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Effect of Simultaneous Administration of Drugs on Absorption and Excretion.
XIII.¹⁾ Effect of Salicylic Acid on the Absorption, Distribution and
Elimination of Carbutamide in Rabbits²⁾

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Salicylic acid reduced the *in situ* intestinal absorption of carbutamide, and enhanced the *in situ* intestinal exsorption of carbutamide. Salicylic acid, however, did not affect the *in vitro* intestinal absorption of carbutamide. These findings indicate that the displacement of carbutamide from its plasma protein binding sites by salicylic acid can become an important factor affecting the intestinal absorption of carbutamide in intact rabbits.

Salicylic acid caused a significant increase in the apparent volume of distribution ($V_{d\beta}$) and in the total body clearance (Cl_T) of carbutamide. In addition, salicylic acid was found to significantly increase the distribution of carbutamide into the pancreas and into the kidney. These findings may be explained on the basis of the displacement of carbutamide from its plasma protein binding sites by salicylic acid.

Keywords—carbutamide; salicylic acid; plasma protein binding; intestinal absorption; apparent volume of distribution; total body clearance; tissue distribution; rabbit

It is well known that plasma protein binding of a drug is an important factor affecting the pharmacokinetics of the drug. For example, Scholtan³⁾ reported a relationship between the degree of plasma protein binding and the tissue distribution of sulfonamides. Anton *et al.*⁴⁾ demonstrated that the displacement of sulfamethoxypyridazine from its plasma protein binding sites by sulfapyrazone causes a significant increase in the tissue distribution of sulfamethoxypyridazine.

In the preceding paper,¹⁾ we presented evidence that salicylic acid markedly altered the hypoglycemic activity and the blood carbutamide concentration after oral administration of carbutamide, by displacing carbutamide from its plasma protein binding sites. The purpose of the present study was to elucidate whether salicylic acid has any effect on the absorption, distribution and elimination of carbutamide in rabbits.

Experimental

Materials—Carbutamide was kindly supplied by Ono Pharmaceutical Industry Co., Ltd. Salicylic acid and other chemicals were obtained commercially.

Animals—Male rabbits weighing 2.5–3.5 kg were fasted for 38–42 h prior to all experiments, but drinking water was allowed *ad libitum*.

***In Situ* Intestinal Absorption and Exsorption Experimental Methods**—*In situ* intestinal absorption experiments were carried out by the method of Goto *et al.*⁵⁾ *In situ* intestinal exsorption experiments were carried out as described previously.⁶⁾

***In Vitro* Intestinal Absorption Experimental Method**—A modified McElnay-type apparatus⁷⁾ was employed for *in vitro* intestinal absorption experiments (Fig. 1). A male rabbit was exsanguinated from the carotid artery and the small intestine (jejunum) was removed carefully. After removal of the small intestine it was placed in a petri dish containing saline solution and everted. The top and end of the intestinal segment were tied on the upper and lower projections of the apparatus with ligatures, respectively. The length of the small intestine used was 11 cm. The chamber was filled with 70 ml of drug solution which was prepared by dissolving carbutamide (500 $\mu\text{g/ml}$) with or without sodium salicylate (500 $\mu\text{g/ml}$ as salicylic acid) in Krebs–Ringer phosphate buffer solution (pH 7.4), and the everted small intestine was filled with 7 ml of Krebs–Ringer phosphate buffer solution (pH 7.4). The apparatus was then kept in a water-bath at 37°C.

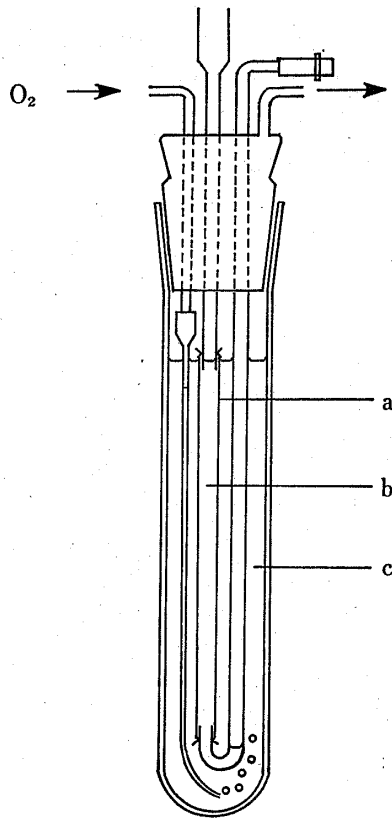


Fig. 1. A Modified McElnay-Type Apparatus for *in Vitro* Intestinal Absorption Experiments

a) everted small intestine, b) buffer solution, c) drug solution.

A 0.1 ml aliquot of the serosal solution was collected and used for the measurement of carbutamide concentration.

In Vivo Experimental Methods—a) Intravenous Injection: Carbutamide (50 mg/kg) alone or in combination with salicylic acid (100 mg/kg) was dissolved in 1–2 ml of saline solution by adding the same molar amount of NaOH and the solution was injected intravenously.

b) Blood Sampling: Blood samples were collected periodically from the ear vein.

Analytical Methods—The carbutamide concentrations in tissues were measured by the Felling–Westheimer method.⁸⁾ The carbutamide concentrations in blood, perfusate and serosal solution were measured by the Bratton–Marshall method.⁹⁾

Statistical Analysis—Statistical analyses were performed by the paired Student *t*-test or by the non-paired Student *t*-test. A *p*-value of 0.05 or less was considered significant.

Results and Discussion

Figure 2 and Table I show the effect of salicylic acid on the *in situ* and *in vitro* intestinal absorption of carbutamide, respectively. In the *in situ* intestinal absorption experiment, salicylic acid evidently increased the amount of carbutamide remaining in the perfusate. In the *in vitro* intestinal absorption experiment, however, salicylic acid had no effect on the transfer of carbutamide from the mucosal to serosal solution. In order to elucidate further the mechanism of carbutamide and salicylic acid interaction during intestinal absorption, the effect of salicylic acid on the *in situ* intestinal exsorption of carbutamide was investigated. As shown in Fig. 3, salicylic acid increased the exsorption rate of carbutamide into the perfusate. All the above results suggest that salicylic acid may reduce the intestinal absorption of carbutamide

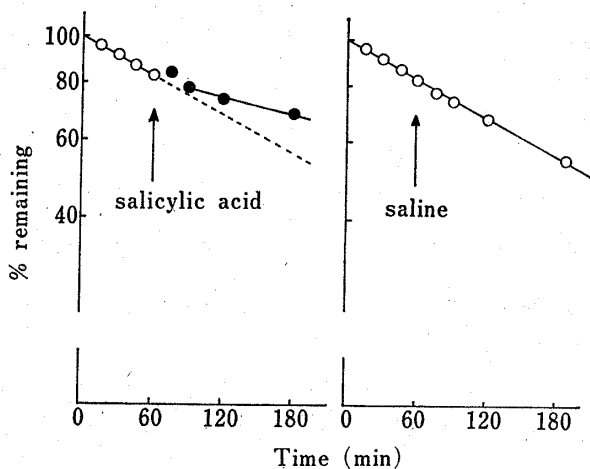


Fig. 2. Effect of Salicylic Acid on the *in Situ* Intestinal Absorption of Carbutamide

Salicylic acid and saline were injected into the ear vein. This figure shows typical data.

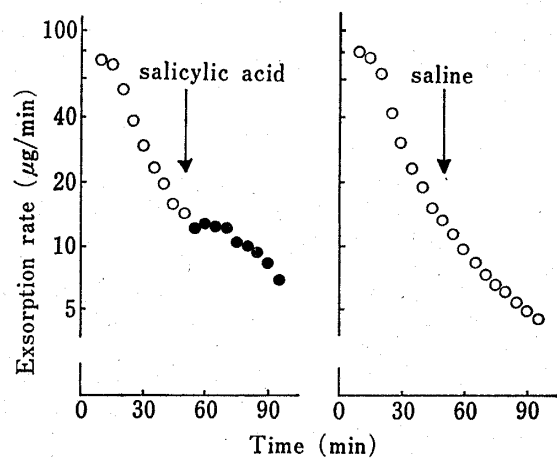


Fig. 3. Effect of Salicylic Acid on the *in Situ* Intestinal Exsorption of Carbutamide

Salicylic acid and saline were injected into the ear vein. This figure shows typical data.

TABLE I. Effect of Salicylic Acid on the *in Vitro* Intestinal Absorption of Carbutamide

Time (h)	Carbutamide transferred (mg)	
	Carbutamide alone	With salicylic acid
0—0.5	0.40 ± 0.05	0.41 ± 0.06
0—1.0	0.77 ± 0.10	0.76 ± 0.11

Values represent the means ± S.E. of 5 rabbits.

in intact rabbits without changing the permeability characteristics of the intestinal membrane.

Our preceding paper¹⁾ showed that salicylic acid strongly displaces carbutamide from its plasma protein binding sites. This implies that salicylic acid affects the concentration gradient of unbound carbutamide across the intestinal membrane. Therefore, it is concluded that the displacement of carbutamide from its plasma protein binding sites by salicylic acid is responsible for the reduced intestinal absorption of carbutamide. The finding that salicylic acid reduces the intestinal absorption of carbutamide may also be discussed from the point of view that salicylic acid increases the distribution of carbutamide into the intestinal tract.

Figure 4 shows the time course of blood carbutamide concentration after intravenous bolus injection of carbutamide alone or in combination with salicylic acid. At early sampling times, the blood carbutamide concentrations were significantly decreased by salicylic acid. Since the plot of blood carbutamide concentration (C) versus time (t) declined biexponentially, the individual data were fitted to the following equation by nonlinear least-squares regression.¹⁰⁾

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

The total body clearance (Cl_T), elimination half-life ($T_{1/2(\beta)}$) and apparent volume of distribution ($V_{d\beta}$) of carbutamide were calculated by means of the following equation using biexponential equation constants,¹¹⁾

$$Cl_T = D/AUC \quad (2)$$

$$T_{1/2(\beta)} = 0.693/\beta \quad (3)$$

$$V_{d\beta} = Cl_T/\beta \quad (4)$$

TABLE II. Pharmacokinetic Parameters of Carbutamide

Rabbit	A μg/ml	α h ⁻¹	B μg/ml	β h ⁻¹	$T_{1/2(\beta)}$ h	$V_{d\beta}$ l/kg	Cl_T l/h/kg
Carbutamide alone							
A	96.2	2.88	50.6	0.90	0.77	0.62	0.56
B	142.3	9.06	159.9	0.78	0.88	0.29	0.23
C	102.5	9.07	99.0	0.95	0.73	0.45	0.43
D	166.4	5.11	75.1	0.92	0.75	0.48	0.44
E	108.2	19.07	139.2	1.22	0.57	0.36	0.44
Mean	123.1	9.04	103.5	0.95	0.74	0.44	0.42
S.E.	13.4	2.77	19.6	0.07	0.05	0.06	0.05
With salicylic acid							
A	70.5	3.31	52.4	1.05	0.66	0.67	0.70
B	157.7	9.50	117.2	1.03	0.67	0.36	0.38
C	54.6	9.16	73.3	1.03	0.67	0.63	0.65
D	146.0	7.35	46.4	0.90	0.77	0.78	0.70
E	87.4	8.99	89.4	1.28	0.54	0.49	0.63
Mean	103.2	7.66	75.7	1.06	0.66	0.59 ^{a)}	0.61 ^{b)}
S.E.	20.6	1.15	12.9	0.06	0.04	0.07	0.06

a) Significantly different from carbutamide alone, $p < 0.05$.

b) Significantly different from carbutamide alone, $p < 0.001$.

where D is the dose and AUC is the area under the blood carbutamide concentration *versus* time curve. The results are summarized in Table II. Salicylic acid caused a significant increase in $V_{d\beta}$ and Cl_T of carbutamide. Salicylic acid, however, caused no significant change in $T_{1/2(\beta)}$ of carbutamide.

Gibaldi *et al.*¹²⁾ proposed the following relationship,

$$V_{d\beta} = V_p + (f_p/f_t)V_t \quad (5)$$

where V_p is the volume of plasma, V_t is the volume of tissues, and f_p and f_t are the fractions of unbound drug in plasma and tissues, respectively. Equation 5 implies that $V_{d\beta}$ is dependent on both plasma and tissue binding. Colburn *et al.*,¹³⁾ for the case when a drug is essentially eliminated by hepatic metabolism and is not limited by liver blood flow, proposed the following relationship,

$$Cl_T = f_p Cl_I \quad (6)$$

where Cl_I is the intrinsic clearance of unbound drug. Equation 6 implies that Cl_T is dependent on plasma binding and hepatic metabolism. Recently, Frigo *et al.*¹⁴⁾ and O'Leary *et al.*¹⁵⁾ have

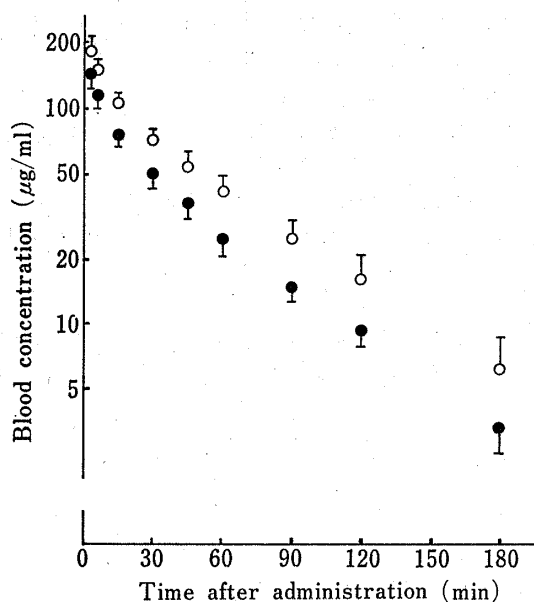


Fig. 4. Time Course of Blood Carbutamide Concentration after Intravenous Bolus Injection of Carbutamide alone or in Combination with Salicylic Acid

Each point represents the mean \pm S.E. of 5 rabbits. At the following sampling times, a significant difference was observed (3 and 30 min, $p < 0.005$; 6 min, $p < 0.01$; 15, 45 and 60 min, $p < 0.05$). —○—, carbutamide alone; —●—, with salicylic acid.

pointed out that the displacement of phenytoin from its plasma protein binding sites by valproic acid can become an important factor increasing $V_{d\beta}$ and Cl_T of phenytoin. Thus, the fact that salicylic acid increases $V_{d\beta}$ and Cl_T of carbutamide may be explained by the displacement of carbutamide from its plasma protein binding sites by salicylic acid, although the effect of salicylic acid on the tissue binding and hepatic metabolism of carbutamide should also be considered.

Table III shows the distribution of carbutamide into the tissues 2.0 h after oral administration of carbutamide alone or in combination with salicylic acid. Salicylic acid significantly increased the distribution of carbutamide into the pancreas and into the kidney. This finding may also be explained by the displacement of carbutamide from its plasma protein binding sites by salicylic acid. Salicylic acid had no significant effect on the distribution of carbutamide into the liver. In rats, however, salicylic acid was found to increase the distribution of warfarin¹⁶⁾ and sulfamethoxazole¹⁷⁾ into the liver. Consequently, a

TABLE III. Effect of Salicylic Acid on the Distribution of Carbutamide into the Tissues

Tissue	$\mu\text{g/g}$ wet weight	
	Carbutamide alone	With salicylic acid
Liver	0.9 ± 0.2	1.0 ± 0.2
Pancreas	4.1 ± 0.4	$7.0 \pm 1.0^a)$
Kidney	16.5 ± 2.3	$23.5 \pm 2.5^a)$

Values represent the means \pm S.E. of 5 rabbits.

a) Significantly different from carbutamide alone, $p > 0.05$.

significant effect of salicylic acid on the distribution of carbutamide into the liver may be observed upon changing the dose or sampling time.

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