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Transformation and Excretion of Drugs in Biological Systems. VIII.[1] Interactions between 5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine and Sulfonamides in Dogs

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Interaction between 5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP), which is one of non-steroidal anti-inflammatory agents, and several sulfonamides was investigated in dogs.

Decline of plasma level of sulfamethizole, sulfamethizole-N^4-acetate and sulfisomezole-N^4-acetate was retarded by simultaneous medication of BCP. By simultaneous medication of BCP, sulfisomezole caused a small sudden drop of plasma level, but no alteration was observed with sulfanilamide. BCP possesses strong displacing activity for binding of certain sulfonamides to dog plasma proteins.

The clearance ratios of sulfamethizole, sulfamethizole-N^4-acetate and sulfisomezole-N^4-acetate were remarkably decreased after BCP infusion. No significant alteration of the clearance ratio of sulfisomezole and sulfanilamide was observed. The clearance ratio of BCP was very low when the urine pH is below 8, but increased in metabolic alkalosis. The renal excretion of BCP was significantly blocked by iodopyracet infusion indicating that BCP is secreted through the proximal tubular route by the PAH transport mechanism.

It became clear that prolongations of plasma levels of the certain sulfonamides by BCP, are mainly due to competitive interactions between the certain sulfonamides and BCP at renal secretory level.

In clinical practices, it is quite general that patients receive more than one drug simultaneously. As the result, many striking examples of interactions among drugs, such as displacement from protein binding of one drug by another, enhancement or inhibition of drug metabolizing enzymes of one drug by another and interaction among drugs at renal level, have been reported.[3–8]

Studies of interactions among drugs have become one of the important problems in areas of drug therapy in order to prevent undesirable side effects.

Recently, many non-steroidal anti-inflammatory agents have been applied to clinical use. 5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) is a well known example.

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2) Location: *a*) *Nishi-5-chome, Kita-14-jo, Sapporo*; *b*) *Nishi-6-chome, Kita-12-jo, Sapporo*. Send reprint requests to: Dr. T. Arita.
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Several reports have already been published concerning biotransformation and biopharmaceutical properties of BCP.[9–15]

The present study is concerned with the interactions in dogs of several sulfonamides with BCP. The results have indicated that the interactions are very complex in nature involving at least competition for binding sites on transporting plasma proteins, and mutual suppression of tubular secretion among drugs. Furthermore, the renal excretory characteristics of BCP was extensively investigated.

Experimental

Preparation of Materials——5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP): Commercially available BCP was recrystallized from *n*-hexane. mp 80—84°. Sulfanilamide (SA): Recrystallized from EtOH. mp 165—167°. Sulfamethizole (SMZ): Recrystallized from EtOH. mp 207—208°. Sulfamethizole-N^4-acetate (SMZ-N^4-AC): Synthesized by acetylation of sulfamethizole.[15] mp 234—237°. Sulfisomezole (SIMZ): Recrystallized from EtOH. mp 168—171°. Sulfisomezole-N^4-acetate (SIMZ-N^4-AC): Synthesized by acetylation of sulfisomezole.[16] mp 223—224°.

Method of Drug Administration——A sulfonamide in dose of 30 mg/kg was administered to the dog through cephalic vein. BCP in dose of 30—60 mg/kg was also administered through cephalic vein with or after the administration of sulfonamides. Blood samples were withdrawn from femoral vein. An aliquot of blood samples was cetrifuged and the plasma was subjected to analysis. Throughout the experiments, dogs were anesthetized with pentobarbital sodium (20—25 mg/kg). Each dog was subjected to the experiment every two or three weeks under vigorously controlled conditions in order to avoid the possibility of drug-induced enzyme inductions.

Binding Experiments——The extent of binding of sulfonamides to dog plasma proteins was determined by the method of equilibrium dialysis as described previously.[17] Interference by BCP on the binding of various sulfonamides to dog plasma proteins was evaluated by the method of Anton.[18] Each sulfonamide was initially added to the external compartment to give a concentration of 100 μg/ml. BCP was also placed at the same concentration in the external compartment. Dialysis was carried out to equilibrium as described in the aforementioned report.[17]

Method of Evaluating the Sulfonamide-displacing Activity of BCP——The method described by Anton[4] was employed in our experiments. The activities of BCP in interfering with the binding of sulfonamides to dog plasma proteins were compared (at equal various sulfonamide concentration by weight). The criterion for this comparison is called Displacing Activity (DA) and is defined as follows: $DA=100-(a/b\cdot 100)$ where DA=Displacing Activity *in vitro*; a=% sulfonamide bound in the presence of a competing drug; b=% sulfonamide bound in the absence of the competing drug. (Sulfonamide was present at 100 μg/ml).

Renal Clearance Experiment——The standard methods for renal clearance were employed.[17] All experiments were carried out in anesthetized (pentobarbital sodium 30 mg/kg) dogs. Male and female dogs weighing 13.0—17.5 kg were used in these experiments. Each substance was injected intravenously and successive infusion was continued throughout the experiments. In order to prevent the proximal tubular secretion of the sulfonamides, iodopyracet (208 mg/kg) or BCP (30—60 mg/kg) was initially given through cephalic vein after 2—5 control clearance periods, and a sustaining infusion of iodopyracet (6.81 mg/kg/min) or BCP (0.3—0.9 mg/min) was continued at a rate of 3 ml/min, until the clearance experiment was performed. The detailed procedure was as described in the previous report.[17] Drug clearance (C) in ml/min is calculated as $C=UV/P$, where U and P indicate the urine and plasma concentrations of drug in mg/ml respectively, and V is the urine flow rate in ml/min. To evaluate the renal handling of the drug, clearance ratio (CR) has been conventionally used and is expressed as $CR=C/GFR$, where GFR represents glomerular filtration rate in ml/min calculated as inulin clearance.

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Analytical Method——Determination of BCP: BCP was estimated in plasma and urine by the spectrophotometric procedure previously described by Mima, *et al.*[9] Determination of other substances: plasma and urine samples were deproteinized with 10% trichloroacetic acid, and then analyzed as follows: sulfonamides by diazotization,[19] inulin by a modification of the method described by Dische, *et al.*,[20] and iodopyracet by the titration method described by Alpert.[21] A Hitachi-Horiba model F-4 pH meter with a glass electrode was used to determine the pH of urine.

Result and Discussion

Alteration of Plasma Level of Sulfonamides by BCP

The plasma level of each sulfonamide in dogs with and without BCP was determined under carefully controlled experimental conditions. The representative examples of time course of each sulfonamide in plasma with or without BCP are presented in Fig. 1—5. In SMZ, SMZ-N^4-Ac and SIMZ-N^4-Ac, as shown in Fig. 2, Fig. 4 and Fig. 5 respectively, the plasma levels of these sulfonamides declined much slower with the simultaneous administration of BCP than those of the control experiments. Particularly, the prolonged SMZ plasma levels by BCP is noteworthy. Thus, by simultaneous medication of BCP, a high plasma level of SMZ is maintained for a long time displaying the prolonged pharmacological effect. It is also demonstrated that the decline of plasma levels of SMZ-N^4-Ac and SIMZ-N^4-Ac is retarded by the simultaneous medication of BCP.

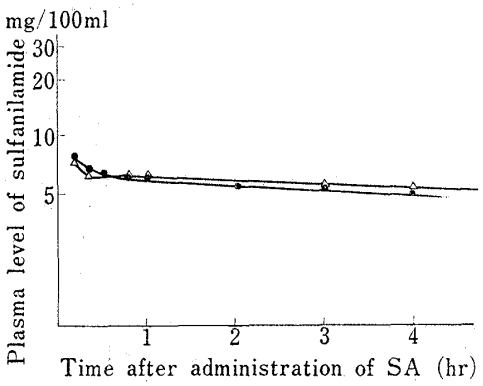
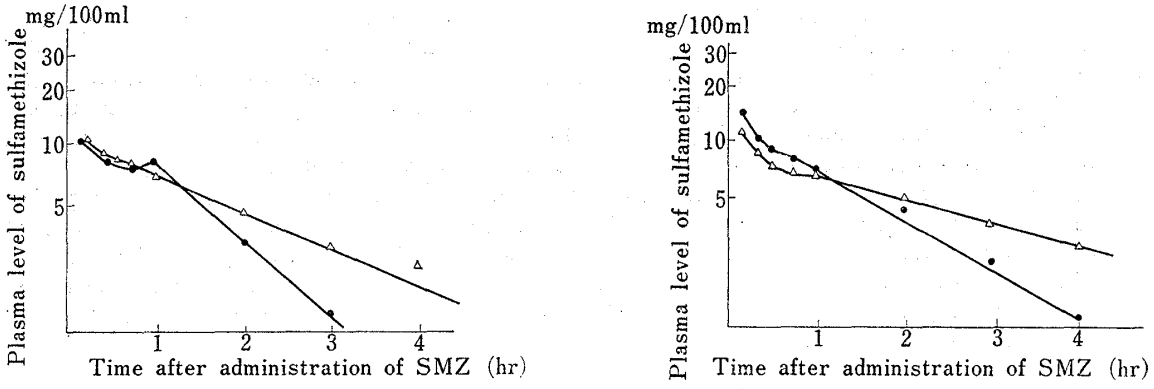


Fig. 1. Effect of BCP on the Plasma Level of Sulfanilamide in Dog

dog: 16.5 kg ♂ (C)
●: SA 30 mg/kg *i.v.*
△: SA 30 mg/kg+BCP 60 mg/kg *i.v.*

It is well known that one of the characteristic toxic effects of sulfonamide is the renal damage due to the biotransformation of the drugs into the N^4-acetyl derivatives.[22–24] Because of the low solubilities of the metabolites in comparison with those of the parent drugs,



dog: 14.0 kg, ♂ (B)
●: SMZ 30 mg/kg *i.v.*
△: SMZ 30 mg/kg+BCP 60 mg/kg *i.v.*

dog: 13.0 kg, ♂ (A)
●: SMZ 30 mg/kg *i.v.*
△: SMZ 30 mg/kg+BCP 30 mg/kg *i.v.*

Fig. 2. Effect of BCP on the Plasma Level of Sulfamethizole in Dogs

19) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
20) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
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23) M. Ueda and K. Kuribayashi, *Yakugaku Zasshi*, **84**, 1104 (1964).
24) M. Ueda, Y. Nakagawa, and N. Murakami, *Yakugaku Zasshi*, **87**, 451 (1967).

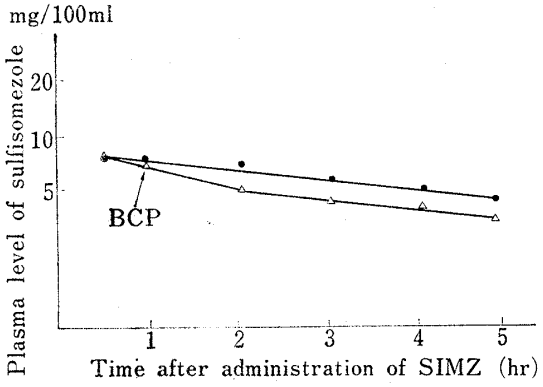

Fig. 3. Effect of BCP on the Plasma Level of Sulfisomezole in Dog

Arrow indicates the administration of BCP.
dog: 18.0 kg, ♂ (F)
●: SIMZ 30 mg/kg *i.v.*
△: SIMZ 30 mg/kg+BCP 30 mg/kg *i.v.*

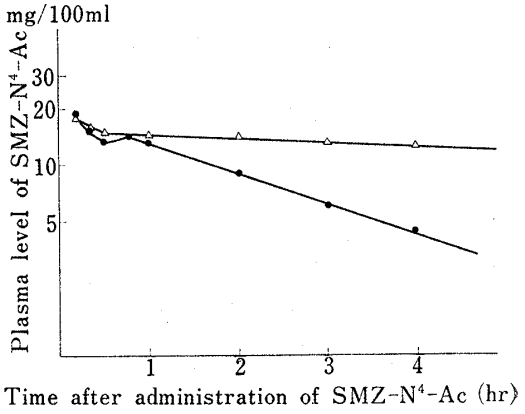

Fig. 4. Effect of BCP on the Plasma Level of Sulfamethizole-N⁴-acetate in Dog

dog: 14.0 kg, ♂ (E)
●: SMZ-N⁴-Ac 30 mg/kg *i.v.*
△: SMZ-N⁴-Ac 30 mg/kg+BCP 30 mg/kg *i.v.*

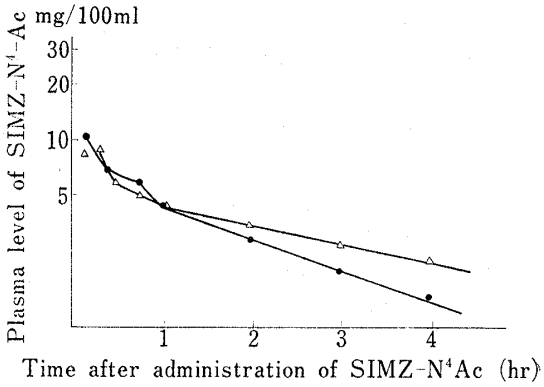

Fig. 5. Effect of BCP on the Plasma Level of Sulfisomezole-N⁴-acetate in Dog

dog: 15.0 kg, ♂ (D)
●: SIMZ-N⁴-Ac 30 mg/kg *i.v.*
△: SIMZ-N⁴-Ac 30 mg/kg+BCP 30 mg/kg *i.v.*

the metabolites tended to crystallize out in the renal tubules. Both SMZ-N⁴-Ac and SIMZ-N⁴-Ac are known to be the main biotransformed product of the original sulfonamides in man. The retarded disappearance of the N⁴-acetylation products of SMZ and SIMZ from dog plasma by BCP drew our special attention concerning the possible change of this undesirable side effect which is attributable to the biotransformation of the sulfonamides.

SIMZ which is one of moderately long acting sulfonamides, acted in dogs in a fashion quite different from SMZ, when BCP was simultaneously medicated. As shown in Fig. 3, BCP caused a small but a sudden drop in the plasma level of SIMZ. After the initial drop, the plasma level of SIMZ declined in a manner rather analogous to that of the control experiment. On the other hand, as shown in Fig. 1, no apparent difference was observed in the SA plasma level with or without BCP.

Displacement of Sulfonamides bound to Dog Plasma Proteins by BCP

Drug activation by the addition of competitive inhibitors to the binding system has been considered by a number of workers and attempts to evaluate such possibilities *in vitro* and *in vivo* have been reported. A number of acidic drugs are known to compete for the same limited number of protein binding sites.[3] Hence one acidic drug may be displaced by another, thereby increasing the concentration of unbound drug at target sites. Thus, highly bound acidic agents such as phenylbutazone, oxyphenbutazone, ethyl biscoumacetate, dicumarol, sulfinpyrazone and salicylic acid are able to displace the long-acting, albumin-bound sulfonamides from the plasma protein.[4] However, such interference with the protein binding of drugs *in vivo* may produce unexpected toxic manifestation due to the rapid rise of concentration of unbound drugs.

BCP, an acidic drug, is well known to bind strongly to serum albumin and possesses a high displacing activity.[10,11] In our experiments, BCP was examined for its activity in displacing bound sulfonamides to dog plasma proteins by the method of Anton.[4] The results

Table I. Interference by BCP with the Binding of Various Sulfonamides to Dog Plasma Protein

Sulfonamide	pK_a	% bound to dog plasma at 100 μg/ml	Displacing activity *in vitro* (%)
Sulfanilamide	10.08	9.72	44.0
Sulfamethizole	5.45	62.92	14.1
Sulfisomezole	6.05	39.02	34.6
Sulfamethizole-N^4-acetate		68.60	7.9
Sulfisomezole-N^4-acetate	5.54	43.72	12.8

The displacing activity (at 100 μg/ml) was determined *in vitro* by equilibrium dialysis. Method of evaluating the sulfonamide-displacing activity of BCP was described under experimental. The pK_a were obtained from the literatures.[25,26]

are shown in Table I. As shown in Table I, BCP possesses a strong displacing activity and significantly alters binding of certain sulfonamides to dog plasma proteins. Particularly, this result seems to relate the phenomenon observed in Fig. 3, in which BCP causes a small sudden drop in the plasma level of SIMZ. It was demonstrated[1] that main route of SIMZ excretion is glomerular filtration which depends on only unbound portion of the sulfonamide. As the result of diminishing bound SIMZ and increasing unbound SIMZ, the equilibrium of unbound SIMZ between plasma and tissues would be disturbed and the redistribution of unbound SIMZ from the plasma to various tissues would cause the decrease in the plasma concentration. Similar phenomena concerning time course of plasma level of sulfonamides have also been reported by Anton.[4] On the contrary, BCP exhibited very little influence against the plasma level of SA which possesses very little affinity to dog plasma proteins.

However a phenomenon of the marked duration of prolonged high plasma level in SMZ, SMZ-N^4-Ac and SIMZ-N^4-Ac by BCP, cannot be explained by the displacement of protein bound sulfonamides by simultaneous medicated BCP. The possibility of other mechanisms can be suggested.

Mutual Suppression of Sulfonamides and BCP at the Renal Level

Competition for tubular transport is well established as a mechanism underlying the depression of the excretion of one compound by another.[27] The aforementioned prolonged effect of the plasma level of certain sulfonamides by BCP suggests the possibility of this competitive inhibition of sulfonamide excretion by BCP at the renal level. Therefore, renal clearance experiments were performed employing seven dogs to determine whether the renal excretion of the sulfonamides could be inhibited by BCP. The results are exemplified in Tables II—VI.

As shown in Fig. 6 and Table III, the difference in the clearance ratio of SMZ before and after BCP infusion was clearly observed. Especially, the marked effect of BCP in suppressing the clearance ratio of SMZ, and the striking similarity between BCP and iodopyracet as for the inhibitory effect of SMZ excretion, suggests that BCP competitively interferes with the proximal tubular secretion of SMZ. Similarly, decrease of clearance ratios of SMZ-N^4-Ac and SIMZ-N^4-Ac after BCP infusion was also observed as shown in Fig. 7. On the contrary, clearance ratios of SA and SIMZ before and after BCP infusion were not altered (Fig. 7).

In our previous report[28] we demonstrated that SMZ, and SMZ-N^4-Ac which is the main product of SMZ biotransformation, are both extensively secreted through the proximal tubular

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28) T. Arita, R. Hori, M. Takada, S. Akuzu, and A. Misawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 937 (1971).

route. Furthermore, it is also demonstrated[1] that SIMZ-N⁴-Ac, which is one of the main metabolites of SIMZ, is considerably secreted by the proximal tubule although SIMZ itself is only slightly secreted by this route. The present results concerning the effect of BCP on

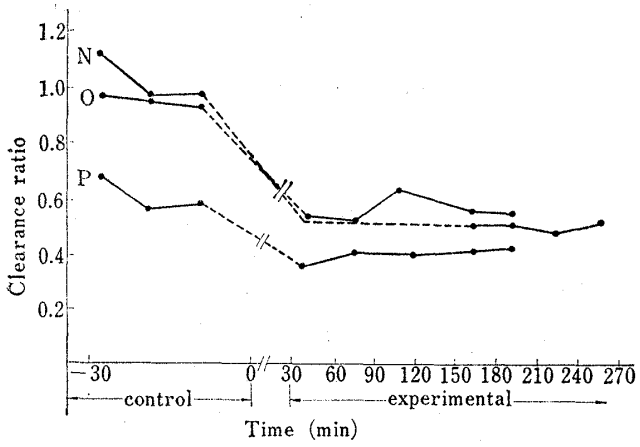

Fig. 6. The Effect of BCP on Renal Clearance of Sulfamethizole

The lines connect the values for each dog.
N: dog ♂ 17.0 kg O: dog ♂ 14.3 kg P: dog ♂ 14.5 kg

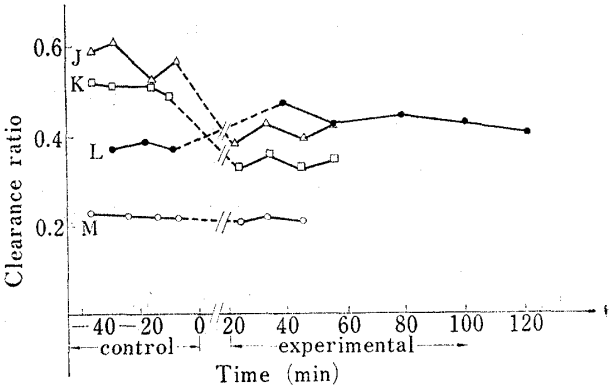

Fig. 7. The Effect of BCP on Renal Clearance of Sulfanilamide, Sulfisomezole, Sulfisomezole-N⁴-acetate and Sulfamethizole-N⁴-acetate

The lines connect the values for each dog.
J: dog ♂ 13.0 kg L: dog ♀ 17.5 kg
K: dog ♂ 15.0 kg M: dog ♂ 14.0 kg
●: SA ○: SIMZ △: SIMZ-N⁴-Ac □: SMZ-N⁴-Ac

Table II. The Effect of BCP on Renal Clearance of Sulfanilamide

	Time (min)	*V* (ml/min)	Urine pH	GFR (ml/min)	Sulfanilamide *U* (mg/ml)	Sulfanilamide *P* (mg/ml)	Sulfanilamide *C* (ml/min)	Sulfanilamide CR	BCP *P* (mg/ml)
Control	30— 20	4.00	7.94	62.6	0.232	0.0395	23.5	0.3754	—
	20— 10	4.08	—	65.0	0.246	0.0391	25.7	0.3954	—
	10— 0	4.04	—	66.2	0.247	0.0400	24.9	0.3761	—
Exptl.[a]	30— 50	5.05	7.82	60.7	0.262	0.0436	30.3	0.4991	0.0828
	50— 70	3.10	—	63.8	0.381	0.0443	26.7	0.4185	0.0924
	70— 90	3.25	—	61.0	0.403	0.0473	27.7	0.4541	0.0872
	90—110	4.50	—	76.1	0.364	0.0496	33.0	0.4336	0.0870
	110—130	4.80	—	75.6	0.344	0.0530	31.1	0.4114	0.0877

dog: ♀ 17.5 kg (dog L in Fig. 7)
a) BCP: 525 mg *i.v.*, 0.9 mg/min infusion

Table III. The Effect of BCP on Renal Clearance of Sulfamethizole

	Time (min)	*V* (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole *U* (mg/ml)	Sulfamethizole *P* (mg/ml)	Sulfamethizole *C* (ml/min)	Sulfamethizole CR	BCP *P* (mg/ml)
Control	30— 20	5.45	6.80	63.9	0.433	0.0380	62.1	0.9718	—
	20— 10	5.72	—	67.9	0.389	0.0344	64.7	0.9529	—
	10— 0	5.64	—	61.0	0.362	0.0359	56.9	0.9328	—
Exptl.[a]	30— 60	3.24	6.91	57.1	0.359	0.0365	31.9	0.5587	0.0785
	60— 90	4.00	—	57.0	0.295	0.0374	31.6	0.5544	0.0714
	90—120	4.28	—	49.6	0.273	0.0345	33.9	0.6835	0.0745
	120—150	4.53	—	52.1	0.261	0.0386	30.6	0.5873	0.0656
	150—180	4.97	7.14	44.9	0.242	0.0458	26.3	0.5857	0.0623

dog: ♂ 14.3 kg (dog 0 in Fig. 6)
a) BCP: 430 mg *i.v.*, 0.3 mg/min infusion

the renal excretory behavior of the sulfonamides agree well with our previous data[1,28] which suggested that the proximal tubular secretion of SMZ, SMZ-N⁴-Ac and SIMZ-N⁴-Ac was markedly blocked by iodopyracet infusion.

It is well known that the major metabolites of most sulfonamides in man are the N⁴-acetates which are less soluble in physiological fluids and have higher affinity for renal tubular

Table IV. The Effect of BCP on Renal Clearance of Sulfisomezole

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfisomezole U (mg/ml)	Sulfisomezole P (mg/ml)	Sulfisomezole C (ml/min)	Sulfisomezole CR	BCP P (mg/ml)
Control	40—30	6.20	7.28	48.1	0.1010	0.0552	11.3	0.2349	—
	30—20	8.00	—	46.7	0.0789	0.0588	10.7	0.2291	—
	20—10	7.60	—	46.1	0.0744	0.0538	10.5	0.2276	—
	10— 0	6.40	7.20	47.8	0.0966	0.0588	10.5	0.2197	—
Exptl.[a]	20—30	3.80	7.96	52.9	0.1690	0.0591	10.9	0.2060	0.1155
	30—40	5.00	—	52.3	0.1530	0.0638	12.0	0.2295	0.1118
	40—50	5.50	8.00	51.7	0.1310	0.0643	11.2	0.2166	0.1091

dog: ♂ 14.0 kg (dog M in Fig. 7)
a) BCP: 420 mg *i.v.*, 0.9 mg/min infusion

Table V. The Effect of BCP on Renal Clearance of Sulfamethizole-N⁴-acetate

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfamethizole-N⁴-acetate U (mg/ml)	Sulfamethizole-N⁴-acetate P (mg/ml)	Sulfamethizole-N⁴-acetate C (ml/min)	Sulfamethizole-N⁴-acetate CR	BCP P (mg/ml)
Control	40—30	2.94	7.56	53.5	0.660	0.0706	27.5	0.5140	—
	30—20	2.76	—	53.3	0.642	0.0657	27.0	0.5066	—
	20—10	2.64	—	51.1	0.625	0.0638	25.9	0.5068	—
	10— 0	2.88	—	53.2	0.564	0.0630	25.8	0.4850	—
Explt.[a]	20—30	2.52	7.58	47.2	0.350	0.0571	15.4	0.3263	0.08373
	30—40	2.64	—	44.3	0.344	0.0556	16.3	0.3679	0.08348
	40—50	2.74	—	44.2	0.311	0.0598	14.2	0.3213	0.08432
	50—60	2.84	7.60	46.5	0.322	0.0572	16.0	0.3441	0.08166

dog: ♂ 15.0 kg (dog K in Fig. 7)
a) BCP: 450 mg *i.v.*, 0.9 mg/min infusion

Table VI. The Effect of BCP on Renal Clearance of Sulfisomezole-N⁴-acetate

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	Sulfisomezole-N⁴-acetate U (mg/ml)	Sulfisomezole-N⁴-acetate P (mg/ml)	Sulfisomezole-N⁴-acetate C (ml/min)	Sulfisomezole-N⁴-acetate CR	BCP P (mg/ml)
Control	40—30	2.68	6.78	39.4	0.396	0.0461	23.0	0.5838	—
	30—20	3.06	—	43.0	0.361	0.0421	26.2	0.6093	—
	20—10	3.48	—	46.1	0.298	0.0440	23.6	0.5119	—
	10— 0	4.00	6.76	47.7	0.280	0.0420	26.7	0.5597	—
Exptl.[a]	20—30	2.88	—	47.2	0.255	0.0407	18.0	0.3814	0.1149
	30—40	3.24	7.05	45.0	0.247	0.0412	19.4	0.4311	0.1044
	40—50	3.18	—	44.1	0.228	0.0422	17.2	0.3900	0.1036
	50—60	3.84	7.18	45.9	0.216	0.0416	19.9	0.4336	0.0984

dog: ♂ 13.0 kg (dog J in Fig. 7)
a) BCP: 390 mg *i.v.*, 0.9 mg/min infusion

transport than the original sulfonamides.[29,30] The present finding that BCP blocks proximal tubular secretion of N^4-acetylated products of some sulfonamides and delays renal excretion of the compounds, lead to a caution concerning the possibility that BCP may depress renal excretion of pharmacologically inactive and toxic metabolites of some acidic drugs including some sulfonamides.

Renal Excretory Behavior of BCP

Our previous reports[1,28] demonstrated that SMZ, SMZ-N^4-Ac and SIMZ-N^4-Ac are considerably secreted through the proximal tubule and the apparent secretory contribution of these sulfonamides are remarkably depressed by iodopyracet as discussed in the previous section.

The present study indicated that BCP similarly depresses the excretion of some sulfonamides. This result strongly suggests that BCP might be actively secreted by the same renal tubular transport mechanism as that proposed for the secretion of iodopyracet and other organic acids, and might compete with the certain sulfonamides in renal proximal tubular secretion. Therefore, for the purpose of clarifying the behavior of BCP in the kidney, an experiment was performed employing dogs. The experiment was to determine whether or not the clearance ratio of BCP could be alter by iodopyracet which has a marked blocking effect on sulfonamide secretion. Furthermore, the effect of urine pH on the renal excretion of BCP was also studied in dogs.

As shown in Fig. 8 and Table VII, the clearance ratio of BCP is extremely low and this observation indicates the predominant distal tubular reabsorption. However, this result was obtained only when the pH of the dog urine is below 8, and BCP excretion increased greatly when metabolic alkalosis was induced by the infusion of 7% $NaHCO_3$. Namely, when the urine pH increased by 0.1—0.4 beyond 8, the clearance ratio of BCP rose to a value of 6—8 times greater than that when the urine is below pH 8 (Fig. 8 and Table VIII).

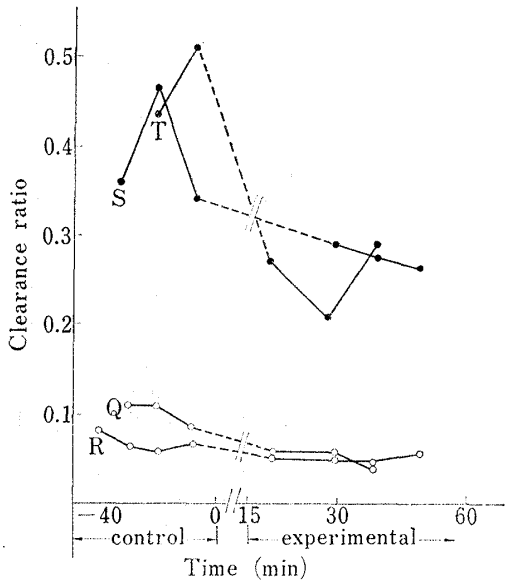

Fig. 8. The Effect of Urine pH and Iodopyracet on Renal Clearance of BCP

The lines connet the values for each dog.
Q: dog ♂ 13.0 kg S: dog ♂ 13.0 kg
R: dog ♂ 14.0 kg T: dog ♂ 15.0 kg
●: metabolic alkalosis
○: urine pH below 8

The marked influence of urinary pH on the excretion of BCP may best be explained on the basis of non-ionic diffusion. The proximal tubular secretion of BCP in dogs was successively examined by blocking the secretion with iodopyracet which is known to be secreted by the PAH excretion mechanism. As shown in Fig. 8 and Tables VII and VIII, in both normal and metabolic alkalosis, the clearance ratio of BCP decreased clearly after iodopyracet infusion. This fact indicates that BCP is considerably secreted through the proximal tubular excretory route. The result can also be interpreted to indicate that BCP competes with iodopyracet for proximal tubular transport. Probably, depending upon the relative concentrations of the two compounds in these experiments, iodopyracet could displace BCP in the transport mechanism. Similar phenomenon has been confirmed in competitive experiments between

29) R.T. Williams, "Detoxication Mechanisms," 2nd ed. by Chapman and Hall Ltd. London, 1959, pp. 510—512.
30) A. Despopoulos, *Theoret. Biol.*, **8**, 163 (1965).
31) I.M. Weiner, J.A. Washington, and G.H. Mudge, *Bull. Johns Hopkins Hosp.*, **106**, 333 (1960).

Table VII. The Effect of Iodopyracet on Renal Clearance of BCP

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	BCP U (mg/ml)	BCP P (mg/ml)	BCP C (mg/min)	BCP CR	Iodopyracet P (mg/ml)
Control	40—30	7.70	7.88	62.1	0.0724	0.113	4.93	0.0794	—
	30—20	6.70	—	63.0	0.0722	0.116	4.17	0.0662	—
	20—10	6.00	—	64.3	0.0730	0.111	3.95	0.0614	—
	10— 0	5.90	—	59.7	0.0708	0.109	3.83	0.0642	—
Exptl.[a]	15—25	7.80	7.90	57.4	0.0502	0.123	3.18	0.0554	0.8983
	25—35	7.00	—	52.2	0.0499	0.124	2.82	0.0540	0.9711
	35—45	6.20	—	52.5	0.0524	0.122	2.66	0.0507	0.9769
	45—55	6.20	7.92	50.3	0.0579	0.120	2.99	0.0594	0.9364

dog: ♂ 14.0 kg (dog R in Fig. 8)
a) iodopyracet: 2.91 g *i.v.*, 95.3 mg/min infusion

Table VIII. The Effect of Iodopyracet on Renal Clearance of BCP during Metabolic Alkalosis[a]

	Time (min)	V (ml/min)	Urine pH	GFR (ml/min)	BCP U (mg/ml)	BCP P (mg/ml)	BCP C (ml/min)	BCP CR	Iodopyracet P (mg/ml)
Control	20—10	21.52	8.44	57.5	0.0486	0.0422	24.8	0.4313	—
	10— 0	20.80	8.46	53.3	0.0468	0.0362	26.9	0.5047	—
Exptl.[b]	15—25	18.80	8.44	46.0	0.0297	0.0443	12.6	0.2739	1.280
	25—35	16.10	8.42	42.5	0.0315	0.0572	8.87	0.2087	1.367
	35—45	12.60	8.44	36.2	0.0350	0.0617	10.8	0.2983	1.646

dog: ♂ 15.0 kg (dog T in Fig. 8)
a) 7% $NaHCO_3$ solution was infused at the rate of 3 ml/min.
b) iodopyracet: 3.12 g *i.v.*, 102.2 mg/min infusion

probenecid and *p*-aminohippurate.[31] The result clearly indicates that BCP is actively secreted by the proximal tubule in large amounts, and its apparent low clearance ratio in urine below pH 8 is a reflection of the almost complete distal tubular reabsorption of BCP which has been introduced into the distal tubular lumen both by glomerular filtration and by secretion.

From the result of our all experiments, it became general concept that plasma levels of the certain sulfonamides which are excreted mainly by proximal tubular secretion, are prolonged by coadministration of BCP which is excreted through the same secretory route with the certain sulfonamides. The mechanism of such prolongation of plasma levels might be competitive interactions between the certain slfonamides and BCP at renal secretory level. Displacement of protein binding of some sulfonamides by BCP seems to cause sudden drop of plasma levels of the sulfonamides, which are extensively bound to plasma protein, being excreted mainly by glomerular filtration.

A more extensive observation of the quantitative aspects of the competition between BCP and other organic acids will, however, be desirable.

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