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Effects of Simultaneous Administration of Drugs on Absorption and Excretion. I. Effect of Phenylbutazone on Absorption and Excretion of Sodium Cyclamate in Rabbits

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The urinary excretion of sodium cyclamate (CHS-Na) in rabbits was accelerated by the simultaneous administration of phenylbutazone, and the excretion rate constant of CHS-Na increased in accordance with an increase in the dose of phenylbutazone. In the experiment of intestinal recirculating perfusion, phenylbutazone did not accelerate the intestinal absorption of CHS-Na in rabbits. And the competitive inhibitory effect of phenylbutazone on the binding between CHS-Na and bovine serum albumin was found in the equilibrium dialysis experiment.

Accordingly, these results demonstrate that the acceleration of the urinary excretion of CHS-Na in the rabbits following simultaneous administration of phenylbutazone is attributed to the competitive inhibitory effect of phenylbutazone in the binding between CHS-Na and rabbit serum albumin.

It has been known that the binding between drugs and serum proteins affects on the absorption, distribution and excretion of various drugs. For example, Anton[2] observed that *in vivo* partial liberation of a drug from its binding sites on serum proteins by another drug provokes an increase of the drug concentration in the tissues as a result of the higher concentration of the free drug. Also, Kakemi, *et al.*[3] have suggested that an interdependence exists between the rate of elimination of salicylic acid derivatives and the degree of binding of those drugs with serum proteins.

In the previous paper,[4] the authors reported that sodium cyclamate (CHS-Na) interacted with bovine serum albumin (BSA). Since the strength of binding of CHS-Na with BSA is considerably weak, it is assumed that the binding of CHS-Na with BSA may be inhibited by other drugs which bind strongly with BSA.

In the present paper we examined the influence of phenylbutazone, which has been known to be strongly bound with serum proteins,[5,6] on the excretion of CHS-Na in rabbits, and investigated *in vitro* the competitive inhibitory effect of phenylbutazone on the binding of CHS-Na with serum proteins.

Experimental

Material——Pure sample of CHS-Na was obtained by repeated recrystallization of reagent grade one, and dried *in vacuo* for 6 hr. BSA (fraction V) and rabbit serum albumin (fraction V), (RSA) were purchased from Armour. Pharm. Co. and Nutritional Biochemicals Corporation respectively. Phenylbutazone and other chemicals used were of reagent grade.

1) Location: *5-1 Oe-honmachi, Kumamoto.*
2) A.H. Anton, *J. Pharmacol. Exptl. Therap.*, **134**, 291 (1961).
3) K. Kakemi, T. Arita, H. Yamashina, and R. Konishi, *Yakugaku Zasshi.*, **82**, 536 (1962).
4) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 1619 (1968).
5) B.B. Brodie and C.A.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957).
6) C. Wunderly, *Arzneimittel-Forsch.*, **6**, 731 (1956).

Animals——Male rabbits weighing 2.6—3.5 kg were fasted for approximately 20 hours prior to the experiments *in vivo* and *in situ*. However, drinking water was allowed *ad libitum*.

***In Vivo* Experimental Methods**——(a) Administration Methods of Drugs: Two hundreds mg of CHS-Na per. kg body weight was administered to rabbits with or without phenylbutazone. CHS-Na and phenylbutazone were administered orally by using Nelaton's catheter in the form of solution or suspension in 100 ml of water.

(b) Collections of Urine: Urinary collections were made by using Nelation's catheter during the following intervals after time zero: 0—1, 1—2, 2—3, 3—4, 4—5, 5—6, 6—8, 8—24 hr. And the urine was collected in a flask containing toluene for preventing putrefaction. The amount of CHS-Na in the urine sample collected was determined by gas liquid chromatography (GLC) method described below.

***In Situ* Intestinal Absorption Procedure**——Rabbit was anesthetized with urethane (1.3 mg/g *i.p.*). The small intestine was exposed by a midline abdominal incision, and two glass cannulae were inserted through small slits at the lower duodenal and upper jejunal portion. The length of the intestine used was about 50 cm. The intestine was flashed with saline solution maintained at 37°, and then with 100 ml of sample solution. The tubing attached to the inflow and outflow glass cannulae were then connected to a flask containing 50 ml of sample solution. The sample solution was then continuously circulated at a flow rate of 30 ml per. min through the small intestine for 2 hr at 37°, using a Tokyo Kagaku Seiki perfusion pump (Type CV-2). A 2 ml aliquot was pipetted out at periodical intervals. Each sample solution was prepared by dissolving CHS-Na and/or phenylbutazone in isotonic phosphate buffer solution (pH 6.7). Phenol red was dissolved in those sample solution to indicate the volume change.

Apparatus and Experimental Condition of GLC——A Shimazu Model GC-3AF dual column gas chromatograph was used. The carrier gas was nitrogen. The column was 300 cm × 3 mm *i.d.* stainless coil-tube containing a packing of 20% PEG 20M and 2.5% NaOH on 60—80 mesh Shimalite. The experimental conditions were as follow: The column temperature was maintained at 130°. The flow rate of carrier gas was kept at 30 ml/min.

Determination Method of CHS-Na by GLC——Sample solution for the preparation of a calibration curve were prepared by dissolving different amounts of CHS-Na in 1.0 ml of rabbit urine. To 1.0 ml of sample solution, 1.0 ml of deinonized water, 0.5 ml of 2% H_2O_2 and 0.5 ml of 3N HCl were added and the mixture was heated in a boiling water-bath for 60 min. After cooling, the mixture was neutralized with 1.0 ml of 10N NaOH and extracted with 3 and 2 ml of $CHCl_3$. The $CHCl_3$ extract was added 0.2 ml of 2N HCl, shaken and evaporated to dryness. The residue was dissolved in 1.0 ml of 30 mg% (w/v) isoamylacetate–$CHCl_3$ solution, and added a small amount of anhyd. K_2CO_3. Two μl of the resulting sample solution was injected into the gas chromatograph at a fixed sensitivity and range. CHS-Na was measured as cyclohexylamine which was produced by hydrolysis of CHS-Na. The peak area of cyclohexylamine and isoamylacetate (internal standard) were determined by triangulation. The calibration curve was obtained by plotting the peak area ratio of cyclohexylamine to isoamylacetate. The amount of CHS-Na in the urine of rabbit was determined by the procedure described above.

Equilibrium Dialysis Experiment——A visking dialysis tubing (24/32 in size) was cut to 100 mm in length, and boiled for 2 hr in deionized water before use. An apparatus for equilibrium dialysis was set up according to the method reported in the previous paper.[4] Eight ml of 0.8% BSA solution, which was dissolved in 0.1M phosphate buffer (pH 7.4), was added inside the dialysis bag, and 20 ml of solution containing CHS-Na (4.0×10^{-3}M) and phenylbutazone (1.0×10^{-3}—5.0×10^{-3}M) was added outside the bag. As a control experiment, 8 ml of 0.1M phosphate buffer (pH 7.4) in place of the 0.8% BSA solution was added inside another bag. The apparatus was kept in a cold chamber of approximately 6° for 72 hr which was a sufficient period of time for attaining to an equilibrium between the solution inside and outside the dialysis bag. The determination of CHS-Na of an external solution of the dialysis bag was carried out by GLC method as described above. The extent of binding of CHS-Na with BSA was calculated from the difference between the concentration of free CHS-Na in the sample tube and that in the control one.

Inhibition of Heat Denaturation of BSA——The inhibitory effect of CHS-Na or other drugs on heat denaturation of BSA was investigated according to the method of Mizushima.[7,8] BSA was dissolved in 1/15M phosphate buffered saline at pH 5.3. CHS-Na, sodium salicylate and sodium *n*-caprylate were dissolved in deionized water into concentration of 10^{-2}M. Phenylbutazone solution was prepared by the addition of 1.1 equivalents of sodium hydroxide to the acid and by adjusting to pH 6.0 with diluted hydrochloric acid. The sample solution was prepared by mixing 2.7 ml of BSA solution and 0.3 ml of 10^{-2}M drug solution. As a control solution, 0.3 ml of deionized water in place of drug solution was added to BSA solution. Then the sample solution or control solution obtained above was placed in a small glass tube and heated in a water-bath at 67° for 3 min. After cooling, the turbidity of those solution was measured photometrically at 660 mμ.

7) Y. Mizushima, *Arch. Int. Pharmacodyn.*, **149**, 1 (1964).
8) Y. Mizushima and H. Suzuki, *Arch. Int. Pharmacodyn.*, **157**, 115 (1965).

Result and Discussion

Effect of Phenylbutazone on Urinary Excretion of CHS-Na

As a preliminary experiment, phenylbutazone (60 mg/kg) was administered orally to rabbits with CHS-Na (200 mg/kg) at the same time and before one hour. The results indicated that no significant difference was observed in the urinary excretion of CHS-Na. Consequently, in the investigation to examine the effect of phenylbutazone on the urinary excretion of CHS-Na in rabbits, phenylbutazone was administered orally with CHS-Na at the same time.

A single dose of 200 mg/kg of CHS-Na was administered orally with or without phenylbutazone to rabbits and CHS-Na in the urine was determined periodically. As shown in Fig. 1, the urinary excretion of CHS-Na in the rabbits receiving CHS-Na together with phenylbutazone was more rapid than that in the control animals given CHS-Na alone. As shown in Fig. 2, semilogarithmic plots of the excretion rate of CHS-Na in the rabbits given CHS-Na with or without phenylbutazone *versus* time were linear after 3—4 hours following oral administration of CHS-Na. These data indicate that the excretion of CHS-Na is the first order. The excretion rate constants and the excretion half-lives for CHS-Na administered orally with different doses of phenylbutazone are shown in Table I.

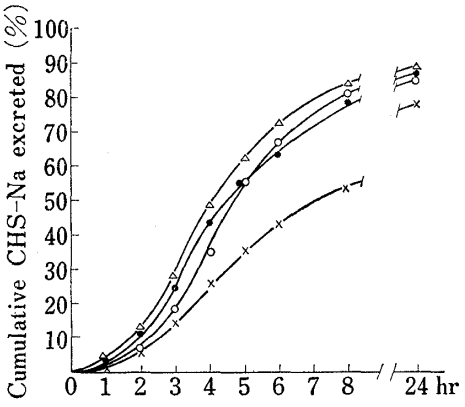

Fig. 1. Mean Cumulative Excretion Curves for CHS-Na following Oral Administration of CHS-Na with Phenylbutazone

—×—: CHS-Na alone
—●—: CHS-Na: phenylbutazone=10:1
—○—: CHS-Na: phenylbutazone=5:1
—△—: CHS-Na: phenylbutazone=1:1
Each value is expressed as the mean of three experiments.

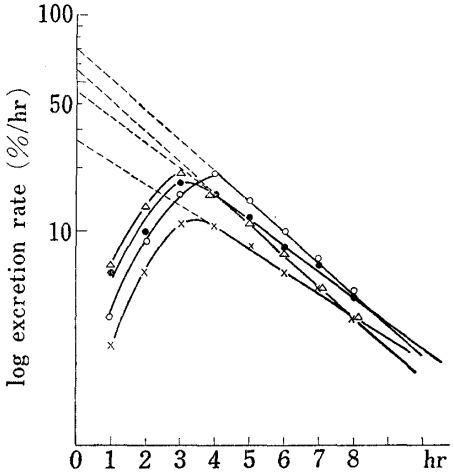

Fig. 2. Logarithm of Excretion Rate *vs.* Time after Oral Administration of CHS-Na with Phenylbutazone

—×—: CHS-Na alone
—●—: CHS-Na: phenylbutazone=10:1
—○—: CHS-Na: phenylbutazone=5:1
—△—: CHS-Na: phenylbutazoue=1:1
Each value is expressed as the mean of three experiments.

Table I. Excretion Rate Constant (K) and Half-life ($t_{1/2}$) for CHS-Na in Rabbit following Oral Administration of CHS-Na with Phenylbutazone

	K (hr^{-1})	$t_{1/2}$ (hr)
CHS-Na alone	0.23	3.0
CHS-Na: phenylbutazone (mole ratio)		
10 : 1	0.28	2.5
5 : 1	0.32	2.2
1 : 1	0.34	2.0

Each value was obtained from semilogarithmic plots of the mean excretion rate of three experiments *vs.* time.

These results suggest that the excretion rate of CHS-Na is promoted by phenylbutazone, and that the rate constant increases in accordance with an increase in the dose of phenylbutazone.

Effect of Phenylbutazone on *in Situ* Intestinal Absorption of CHS-Na

It has been known that the gastrointestinal absorption of a drug is influenced by other drug.[9,10] Accordingly, it may be also considered that the accelerating effect of phenylbutazone on the intestinal absorption of CHS-Na is responsible for the increase in the excretion rate of CHS-Na. In order to examine the effect of phenylbutazone on the intestinal absorption of CHS-Na, the *in situ* small intestinal absorption of CHS-Na administered together with phenylbutazone was compared with that of single administration at pH 6.7 in rabbits, using an intestinal recirculating perfusion method. As shown in Table II, it is apparent that there is no significant difference between single and simultaneous administration, although these absorbed percentages are small. These data indicated that phenylbutazone does not accelerate the intestinal absorption of CHS-Na in rabbits.

TABLE II. Effect of Phenylbutazone on the Absorption of CHS-Na from Rabbit Small Intestine, *in Situ*

Time	Exp. No.	Percentage absrobed	
		Single administration[a]	Simultaneous administration[b]
1 hr	1	2.3	4.2
	2	2.3	4.8
	3	5.5	
	mean±SD[c]	3.4±1.5	4.5±0.3[d]
2 hr	1	9.8	8.0
	2	12.5	8.9
	3	6.4	
	mean±SD	9.6±2.5	8.5±0.5[d]

initial concentration
a) CHS-Na: 4.0×10^{-3}M
b) CHS-Na: 4.0×10^{-3}M+phenylbutazone: 2.0×10^{-3}M
c) SD: standard deviation
d) non-significant difference to single administration, $p>0.05$

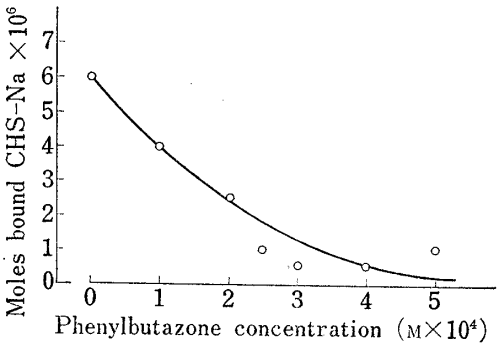

Fig. 3. Inhibitory Effect of Phenylbutazone on the Binding of CHS-Na with BSA

initial concentration of CHS-Na: 4.0×10^{-3}M, albumin concentration: 0.8 (w/v) %, 0.1M phosphate buffer at pH 7.4

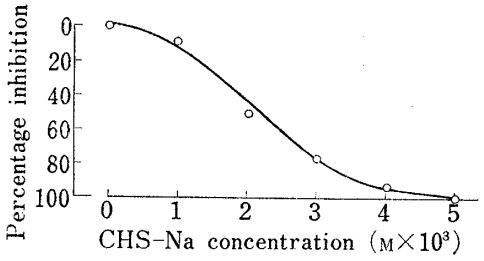

Fig. 4. Inhibitory Effect of CHS-Na on Heat Denaturation of BSA

final concentration of BSA: 0.5 (w/v) %, The samples were heated at 67° for 3 min.

9) S. Kojima, H. Ichibagase, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 965 (1966).
10) P.W. Ragozzino and M.H. Malone, *J. Pharmacol. Exptl. Therap.*, **141**, 363 (1963).

Effect of Phenylbutazone on Binding between CHS-Na and BSA

It has been known that many drugs are bound to serum proteins, and that the interactions are inhibited by other drugs.[11,12] As can be understood from the association constants of CHS-Na[13] and phenylbutazone[5] to serum albumin, the strength of binding between CHS-Na and serum albumin is weak as compared with that between phenylbutazone and serum albumin. Accordingly, it may be considered that the binding of CHS-Na with BSA can be inhibited by phenylbutazone. In order to solve this problem, an investigation was carried out by using the equilibrium dialysis method. As shown in Fig. 3, the extent of binding of CHS-Na with BSA decreased gradually in accordance with an increase in the concentration of phenylbutazone. These results evidently indicate that the binding of CHS-Na with BSA is inhibited by the coexistence of phenylbutazone. Furthermore, the extent of binding of CHS-Na with BSA and rabbit serum albumin (RSA) was examined by the equilibrium dialysis method. As shown in Table III, there was no evident difference between BSA and RSA.

Table III. Binding of CHS-Na with BSA and RSA

Serum albumin	Amount of CHS-Na bound (mole)
BSA	1.26×10^{-5}
RSA	0.85×10^{-5}

initial concentration of CHS-Na: 8.0×10^{-3}M, albumin concentration: 1.0 (w/v) %, 1/15M phosphate buffer at pH 7.4

Accordingly, the results obtained above demonstrate that the increase in the urinary excretion rate of CHS-Na in the rabbits following oral administration of CHS-Na together with phenylbutazone is attributed to the competitive inhibitory effect of phenylbutazone to the binding between CHS-Na and RSA.

To clarify futher the binding properties of CHS-Na and phenylbutazone with BSA, the inhibitory effects of CHS-Na and phenylbutazone on the heat denaturation of BSA were investigated according to the method reported by Mizushima.[7,8] As can be seen from Table IV and Fig. 4, CHS-Na inhibited the heat denaturation of BSA, although the inhibitory effect of CHS-Na on the heat denaturation of BSA was small as compared with that of phenylbutazone. In the previous paper,[4] also, the authors reported that the extent of binding between CHS-Na and N-acetyl BSA distinctly decreased as compared with that observed between CHS-Na and BSA. And Skidmore, *et al.*[14] have been recently reported that the heat denaturation of N-acetyl BSA and trinitrophenyl BSA is not inhibited by phenylbutazone. Thus, these data suggest that the amino groups in BSA participate in CHS-Na or phenylbutazone binding.

Table IV. Inhibitory Effect of Drug on Heat Denaturation of BSA

Drugs	Percentage inhibition
CHS-Na	9
Phenylbutazone	100
Sodium salicylate	55
Sodium *n*-caprylate	100

final concentration of drugs: 1.0×10^{-3}M, final concentration of BSA: 0.5 (w/v) %, The samples were heated at 67° for 3 min.

11) A.H. Anton, *J. Pharmacol. Exptl. Therap.*, **129**, 282 (1960).
12) P.M. Keen, *Brit. J. Pharmacol. Chemotherapy.*, **26**, 704 (1966).
13) The association constant of CHS-Na to BSA, 0.46×10^{3} (pH 7.5) was calculated from the data in the previous paper; S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 1619 (1968).
14) I. F. Skidmore and M.W. Whitehouse, *J. Pharm. Pharmacol.*, **17**, 671 (1961).

Futhermore, Bluestone, *et al.*[15] reported that the hydrophobic groups in BSA participate in the binding between BSA and phenylbutazone. It has been also known that fatty acids and aliphatic sulfates inhibit the heat denaturation of BSA, and that the inhibitory effect of those compounds is correlated to the length of aliphatic chains of those compounds.[16-18] Since CHS-Na is regarded as an unique aliphatic sulfate with cyclohexane ring, it is presumed that the hydrophobic groups in BSA also participate in CHS-Na binding.

From the results described above, it is presumed that CHS-Na and phenylbutazone are bound at the same sites in BSA molecule, and that since the strength of binding of CHS-Na with BSA is weak as compared with that of CHS-Na with phenylbutazone, the binding of CHS-Na with BSA is inhibited by phenylbutazone. Accordingly, it may be considered that the urinary excretion of CHS-Na in rabbits is accelerated by simultaneous administration of phenylbutazone.

15) R. Bluestone, I. Kippen, and J.R. Klinenberg, *J. Lab. Clin. Med.*, **76**, 85 (1970).
16) P.D. Boyer, G.A. Bollou, and J.M. Luck, *J. Biol. Chem.*, **167**, 407 (1947).
17) J.D. Teresi and J.M. Luck, *J. Biol. Chem.*, **194**, 823 (1952).
18) F. Karush and M. Sonenberg, *J. Am. Chem. Soc.*, **71**, 1369 (1949).