

its protein binding sites. However, further studies are necessary to prove fully this problem.

comitant administration of PBZ (see Table I). Anton<sup>9)</sup> have shown that the displacement of drugs from plasma albumin results in an increased rate of metabolism and excretion. As shown in Fig. 5, however, the urinary excretion of unchanged and total SDM were conversely reduced by PBZ. In addition, the increase in the value of  $k_{el}$  for sulfanilamide in rabbits after concomitant administration of PBZ was not recognized (see Table II). Accordingly, it may be considered that the metabolism for SDM can be markedly increased in rabbits as the result of the displacement of SDM by PBZ from

8) A.H. Anton and R.W. Rodriguez, *Science*, **180**, 947 (1973).

9) A.H. Anton, *J. Pharm. Exptl. Therap.*, **134**, 291 (1961).