Drug Biotransformation Interactions in Man II: A Pharmacokinetic Study of the Simultaneous Conjugation of Benzoic and Salicylic Acids with Glycine

LEWIS P. AMSEL and GERHARD LEVY

Abstract □ The major route of biotransformation of benzoic acid and salicylic acid in man is conjugation with glycine, resulting in the formation of hippuric acid and salicyluric acid, respectively. Both processes are capacity-limited in the usual dose range and approach a maximum rate following administration of sufficiently high doses of precursor. The rate of hippuric acid formation after oral administration of 5 g. benzoic acid in solution is increased markedly by the concomitant administration of glycine. In contrast, the administration of glycine has no effect on the formation of salicyluric acid. Salicylic acid in doses of 1 to 3 g. given orally 2 or 3 hr. prior to 2.0 to 5.0 g. benzoic acid had no measurable effect on the formation of hippuric acid. However, benzoic acid has a pronounced inhibitory effect on the formation of salicyluric acid from salicylic acid. This effect was not prevented by the co-administration of glycine. These results indicate that in man the availability of glycine is rate-limiting in the formation of hippuric acid, but not in the formation of salicyluric acid. Apparently, the inhibitory effect of benzoic acid on the formation of salicyluric acid is not due to competition for glycine but involves another phase in the biotransformation process.

Keyphrases □ Biotransformation interactions—drugs □ Pharmacokinetics—benzoic, salicylic acids, simultaneous glycine conjugation □ Salicylic acid effect—hippurate, benzoyl glucuronide formation, benzoic acid □ Benzoic acid effect—salicyluric acid formation, salicylic acid □ Glycine effect—benzoic, salicylic acid elimination □ UV spectrophotometry—analysis

The elimination of salicylate in man is due mainly to its conjugation with glycine, *i.e.*, the formation of salicyluric acid (1). Since man has a limited capacity for salicylurate formation, the time necessary to eliminate a given fraction of a dose of salicylate increases with increasing dose except in the very low dose range (1, 2). These pharmacokinetic characteristics of salicylate cause this drug to be readily accumulated in the body so that chronic administration may result in intoxication, particularly in young children (3). Treatment of salicylate intoxication is based largely on measures which accelerate the elimination of this drug from the body. It is evident, therefore, that a better understanding of the mechanism and pharmacokinetics of salicylurate formation may lead to safer salicylate therapy and to more effective treatment of salicylate intoxications.

The elimination of benzoic acid in man is due almost exclusively to the conjugation of this drug with glycine, resulting in the formation of hippuric acid (4). This involves the following mechanism:

$$\text{benzoic acid} + \text{ATP}^1 \rightarrow \text{adenylbenzoate} + \text{pyrophosphate}$$
$$\text{adenylbenzoate} + \text{CoA}^2 \rightarrow \text{benzoyl-CoA} + \text{adenylic acid}$$
$$\text{benzoyl-CoA} + \text{glycine} \rightarrow \text{hippuric acid} + \text{CoA}$$

[1] Adenosine triphosphate.
[2] Coenzyme A.

Presumably, the same sequence of reactions are involved in the formation of salicylurate from salicylate. There are, however, some significant differences in the formation of hippurate and salicylurate. The maximum formation rate of hippurate in man is about 20 times higher than the maximum formation rate of salicylurate (1). The formation of hippurate from benzoate is rate limited by the availability of glycine when benzoate levels exceed about 2 g. in healthy adult man so that administration of glycine with benzoate increases the formation rate of hippurate (5). On the other hand, administration of glycine does not increase the maximum formation rate of salicylurate in man (6). A study of the mutual effects of benzoate and salicylate on the formation of their respective glycine conjugates should be helpful, therefore, in elucidating the rate-limiting step in the formation of salicylurate. This report deals with studies in man; the results of parallel studies in rats will be reported subsequently (7).

THEORETICAL

The formation of hippurate from small doses of benzoate is extremely rapid in man. According to Wu and Elliott (8), the average apparent first-order rate constant for hippurate formation is 10.5 $hr.^{-1}$ while the average apparent first-order rate constant for hippurate excretion is 2.7 $hr.^{-1}$. On the other hand, the formation of hippurate in man occurs at an essentially constant rate for an appreciable length of time after administration of relatively large doses (≥ 5 g.) of benzoic acid (5, 9). Since, as will be shown subsequently, the renal excretion of hippurate is not rate limited by the capacity of the renal tubular transport system even at the highest excretion rates obtained after administration of benzoate, the following pharmacokinetic model may be applied:

$$\text{benzoate in body} \xrightarrow{R} \text{hippurate in body} \xrightarrow{k} \text{hippurate in urine}$$

where R is the essentially constant rate of hippurate formation and k is the apparent first-order rate constant for hippurate elimination. Since there is no further metabolism of hippuric acid, the rate constant for hippurate elimination (k) is equivalent to the rate constant for hippurate excretion (k_h). The kinetics are similar to those of constant infusion (10), where

$$C = \frac{R}{Vk}(1 - e^{-kt}) \qquad \text{(Eq. 1a)}$$

or

$$A = \frac{R}{k}(1 - e^{-kt}) \qquad \text{(Eq. 1b)}$$

In these equations, C is the concentration of drug (in this case, hippurate) in the blood, V is the apparent volume of distribution of the drug, t is time, and A is the amount of drug in the body. The term $1 - e^{-kt}$ approaches unity when t becomes large. Equation 1*b* then reduces to

$$A = \frac{R}{k} \qquad \text{(Eq. 2)}$$

which shows that the amount of drug in the body attains a constant

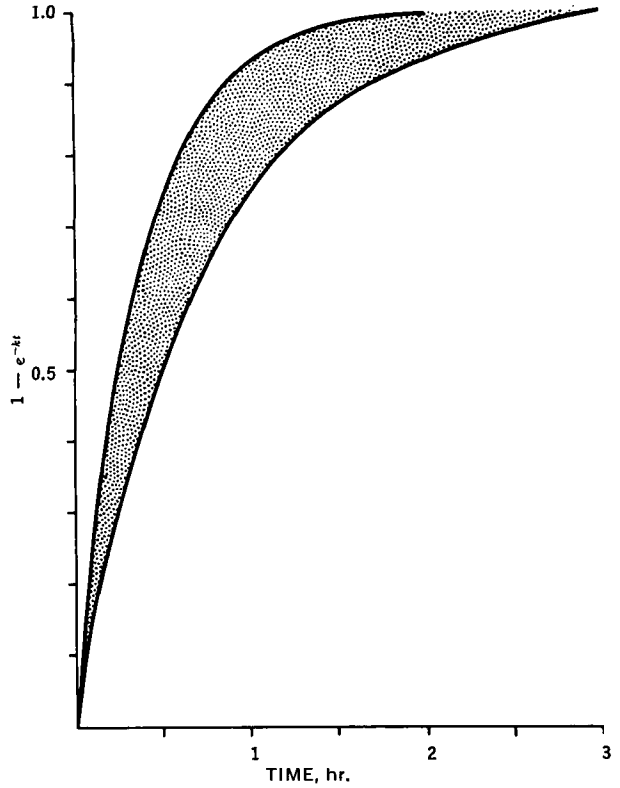

Figure 1—*Plot of* $1\text{-}e^{-kt}$ *as a function of time. Values for* k *of 2.7 and 1.4 hr.*$^{-1}$ *were used to construct the upper and lower curves, respectively.*

level independent of time. Since, in the case of hippurate elimination, k is equivalent to the hippurate excretion rate constant k_h

$$\text{excretion rate of hippurate} = kA \qquad \text{(Eq. 3)}$$

Therefore, by substituting R/k from Eq. 2 in Eq. 3,

$$\text{excretion rate of hippurate} \simeq R \qquad \text{(Eq. 4)}$$

when e^{-kt} approaches zero. This indicates that the rate of formation of hippurate can be estimated from urinary excretion data some time after benzoate administration.

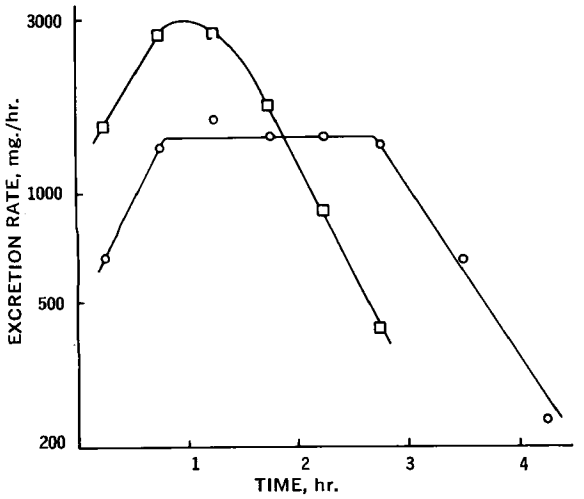

Figure 2—*Effect of glycine (5 g. at −1 hr. and 2 g. every hour thereafter) on the elimination of benzoic acid (Subject A). Shown are the urinary excretion rates of hippuric acid (in terms of benzoic acid) as a function of time after oral administration of 5 g. benzoic acid alone* (○), *and with glycine* (□).

Figure 1 shows theoretical curves for $1 - e^{-kt}$ based on a range of k values from 1.4 to 2.7 hr.$^{-1}$. The high value of k was taken from Wu and Elliott (8); the low value was found in the present study. It is evident that $1 - e^{-kt} \simeq 0.9$ in 1.5 hr. on the average. From that time on, almost constant rates of hippurate excretion should be observed as long as hippurate formation proceeds at an essentially constant rate. During this time, the rate of hippurate excretion will reflect the rate of formation of this metabolite (Eq. 4). An inhibition of the hippurate formation process should therefore be readily apparent from the urinary excretion data. In addition to a lower rate of hippurate excretion, there should occur an increase in the fraction of benzoate which is converted to benzoyl glucuronide. The latter accounts for less than 1% of a dose in the 1 to 2-g. dose range but increases with dose when the dose of benzoate exceeds 2 g. (9).

EXPERIMENTAL

Three healthy male ambulatory human subjects received 2.0 to 5.0 g. benzoic acid as sodium benzoate in aqueous solution. In some of the tests, benzoate was administered 2 hr. after oral administration of 1 to 3 g. salicylic acid as the sodium salt in aqueous solution. The experiments were initiated in the morning after an overnight fast and food was withheld for at least 2 or 3 hr. after benzoate administration. Urine was collected every 30 min. for the first 4 hr., then at longer intervals for a total of at least 8 hr. From 50 to 100 ml. water was ingested after each urine collection to assure adequate urine output. Urine samples were stored in a refrigerator and were usually assayed within 1 day. In experiments with glycine, 5 g. were taken orally in aqueous solution 1 hr. before drug administration and 2 g. every hour thereafter. The experiments involving the administration of hippuric acid were carried out in the same manner as the benzoate studies.

Determination of Benzoate and Hippurate in the Urine—Benzoic acid in the urine was determined by acidifying 2 ml. urine with 1 ml. 6 *N* hydrochloric acid and extracting with 30 ml. reagent grade carbon tetrachloride. Ten milliliters of the organic phase was then extracted with 10 ml. of 5% sodium bicarbonate solution. To 5 ml. of the latter was added 1 ml. of concentrated hydrochloric acid. This solution was shaken in a test tube until bubbles of carbon dioxide disappeared, and the absorbance of the solution was then determined at 232 mμ. The absorbance values were corrected for blanks obtained by carrying a sample of water through the same procedure. No measurable hippuric acid is extracted by this procedure. Concentrations of benzoic acid in the presence of salicylic

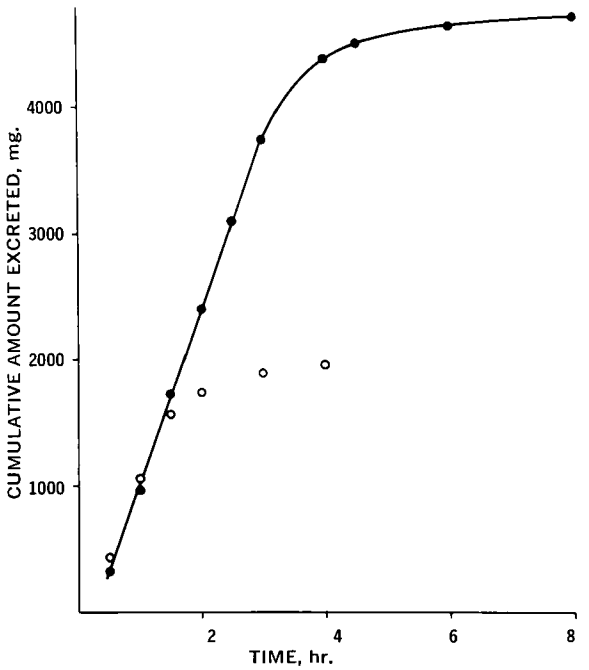

Figure 3—*Cumulative urinary excretion of hippuric acid (in terms of benzoic acid) as a function of time after oral administration of 2 g.* (○) *and 5 g.* (●) *benzoic acid (Subject A).*

Table I—Effect of Dose and Co-administration of Glycine on Benzoic Acid Elimination in Subject A

Dose, g.	Percent of Dose Excreted as Hippuric Acid	Percent of Dose Excreted as Benzoyl Glucuronide	Maximum Excretion Rate of Hippuric Acid, mg./hr.	Hippuric Acid Excretion Rate Constant, hr.$^{-1}$
2.0	95	1.8	1,730	1.2
5.0	94	3.4	2,090	1.1
5.0[a]	100	3.2	2,100	1.2
5.0 + glycine[b]	100	0.6	4,050	1.4
5.0 + glycine[b] + 1.5 g. salicylic acid	98	0.5	3,710	1.4

[a] This experiment was carried out 16 months after the other experiment with the same dose of benzoic acid. [b] See *Experimental.*

acid were determined by two component spectrophotometry (11), at 232 and 305 mμ.

For the determination of hippuric acid, 5 ml. of suitably diluted urine and 5 ml. of concentrated hydrochloric acid were placed in a 20-ml. capacity glass ampul which was sealed and placed in an oven at 100° for 16 hr. A 2-ml. aliquot of this solution was then assayed for benzoic acid as described above. The concentration of hippuric acid in the sample was obtained by subtracting the separately determined concentrations of free benzoic acid and benzoyl glucuronide (expressed as benzoic acid) from the concentration of total benzoic acid. This yielded the hippuric acid concentration in terms of benzoic acid.

Determination of Benzoyl Glucuronide—Five milliliters of urine (diluted with water if necessary) and 2 ml. of 0.4 *M* acetate buffer, pH 4.5 were placed in each of two 25-ml. capacity glass-stoppered flasks. One milliliter β-glucuronidase[3] was added to one flask and 1 ml. water to the other. The solutions were then incubated at 37° for 16 hr. Two milliliters of the solution was acidified with 1 ml. 6 *N* hydrochloric acid and extracted immediately into carbon tetrachloride. The assay then proceeded as described above for benzoic acid. The results thus obtained represent both free benzoic acid and benzoyl glucuronide; subtraction of the separately determined free benzoic acid concentration yields the concentration of benzoyl glucuronide.

Recoveries and Blank Values—The recoveries of hippuric acid and benzoic acid in solutions containing various known concentrations of these two substances as well as salicylic acid were generally between 98 to 100%. Apparent hippuric acid output in nonmedicated subjects ranged from 15 to 30 mg. benzoic acid equivalent/hr., which is in the range of normal values (12). The apparent excretion rate of benzoic acid in nonmedicated subjects was less than 0.4 mg./hr.

Determination of Salicylate and Metabolites—Salicylate and its metabolites were determined in the urine as previously described (13).

RESULTS

Formation of Hippurate from Benzoate—Figure 2 shows the urinary excretion rate of hippurate as a function of time after oral administration of 5 g. benzoate. Hippurate was excreted at an essentially constant rate of about 2.1 g./hr. (equivalent to 1.4 g. benzoic acid/hr.), from about 1 to about 3 hr. after benzoate administration. When benzoate was administered together with glycine (5 g. at −1 hr. and 2 g. every hour thereafter), the rate of hippurate excretion was increased to 4.1 g./hr. (equivalent to 2.8 g. benzoic acid/hr.). However, the slope of the terminal exponential excretion phase of hippurate was the same in each instance.

Figure 3 is a plot of cumulative amount of hippurate excreted as a function of time after oral administration of 2 and 5 g. benzoate, respectively. It is apparent that the initial excretion rate of hippurate following the lower dose is quite similar to that after the higher dose of benzoate. Benzoate was almost quantitatively recovered in the urine as hippurate, except for a minor fraction which was excreted as benzoyl glucuronide (Table I). No free benzoic acid was detected in the urine; the sensitivity of the analytical method used in this study was sufficient to detect 0.5% benzoyl glucuronide of a total dose of 2 g. benzoic acid under the experimental conditions. The

[3] Ketodase containing 5,000 units/ml., Warner-Chilcott, Morris Plains, N. J.

Table II—Elimination Kinetics of Hippuric Acid

Subject	Dose, g.	Percent Recovered in Urine	Maximum Excretion Rate, mg./hr.	Hippuric Acid Excretion Rate Constant, hr.$^{-1}$
A	2.9	92	1,380	1.1
A	5.0	106	2,030	(0.74)[a]
A	7.5	96	2,600	(0.92)[a]
B	2.9	92	1,790	1.3

[a] Questionable value, probably affected by prolonged absorption phase.

2-g. dose of benzoate yielded relatively less benzoyl glucuronide than the 5-g. doses; concomitant administration of glycine with 5-g. benzoic acid resulted in a decrease in benzoyl glucuronide output (Table I).

The excretion rate constant of hippuric acid was essentially the same when this metabolite was formed in the body from benzoate or when it was administered as such (Tables I and II). The maximum excretion rate of hippurate increased with increasing oral dose of this metabolite, but this relationship was not linear (Table II).

Effect of Salicylate on the Formation of Hippurate from Benzoate—Administration of 2 g. salicylic acid 2 hr. before 2 g. benzoic acid had no apparent effect on the elimination of the latter (Fig. 4). A higher dose of salicylate (3 g.) also did not affect hippurate output (Fig. 5). Furthermore, salicylate did not prevent the enhancement of hippurate formation by exogenous glycine (Fig. 6). Salicylate had no measurable effect on the proportion of hippuric acid and benzoyl glucuronide excreted in the urine after benzoate administration, and on the excretion rate constant of hippuric acid (Fig. 5, Tables III and IV).

Effect of Benzoate on Salicyluric Acid Formation from Salicylate—

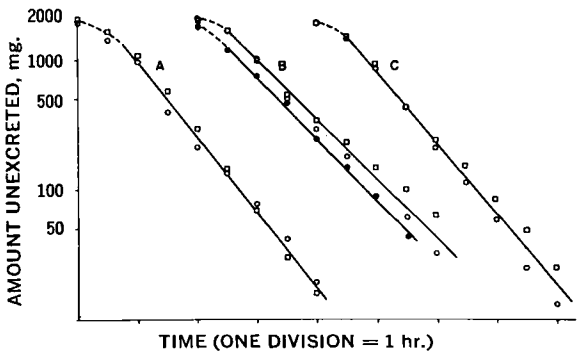

Figure 4—*Effect of salicylic acid on the elimination of benzoic acid in three human subjects. Plotted are the total amounts of benzoate metabolites (mainly hippuric acid) remaining unexcreted as a function of time after oral administration of 2 g. benzoic acid alone* (○), *and 2 hr. after oral administration of 2 g. salicylic acid* (□). (●), *amount of hippurate unexcreted following oral administration of 2.9 g. hippuric acid (equivalent to 2 g. benzoic acid). Amounts unexcreted are expressed in terms of benzoic acid.*

Table III—Effect of Salicylic Acid (SA) on Elimination of Benzoic Acid (BA)

Subject[a]	Dose of BA, g.	Percent of Dose Excreted as Hippuric Acid		Percent of Dose Excreted as Benzoyl Glucuronide		Hippuric Acid Excretion Rate Constant, hr.$^{-1}$	
		Control	With SA	Control	With SA	Control	With SA
A(M,25,90)	2.0	95	100[c]	1.8	1.3	1.2	1.4
B(M,24,84)	2.0	105	102[c]	0.4	0.8	1.2	1.0
C(M,34,72)	2.0	95	94[c]	2.5	1.0	1.3	1.0
A	5.0 and glycine[b]	100	98[d]	0.6	0.5	1.4	1.4

[a] In parentheses: sex, age in years, body weight in kilograms. [b] See *Experimental.* [c] 2.0 g. SA at −2 hr. [d] 1.5 g. SA at −3 hr.

Figure 7 depicts the time course of salicyluric acid excretion in Subject A after oral administration of 2 g. salicylate when 2 g. benzoate was given 2 hr. later. Similar results were obtained in Subjects B and C.[4] The salicyluric acid excretion rate reached a plateau at about 1 hr. and decreased precipitously immediately after benzoate administration. It then increased again and returned to the plateau level at about 5 hr. It is noteworthy that the excretion rate of salicyluric acid at 6 to 8 hr. was essentially the same as at 1 hr. even though the amount of salicylate in the body had decreased by more then one third. Co-administration of a large amount of glycine did not prevent the inhibition of salicyluric acid formation by 2 g. benzoate (Fig. 8). Figure 9 shows the excretion rate of salicylurate as a function of time after administration of salicylate and a larger dose (3.2 g.) of benzoate with and without glycine. Glycine reduced, but did not prevent, the inhibitory effect of benzoate on salicylurate formation.

DISCUSSION

Capacity-Limited Elimination of Benzoate—The data in Table I and in Figs. 2 and 3 show that the formation of hippurate from benzoate is capacity limited, the availability of glycine being the rate-limiting factor. Consistent with this is the increase with increasing dose in the fraction of benzoate excreted as the glucuronide, and the decrease in this fraction when glycine is administered together with benzoate (Table I). Even with as low a dose as 2 g. of benzoic acid, hippurate formation is approaching its maximum rate (Fig. 3, Table I). Following an oral dose of 5 g. benzoic acid, hippurate excretion reached its maximum level somewhat earlier than predicted by theory (Fig. 1). This was observed also in other experiments and is probably due to the presence of some endogenous glycine at the site of biotransformation. Only after this initial "pool" of glycine is depleted somewhat will the rate of supply of additional glycine become the limiting factor in the synthesis of hippurate. The maximum rate of hippurate formation, as reflected by the urinary excretion data, is similar to that observed by other workers (reviewed in *Reference 1*). Since the results of the benzoate elimination experiments agreed with and confirmed the results and conclusions of other investigators, this aspect of the investigation was not pursued further.

Hippurate Elimination Kinetics—The rate constant for hippurate excretion ranged from 1.1 to 1.4 hr.$^{-1}$ regardless if the metabolite was formed in the body or administered as such (Tables I–IV). Salicylate had no measurable effect on hippurate excretion in the dose range studied (Table IV). Wu and Elliott (8) observed a considerably higher average hippurate excretion rate constant (2.7 hr.$^{-1}$) in 25 subjects, while Hirsheimer (14), in a study of 58 subjects obtained data following intravenous administration of hippurate which yielded an average value of 1.6 hr.$^{-1}$, in good agreement with the results of the present study. The maximum excretion rate of hippurate did not increase linearly with increasing dose (Table II). This may have been due to partial precipitation of hippuric acid

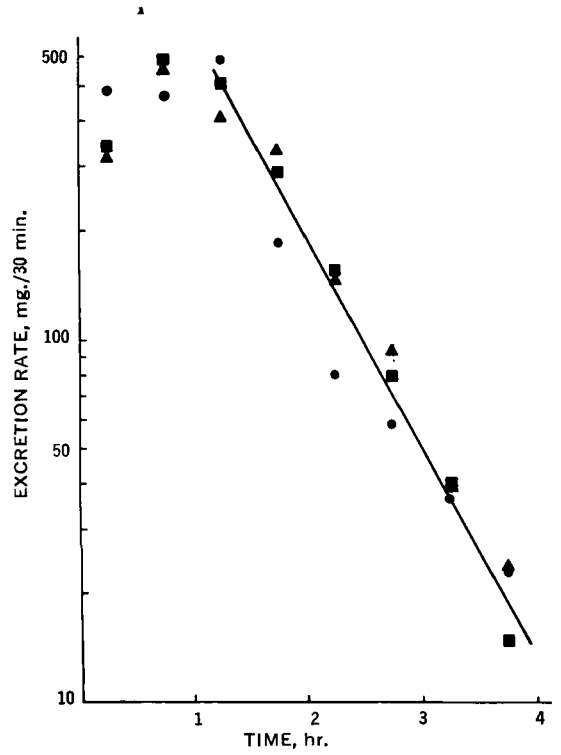

Figure 5—*Effect of different doses of salicylic acid on the elimination of benzoic acid. Shown are the urinary excretion rates of hippuric acid (expressed in terms of benzoic acid) after oral administration of 2 g. benzoic acid alone* (●), *2 hours following 2 g. salicylic acid* (◨), *and 2 hours following 3 g. salicylic acid* (▲). *Subject A.*

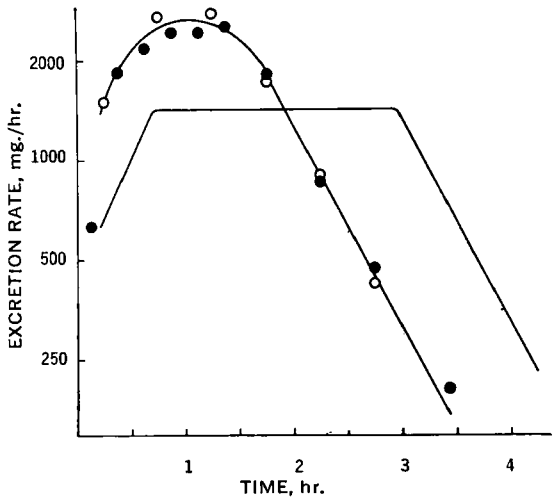

Figure 6—*Effect of salicylic acid on the availability of exogenous glycine for hippurate formation (Subject A). Excretion rates of hippurate (in terms of benzoic acid) as a function of time after oral administration of 5 g. benzoic acid and glycine (5 g. at −4 hr. and 2 g. every hour thereafter) with* (●), *and without* (○), *salicylic acid (1.5 g. at −3 hr.). The lower curve is taken from Fig. 2 and represents the urinary excretion rates of hippurate after administration of 5 g. benzoic acid without glycine in the same subject.*

[4] These data are available from the authors upon request.

Table IV—Effect of Different Doses of Salicylic Acid on Elimination of Benzoic Acid in Subject A

Dose, g. BA	Dose, g. SA	Percent Recovered in Urine	Percent of Dose Excreted as Hippuric Acid	Benzoyl Glucuronide	Hippuric Acid Excretion Rate Constant, hr.$^{-1}$
2.0	—	97	95	1.8	1.2
2.0	1.0	96	94	1.3	1.3
2.0	2.0	101	100	1.3	1.4
2.0	2.0 + glycine[a]	91	89	1.1	1.2
2.0	3.0	99	98	1.4	1.1

[a] See *Experimental.*

in the stomach and/or delayed gastric emptying,[5] resulting in relatively slower absorption of the large doses.

Effect of Salicylate on Benzoate Elimination—Administration of 2 g. salicylic acid, a dose at which salicylurate formation is capacity limited and proceeding at an essentially constant and maximum rate (1), had no measurable effect on hippurate formation (Fig. 4). Consistent with this is the fact that benzoyl-glucuronide formation, a parallel and competing process for benzoate elimination, did not increase during salicylate administration (Table III). Even when the dose of salicylate was increased to 3 g., there was no apparent inhibition of hippurate formation. It is particularly interesting that salicylate also does not interfere with the availability of exogenous glycine for hippurate formation (Fig. 6), in view of the fact that there exists a metabolic inhomogeneity of glycine in man and that plasma glycine is used preferentially for hippurate synthesis (15). These results are contrary to those of Little *et al.* (16) who found that salicylate inhibits the formation of *para*-aminohippurate in liver and/or kidney slices of rats, dogs, and rabbits. On the other hand, it has been stated by Cummings *et al.* (17) that salicylate does not affect the formation of *para*-aminohippurate from *para*-aminobenzoate in man, although no experimental data were presented in support of this statement.

Effect of Benzoate on Salicylurate Formation—Benzoate had a rapid and pronounced inhibitory effect on salicylurate formation (Fig. 7). The duration of this effect was short, consistent with the relatively rapid elimination of benzoate. It has been shown previously that repeated administration of small doses of benzoate (0.5 g. every 30 min.) can inhibit salicylurate formation for extended periods of time (18). The magnitude of the inhibitory effect was dose-dependent (Fig. 7 *versus* Fig. 9). The essentially constant excretion rate of salicylurate before and after benzoate, in a period of time when the amount of salicylate in the body decreased by nearly 50%, reflects the capacity-limited nature of the salicylurate formation process (1, 19). Concomitant administration of large amounts of glycine did not prevent the inhibitory effect of benzoate on salicylurate formation (Figs. 8 and 9), but reduced the magnitude and duration of inhibition. This is readily apparent in Fig. 9 and is due to the more rapid elimination of benzoate when glycine is given. Glycine did not increase the formation rate of salicylurate before benzoate administration or after benzoate elimination. This is consistent with the results of previous studies in this laboratory (6). It has also been shown previously that benzoate has no apparent effect on salicylurate excretion (18).

Rate-Limiting Factors in Hippurate and Salicylurate Formation, and Mechanism of Interaction Between Benzoate and Salicylate—The results of this and previous studies (5) show that the availability of glycine is rate limiting in the formation of hippurate, but that this is not the rate-limiting factor in the formation of salicylurate. It is evident also (Figs. 4, 5, and 9) that salicylate does not inhibit the access of endogenous or exogenous glycine to the site of hippurate formation. The apparent lack of effect of salicylate on hippurate formation may be due to a greater affinity of benzoate to the enzyme responsible for the conjugation with glycine; it could also reflect a greater relative distribution of benzoate, as compared to salicylate, to the site of biotransformation. On the other hand, the formation of salicylurate and hippurate may involve different enzyme systems, similar to what has been found with respect to glucuronide formation (20, 21). Thus, Schachter and Taggart have found that the activating enzyme involved in the formation of benzoyl CoA does not form salicyl-CoA (22). However, attempts to obtain salicylurate synthesis even in liver and kidney slices (16) or homogenates (23) have not been entirely successful. Until salicylurate formation can be consistently demonstrated *in vitro*, the results of Schachter and Taggart cannot be taken as definite proof of

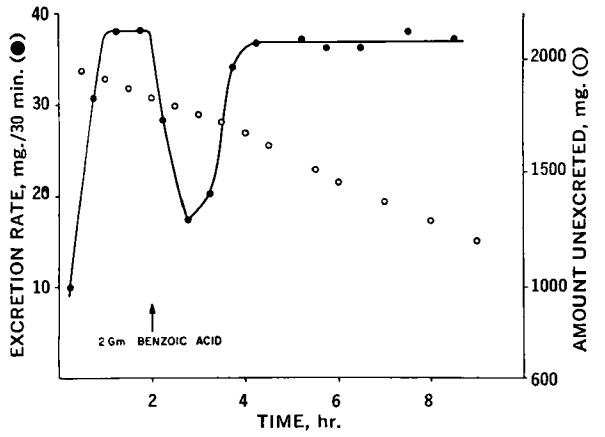

Figure 7—*Effect of benzoic acid on the formation of salicyluric acid from salicylic acid by Subject A. Shown are the urinary excretion rates of salicyluric acid* (●) *and the amounts of salicylate remaining in the body* (○) *as a function of time after oral administration of 2 g. salicylic acid. Two grams benzoic acid was given 2 hr. after administration of salicylic acid.*

[5] The larger doses caused some nausea, which in turn retards gastric emptying (unpublished observations).

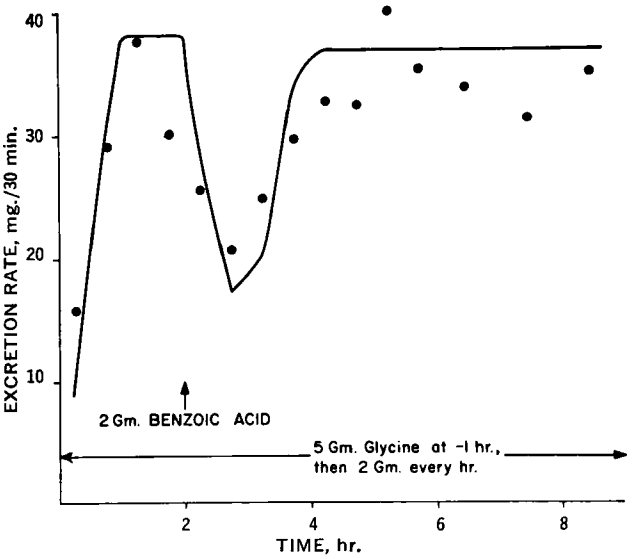

Figure 8—*Effect of benzoic acid on the formation of salicyluric acid from salicylic acid during co-administration of glycine (Subject A). Shown are the urinary excretion rates of salicyluric acid as a function of time after oral administration of 2 g. salicylic acid. The curve is taken from Fig. 6 and represents the excretion rates observed when glycine was not given.*

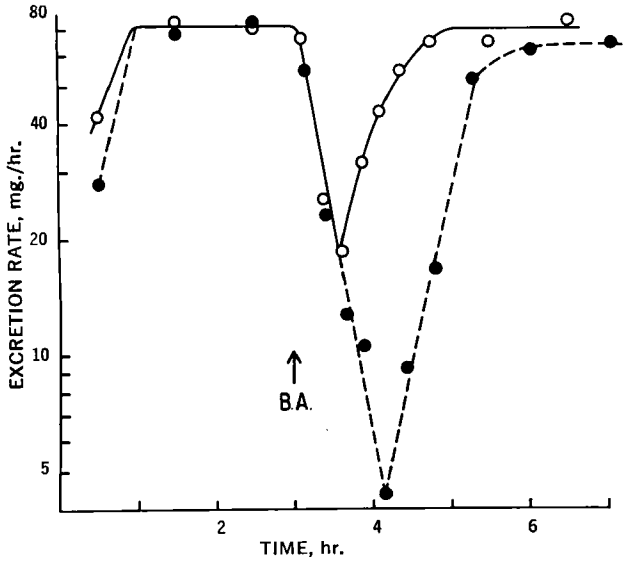

Figure 9—*Effect of benzoic acid on the formation of salicyluric acid from salicylic acid with and without administration of glycine (Subject A). Shown are the urinary excretion rates of salicyluric acid as a function of time after oral administration of 1 g. salicylic acid and 3.2 g. benzoic acid 3 hr. later, with* (○), *and without* (●), *glycine.*

the existence of an enzyme capable of activating benzoate, but not salicylate. If, however, there are more than one substrate-activating and/or glycine-transferring (*N*-acylase) enzymes with different substrate specificity, it may also be that one substrate inhibits the glycine conjugation of another substrate, but not the reverse. This is known to occur in glucuronide conjugation (20), where there is increasing evidence for the existence of two or more enzyme systems with different substrate specificity (20, 21). The results of the present study suggest that the same may be the case with respect to glycine conjugation.

REFERENCES

(1) G. Levy, *J. Pharm. Sci.*, **54,** 959(1965).

(2) C. Bedford, A. J. Cummings, and B. K. Martin, *Brit. J. Pharmacol.*, **24,** 418(1965).

(3) G. Levy and S. J. Yaffe, *Clin. Toxicol.*, in press.

(4) R. T. Williams, "Detoxication Mechanisms," 2nd ed., Chapman and Hall, London, England, 1959, Chap. 11.

(5) A. J. Quick, *J. Biol. Chem.*, **92,** 65(1931).

(6) E. Nelson, M. Hanano, and G. Levy, *J. Pharmacol. Exptl. Therap.*, **153,** 159(1966).

(7) L. P. Amsel and G. Levy, to be published.

(8) H. Wu and H. C. Elliott, Jr., *J. Appl. Physiol.*, **16,** 553 (1961).

(9) D. Schachter, *J. Clin. Invest.*, **36,** 297(1956).

(10) J. G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53,** 1392 (1964).

(11) H. H. Willard, L. L Merritt, and J. A. Dean, "Instrumental Methods of Analysis," 3rd ed., Van Nostrand, Princeton, N. J., 1958, p. 120.

(12) "Documenta Geigy," 6th ed., K. Diem, Ed., Geigy Pharmaceuticals, New York, N. Y., p. 530.

(13) G. Levy and J. A. Procknal, *J. Pharm. Sci.*, **57,** 1330(1968).

(14) A. Hirsheimer, *Am. J. Obstet. Gynecol.*, **37,** 363(1939).

(15) D. Garfinkel and A. Lajtha, *J. Biol. Chem.*, **238,** 2429 (1963).

(16) J. M. Little, E. A. Angell, and A. M. Morgan, *Arch. Intern. Pharmacodyn.*, **111,** 259(1957).

(17) A. J. Cummings, B. K. Martin, and R. Renton, *Brit. J. Pharmacol.*, **26,** 461(1966).

(18) G. Levy and L. P. Amsel, *Biochem. Pharmacol.*, **15,** 1033 (1966).

(19) G. Levy, "Symposium on Importance of Fundamental Principles in Drug Evaluation," APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, 1968, in press.

(20) G. A. Tomlinson and S. J. Yaffe, *Biochem. J.*, **99,** 507(1966).

(21) A. R. Temple, M. S. Clement, and A. K. Done, *Proc. Soc. Exptl. Biol. Med.*, **128,** 307(1968).

(22) D. Schachter and J. V. Taggart, *J. Biol. Chem.*, **208,** 263 (1954).

(23) R. Gorodischer, personal communication.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1968, from the *Biopharmaceutics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication November 15, 1968.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

Supported in part by grant No. 1R01 DS00021-01 from the U. S. Public Health Service, Bethesda, Md., and by a fellowship for L.P.A. from the American Foundation for Pharmaceutical Education.

Previous paper: G. Levy and J. A. Procknal, *J. Pharm. Sci.*, **57,** 1330(1968).