Factors influencing Absorption and Excretion of Drugs. III.[1] Effect of Fasting on Absorption and Excretion of Sodium Salicylate and Aspirin in Rabbits

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The effect of fasting on the oral absorption and excretion of sodium salicylate and aspirin was studied in rabbits. The attainment of the maximum blood levels of those drugs in the fasted rabbits was somewhat slower than in the nonfasted rabbits. However, the extents of absorption of those drugs were the same in the fasted rabbits as in the nonfasted rabbits. The elimination rates of sodium salicylate and aspirin in the nonfasted rabbits were markedly enhanced by the increase in the urine pH owing to the food, resulting in the decrease in the blood levels of those drugs in the nonfasted rabbits. Also, the absorption rate constant for sodium salicylate in the nonfasted rabbits, in which the elimination rate constant was almost the same with that in the fasted rabbits, was significantly greater than that in the fasted rabbits. The percent of sodium salicylate remaining in the stomach after oral administration of the drug was significantly greater in the fasted rabbits. Therefore, it is concluded that the rates of stomach emptying of sodium salicylate and aspirin in the fasted rabbit are much slower than in the nonfasted rabbit, resulting in a decrease in the gastrointestinal absorption of those drugs.

It has been generally known that the presence of food in the gastrointestinal tract influences significantly the rate or extent of oral absorption of drugs on the basis of decreased diffusion rate of drugs to the mucosal absorption surface, decreased dissolution rate of solid dosage forms, or delayed gastric emptying for drugs absorbed primarily in the small intestine. A number of examples of the effect of food on the oral absorption of various drugs such as penicillin derivatives,[3,4] various tetracycline antibiotics,[5] erythromycin,[6] sulfonamides,[7] barbiturates,[8,9] etc. can be found in the literatures. In contrast to these studies, some very interesting findings have been reported of cases in which certain foods preceding administration of the drugs such as riboflavin,[10] riboflavin-5'-phosphate,[11] griseofulvin,[12] and clindamycin palmitate[13] enhance the absorption of those drugs from the gastrointestinal tract.

Although there are a number of investigations concerning with the characteristics of absorption and excretion of salicylic acid derivatives, however, little work has been done on the differences of absorption and excretion between fasted and nonfasted states. The present study reports the effect of fasting on the rates of absorption, extents of absorption, and rates of excretion of sodium salicylate and aspirin when administered orally to rabbits.

1) Part II: S. Kojima, T. Tenmizu, T. Shino, and M. Cho, *Chem. Pharm. Bull.* (Tokyo), **22**, 952 (1974).
2) Location: *5-1 Oehon-machi, Kumamoto.*
3) J.T. Doluisio, J.C. LaPiana, G.R. Wilkinson, and L.W. Dittert, *Antimicrob. Ag. Chemother.,* **1969,** 49.
4) J.O. Klein and M. Finland, *New England J. Med.,* **269,** 1019 (1963).
5) W.M.M.Kirby, C.E. Roberts, and R.E. Bardick, *Antimicrob. Ag. Chemother.,* **1961,** 286.
6) H.A. Hirsch and M. Finland, *Amer. J. Med. Sci.,* **237,** 693 (1959).
7) H. MacDonald, V.A. Place, H. Falk, and M.A. Darken, *Chemotherapia,* **12,** 282 (1967).
8) S. Kojima, R.B. Smith, and J.T. Doluisio, *J. Pharm. Sci.,* **60,** 1639 (1971).
9) S. Kojima, *Chem. Pharm. Bull.* (Tokyo), **21,** 2432 (1973).
10) G. Levy and W.J. Jusko, *J. Pharm. Sci.,* **55,** 285 (1966).
11) W.J. Jusko and G. Levy, *J. Pharm. Sci.,* **56,** 58 (1967).
12) R.R. Crounse, *J. Invest. Dermatol.,* **37,** 529 (1961).
13) R.M. Dehaan, W.D. Vandenbosch, and C.M. Metzler, *J. Clin. Pharmacol.,* **12,** 205 (1972).

Experimental

Materials and Equipment——Sodium salicylate and aspirin were of JP VIII grade. Other chemicals were of reagent grade.

A Shimadzu QV-50 spectrophotometer and a Hitachi-Horiba F-5 pH meter were utilized.

Test Animals——Male rabbits weighing between 2.4 and 3.4 kg were used. The rabbits were fasted 24 hr prior to use, but drinking water was allowed *ad libitum*. For studies on the effect of food, the rabbits were allowed food[14] for about 18 hr after fasting 24 hr. The rabbits consumed about 60—80 g food/kg body weight.

Administration of Drugs——Sodium salicylate (100 mg/kg) and aspirin (100 mg/kg) were administered to rabbit orally by a stomach tube in the forms of solution and suspension in about 80 ml of water, respectively. Also, a single intravenous injection of 60 mg/kg of sodium salicylate was given in the form of 6% (w/v) aqueous solution through ear-vein of rabbit.

Collections of Blood and Urine Specimens——After oral administration of each drug to rabbit secured on its back on an animal board, 0.5 ml of blood specimen was drawn from ear-vein at periodic intervals. Urine specimens were collected by using a catheter every 1 hr up to 8 hr after administration of each drug and then in the flask up to 24 hr.

Analytical Procedures——A. Total Salicylate in Blood and Urine: Total salicylate was determined by hydrolyzing salicyluric acid and salicyl glucuronides to salicylic acid according to a minor modification of the Kakemi, *et al.*[15] modification of the method of Brodie, *et al.*[16] To 0.5 ml of blood brought to 1.5 ml with water or 1.5 ml of appropriately diluted urine was added 1.0 ml of concentrated HCl. The mixture was heated in a boiling-water bath for 1 hr and again heated for 15 min after adding 1.5 ml of 40% NaOH. The resulting hydrolysate was acidified by adding 1.0 ml of concentrated HCl and extracted with 5 ml of $CHCl_3$. Four milliliters of the organic solvent phase was then re-extracted with 5 ml of ferric nitrate reagent (1% $Fe(NO_3)_3$ in 0.07N HNO_3). The absorbance of the ferric nitrate reagent phase was determined at 530 nm.

B. Free Salicylic Acid and Salicyluric Acid in Urine: Free salicylic acid and salicyluric acid in the urine of rabbit receiving sodium salicylate were determined according to the method of Levy, *et al.*[17]

C. Determination of Salicylic Acid remaining in Rabbit Stomach after Oral Administration of Sodium Salicylate: At 30 min after oral administration of sodium salicylate (100 mg/kg) to fasted and nonfasted rabbits, a stomach tube (Nelaton's catheter, No. 12) was inserted into rabbit stomach and the stomach content was withdrawn by suction with a syringe. To remove any residual stomach content, about 100 ml of warm water was instilled into the stomach through the stomach tube and the fluid was withdrawn. This procedure was repeated about 10 times in order to remove the stomach content as completely as possible. The combined washings were neutralized with 10N NaOH and centrifuged. After removing the supernatant fluid, the precipitate was washed out with water several times and the clear washings were combined to the supernatant fluid obtained above. The resulting solution was submitted to the determination of salicylic acid. Salicylic acid was extracted from the sample solution acidified with concentrated HCl with $CHCl_3$. The $CHCl_3$ phase was then extracted with the ferric nitrate reagent described above and the absorbance of the reagent phase was measured at 530 nm. When sodium salicylate was added to the stomach content obtained from control rabbit, the analytical method described above gave the recoveries of 90 to 93%.

Result and Discussion

In order to investigate the effect of fasting on the oral absorption and excretion of sodium salicylate, a single dose of 100 mg/kg of sodium salicylate was administered orally to fasted and nonfasted rabbits. As shown in Fig. 1, the blood level of the drug significantly changed between the fasted and nonfasted rabbits. The blood level curves demonstrated that the attainment of the maximum blood level of the drug in the fasted rabbits was somewhat slower than in the nonfasted rabbits. However, the blood level of total salicylate in the fasted rabbits was considerably greater than that in the nonfasted rabbits. Similarly, a single dose of 100 mg/kg of aspirin was administered orally to the fasted and nonfasted rabbits.

14) "ORC-5", Oriental Yeast Industry Co., Ltd. was used. This food consisted of moisture (7.0%), crude protein (22.1%), crude fat (3.6%), crude fiber (11.8%), crude ash (7.8%), and nitrogen free extract (47.7%).
15) K. Kakemi, T. Arita, H. Yamashina, and R. Konishi, *Arch. Pract. Pharm.*, **21**, 103 (1961).
16) B.B. Brodie, S. Udenfriend, and A.F. Coburn, *J. Pharmacol. Exptl. Therap.*, **80**, 114 (1944).
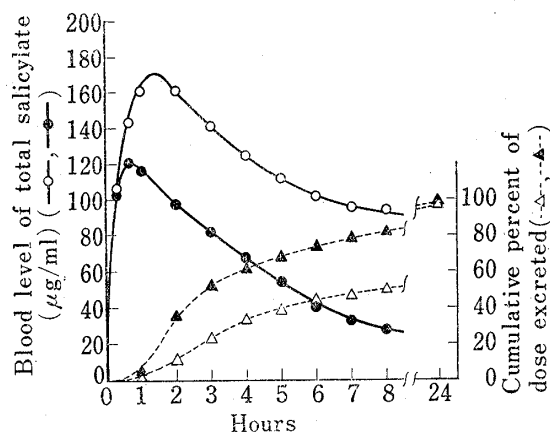17) G. Levy and J.A. Procknal, *J. Pharm. Sci.*, **57**, 1330 (1968).

Fig. 1. Mean Blood Levels and Mean Cumulative Urinary Recoveries of Total Salicylate following Oral Administration of Sodium Salicylate (100 mg/kg) in Fasted and Nonfasted Rabbits

The values represent the mean of 3 experiments.
—○—, ---△---: fasted rabbits
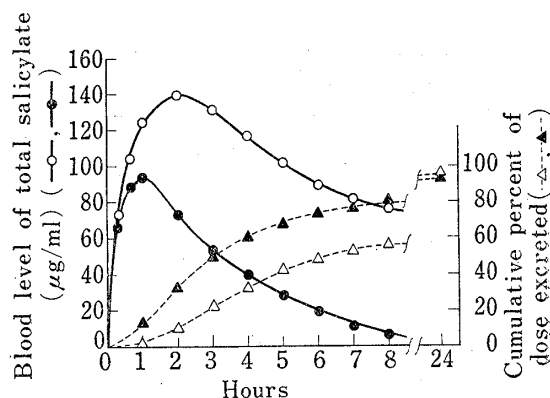—●—, ---▲---: nonfasted rabbits

Fig. 2. Mean Blood Levels and Mean Cumulative Urinary Recoveries of Total Salicylate following Oral Administration of Aspirin (100 mg/kg) in Fasted and Nonfasted Rabbits

The values represent the mean of 3 experiments.
—○—, ---△---: fasted rabbits
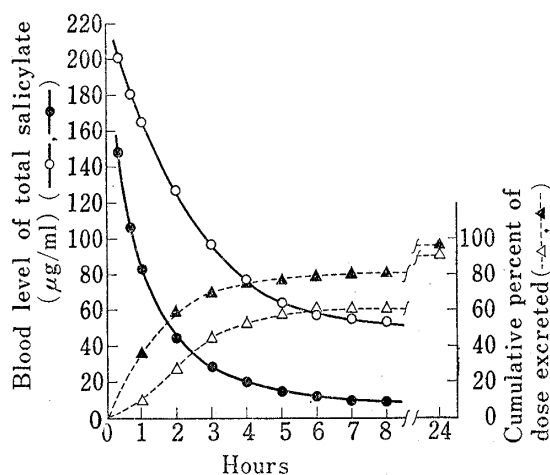—●—, ---▲---: nonfasted rabbits

Fig. 3. Mean Blood Levels and Mean Cumulative Urinary Recoveries of Total Salicylate following Intravenous Injection of Sodium Salicylate (60 mg/kg) in Fasted and Nonfasted Rabbits

The values represent the mean of 3 experiments.
—○—, ---△---: fasted rabbits
—●—, ---▲---: nonfasted rabbits

As indicated in Fig. 2, the presence of food in the gastrointestinal tract hastened the attainment and decreased the magnitude of the maximum blood level of aspirin as well as the case of sodium salicylate.

The elimination rate constant of each drug was calculated from the slope of the line on the semilogarithmic plots of blood level vs. time. As can be seen from Table I, sodium salicylate and aspirin in the nonfasted rabbits were eliminated 2 to 2.5 times more rapidly than those in the fasted rabbits. In order to confirm such a phenomenon, moreover, a single dose of 60 mg/kg of sodium salicylate was administered intravenously to the fasted and nonfasted rabbits. The result showed that the elimination rate of the drug in the nonfasted rabbits was much faster than that in the fasted rabbits (see Fig. 3 and Table I). It is demonstrated from the above results that food preceding oral administration of sodium salicylate and aspirin enhances the elimination of those drugs from rabbit body.

In order to clarify further the effect of food on the elimination of sodium salicylate and aspirin, it was considered desirable to determine whether food increases the formation rate of salicyluric acid, as a metabolite of those drugs, and therefore presumably increases the overall rate of salicylate elimination from rabbit body. After oral administration of sodium salicylate to the fasted and nonfasted rabbits, free salicylic acid and salicyluric acid in the urines were determined separately. As shown in Fig. 4, a large proportion of sodium salicylate was excreted unchanged, and food had little effect on the formation rate and urinary excretion rate of salicyluric acid. Furthermore, the pH of the urines in the fasted and nonfasted rabbits following oral administration of sodium salicylate and aspirin was measured every 1 hr during

TABLE I.   Elimination Rate Constants for Sodium Salicylate
and Aspirin in Fasted and Nonfasted Rabbits

		Dose (mg/kg)	Elimination rate constant[a] (hr^{-1})
Sodium salicylate (*p.o.*)	fasting	100	0.104±0.002 (3)
	nonfasting	100	0.220±0.021 (3)
Sodium salicylate (*i.v.*)	fasting	60	0.273±0.063 (3)
	nonfasting	60	0.739±0.042 (3)
Aspirin (*p.o.*)	fasting	100	0.114±0.007 (3)
	nonfasting	100	0.295±0.020 (3)

a) The value is expressed as the mean±standard deviation with the number of experiments in parentheses.

an 8 hr period. As shown in Fig. 5, the pH values of the urines in the nonfasted rabbits were significantly larger than those in the fasted rabbits. It has been reported[18] that salicylate can be secreted and reabsorbed by the renal tubules, as well as filtered at the glomerulus.   Tubular reabsorption predominates when the urine is acid and alkalinization of the urine causes a marked increase in the clearance of salicylate by diminishing reabsorption of salicylate by the tubules.[18,19] Thus, it is demonstrated that the increase in the urine pH in the nonfasted rabbits is presumably attributable to the food given to the animals in this investigation and, consequently, that the higher concentration of ionized salicylate is unfavorable to reabsorption from the tubular fluid which results in a shorter biological half-life for salicylate in the nonfasted rabbits receiving sodium salicylate or aspirin.

In addition, it is evident from Figs. 1 and 2 that the cumulative urinary recoveries in a 24 hr period of sodium salicylate and aspirin scarcely change between the fasted and nonfasted rabbits and, thereby, that the extents of absorption of those drugs are the same in the nonfasted rabbits
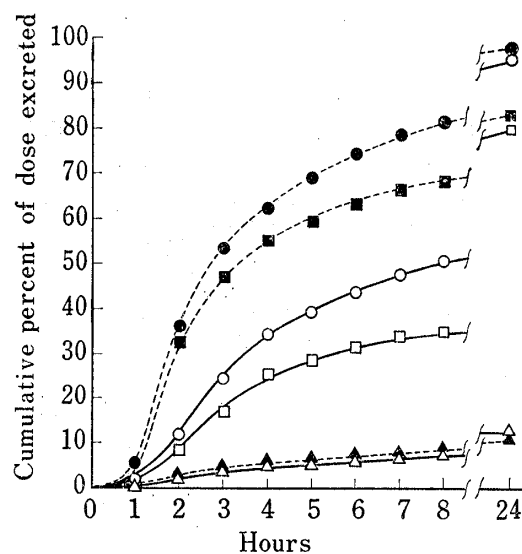

Fig. 4.   Cumulative Urinary Recoveries of Free Salicylic Acid, Salicyluric Acid and Total Salicylate following Oral Administration of Sodium Salicylate (100 mg/kg) in Fasted and Nonfasted Rabbits

The values represent the mean of 3 experiments.
—□—: free salicylic acid
—△—: salicyluric acid   } fasted rabbits
—○—: total salicylate
---■---: free salicylic acid
---▲---: salicyluric acid   } nonfasted rabbits
---●---: total salicylate

as in the fasted rabbits.   Accordingly it is suggested that the decrease in the blood levels of sodium salicylate and aspirin in the nonfasted rabbits may be due to the enhanced elimination of those drugs.

To understand in detail the gastrointestinal absorption of sodium salicylate in the presence of food in the gastrointestinal tract, further investigations were carried out as follows.   In order to equalize approximately the elimination rate in the nonfasted rabbits to that in the fasted rabbits, ammonium chloride (500—700 mg/kg) was given orally to assure acidity of the urine in the nonfasted rabbits.   After the urine pH in the nonfasted rabbits fell to about 7,

18) D. Schachter and J.G. Manis, *J. Clin. Invest.*, **38**, 800 (1958).
19) P.K. Smith, H.L. Gleason, C.G. Stoll, and S. Ogorzalek, *J. Pharmacol. Exptl. Therap.*, **87**, 237 (1946); D. Lester, G. Lolli, and L.A. Greenberger, *J. Pharmacol. Exptl. Therap.*, **87**, 329 (1946); A.B. Gutman, T.F. Yu, and J.H. Sirota, *J. Clin. Invest.*, **34**, 711 (1955); I.M. Weiner, J.A. Washington, and G.H. Mudge, *Bull. Johns Hopkins Hosp.*, **105**, 284 (1959).
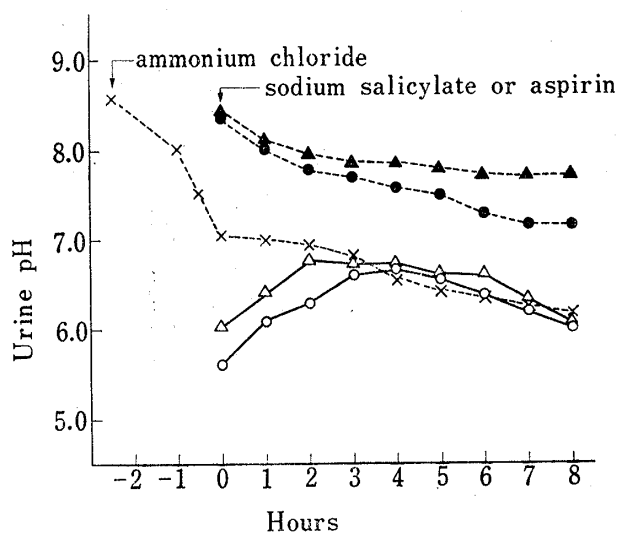
Fig. 5. Changes in Urine pH in Fasted and Non-
fasted Rabbits following Oral Administration
of Sodium Salicylate or Aspirin

The values represent the mean of 3 experiments.
sodium salicylate —○—: fasted rabbits
　　　　　　　　—●—: nonfasted rabbits
　　　　　　　　---×---: nonfasted rabbits pretreated
　　　　　　　　　　　 with ammonium chloride
aspirin　　　　—△—: fasted rabbits
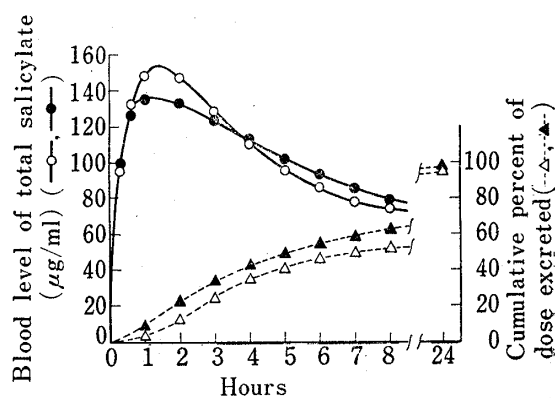　　　　　　　　---▲---: nonfasted rabbits

Fig. 6. Mean Blood Levels and Mean Cumu-
lative Urinary Recoveries of Total Salicylate
following Oral Administration of Sodium
Salicylate (100 mg/kg) in Fasted Rabbits
and Nonfasted Rabbits pretreated with
Ammonium Chloride (500—700 mg/kg)

The values represent the mean of 3 experiments.
—○—, ---△---: fasted rabbits
—●—, ---▲---: nonfasted rabbits pretreated with
　　　　　　　 ammonium chloride

TABLE II. Absorption and Elimination Rate Constants for Sodium Salicylate following
Oral Administration of the Drug in Fasted Rabbits and Nonfasted Rabbits
pretreated with Ammonium Chloride (500—700 mg/kg)

	Dose (mg/kg)	Elimination rate constant[a] (hr^{-1})	Absorption rate constant[a] (hr^{-1})
Fasting	100	0.130 ± 0.035 (3)[b]	2.263 ± 0.034 (3)[c]
Nonfasting	100	0.097 ± 0.023 (3)	2.946 ± 0.166 (3)

a) The value is expressed as the mean ± standard deviation with the number of experiments in parentheses.
b) not significantly different from nonfasting, $p > 0.05$
c) significantly different from nonfasting, $p < 0.01$

TABLE III. Percent of Sodium Salicylate Remaining in the Stomach
following Oral Administration of the Drug in Fasted
and Nonfasted Rabbits

	Dose (mg/kg)	Percent remaining in Stomach[a]
Fasting	100	49.0 ± 6.9 (4)[b]
Nonfasting	100	34.1 ± 6.7 (4)

Sodium salicylate remaining in rabbit stomach was measured at 30 min after oral admini-
stration of the drug.
a) The value indicates the mean ± standard deviation with the number of experiments in
parentheses.
b) significantly different from nonfasting, $p < 0.05$

a single dose of 100 mg/kg of sodium salicylate was administered orally to the rabbits. As
shown in Fig. 5, the urine pH in the nonfasted rabbits pretreated with ammonium chloride
was somewhat higher up to about 3 hr after oral administration of sodium salicylate and

thereafter almost the same with compared to that in the fasted rabbits. The blood level and cumulative urinary excretion curves of total salicylate in the nonfasted rabbits pretreated with ammonium chloride were shown in Fig. 6. The elimination rate constants of sodium salicylate calculated from the slope of the line on the semilogarithmic plots of blood level vs. time and the absorption rate constants for the drug obtained by the method of Wagner and Nelson[20] using the blood level data shown in Fig. 6 were given in Table II. The results indicated that the elimination rate constant for sodium salicylate did not show a significant difference between the nonfasted rabbits pretreated with ammonium chloride and fasted animals but that the absorption rate constant for the drug was significantly smaller in the fasted rabbits. Moreover, the percent of sodium salicylate remaining in the stomach at 30 min after oral administration of the drug was determined in the fasted and nonfasted rabbits. The result showed that the percent of sodium salicylate remaining was significantly greater in the fasted rabbits (see Table III). Chiou, et al.[21] investigated the stomach emptying of barium sulfate after the administration of an aqueous suspension of the compound to fasted and nonfasted rabbits. They found that the rate of stomach emptying of barium sulfate in the nonfasted state was much faster than in the fasted state.

From the data described above, it is concluded that the rates of stomach emptying of sodium salicylate and aspirin in the fasted rabbit are much slower than in the nonfasted rabbit and, consequently, that the fasted state decreases the gastrointestinal absorption of those drugs.

20) J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53**, 1392 (1964).
21) W.L. Chiou, S. Riegelman, and J.R. Amberg, *Chem. Pharm. Bull.* (Tokyo), **17**, 2170 (1969).