Drug Biotransformation Interactions in Man I

Mutual Inhibition in Glucuronide Formation of Salicylic Acid and Salicylamide in Man

By GERHARD LEVY and JOSEPHINE A. PROCKNAL

Co-administration of salicylamide (SAM) and sodium salicylate to healthy adult humans resulted in a pronounced decrease in the formation of the glucuronides of these two drugs. This is thought to be due to a limited capacity for glucuronide conjugation in man and/or to the uncoupling action of salicylate on oxidative phosphorylation. Salicylate *may* inhibit also the formation of SAM sulfate; it has no effect on the excretion rate constants of SAM glucuronide and SAM sulfate in the doses used in the investigation. The results of this study indicate that concomitant use of certain drugs which are readily available to the public may lead to appreciable interactions in biotransformation and to enhanced pharmacologic effects.

CLINICAL PHARMACOLOGIC STUDIES in recent years have led to a recognition of the important role of drug interactions in therapy and in toxicology (1). Most of these studies have dealt with enzyme induction (2), but the role of competitive and noncompetitive inhibitory effects on drug biotransformation and excretion processes is also being recognized. It is now evident that a number of important drug biotransformation processes in man have a relatively limited capacity (3–6). This in itself is not surprising since drug biotransformation involves enzymic reactions, but it was not realized until recently that "saturation" effects may be encountered when drugs are given in the usual therapeutic dose range. It may be expected that co-administration of two or more drugs which share a biotransformation pathway of low capacity will lead to competitive inhibition of the formation of the respective metabolites. Consequently, there will be a change in quantitative composition of metabolites and a decrease in the elimination rate of one or both of the drugs. This can result in enhanced pharmacologic activity or, if the metabolite produced by the capacity-limited process is the pharmacologically active species, in a decrease of the pharmacologic activity of a drug. An exploration of the mutual pharmacokinetic effects of drugs sharing a biotransformation route of low capacity in man is therefore highly pertinent. Such effects have obvious implications with respect to drug safety since they may lead to adverse reactions and intoxications; it should be recognized, however, that the judicious and informed use of such drug combinations may also result in safer and more effective medication. Finally, the study of drug metabolism interactions represents an effective research approach for identifying possible differences in the rate-limiting step in the formation of metabolites of different drugs which apparently share the same biotransformation pathway. This report is the first of a series from this laboratory in which studies of such drug interactions will be described. It is concerned with what is generally considered to be the most important, versatile, and ubiquitous drug biotransformation pathway in man, the formation of glucuronides. The relevance of such studies is emphasized further by the nature of the two drugs utilized in the investigation—salicylate and salicylamide—since they are among the most widely used and most readily available to the public.

Received March 4, 1968, from the Biopharmaceutics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214

Accepted for publication April 23, 1968.

Presented at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 1968.

Supported in part by grant No. 1 R01 DS00021-01 from the U. S. Public Health Service, Bethesda, Md.

EXPERIMENTAL

Healthy, male, ambulatory human subjects received orally 0.6 g. salicylamide in one test, and the same dose of salicylamide 2 hr. following oral administration of 2.32 g. sodium salicylate (equivalent to 2 g. salicylic acid) in another test. Both tests were initiated in the morning on an empty stomach. Food was withheld for at least 2 hr. after drug administration. Both drugs were given in aqueous solution. Urine was collected every 30 min. for the first 4–8 hr., then at longer intervals for a total of 24 hr. From 50 to 100 ml. water was ingested after each urine collection to assure adequate urine output. The urine samples were stored in a freezer until assayed.

Assay Methods for Salicylic Acid and Its Metabolites—Salicylic acid and its major metabolites in urine were determined by a further modification of the Smith *et al.* (7) modification of the colorimetric method of Brodie *et al.* (8) for the determination of salicylic acid in plasma. In essence, the procedure as used in the study reported here consists of (*a*) determination of salicylic acid (SA) by extraction of the sample with carbon tetrachloride and reextrac-

tion into ferric nitrate reagent solution, (*b*) determination of the absorbance due to both SA and salicyluric acid (SU) by extraction of the sample with ethylene dichloride and reextraction into ferric nitrate solution, and (*c*) determination of total salicylates [*i.e.*, SA, SU, and salicylic glucuronides (SAG)] after complete acid hydrolysis of these metabolites to SA, followed by step *a*. SU content is calculated by difference of *a* from *b*, and SAG content is obtained by subtracting SA and SU values from *c*.

The major difference between the method of Smith *et al.* (7) and the method described here is based on studies in this laboratory which have shown that SU does not partition to any significant extent into carbon tetrachloride. Partition coefficients of SU between 0.1 *N* HCl and various organic solvents were determined with a pure sample of SU obtained after several recrystallizations from water and ethanol–chloroform mixture and found free of impurities by thin-layer chromatography, elemental analysis, and by examination of the infrared spectrum. These partition coefficients are listed in Table I. The lack of significant partitioning of SU into carbon tetrachloride even in the presence of SA is evident from the data shown in Table II. The apparent extraction of SU by carbon tetrachloride reported by Brodie *et al.* (8) and Smith *et al.* (7) was probably due to contamination of their sample (both groups used material from the same source) by about 5% SA. The other modification of the method of Smith *et al.* (7) was to hydrolyze SA metabolites totally rather than partially, since differences in heating and cooling rates can affect the degree of partial hydrolysis, and thus the reproducibility of the assay results.

Procedure A—The concentration of SA in urine is determined as follows: 2 ml. of the urine sample (diluted with water if necessary), 0.5 ml. 6 *N* HCl, and 30 ml. carbon tetrachloride are placed in a glass-stoppered bottle. After shaking vigorously for 5 min., the mixture is transferred to a centrifuge tube, centrifuged to separate the phases, and the urine phase is aspirated and discarded. Ten milliliters of the organic phase and 5 ml. of ferric nitrate reagent solution are placed in a glass-stoppered centrifuge tube which is shaken vigorously for 5 min. After centrifuging if necessary to separate the layers, a portion of the aqueous phase is removed and its absorbance is determined at 530 mμ. A reagent blank value is obtained by using distilled water in place of the urine sample and applying the same procedure. The ferric nitrate reagent solution is freshly prepared by diluting 5 ml. of ferric nitrate stock solution [1.0% $Fe(NO_3)_3$ in 0.07 *N* HNO_3] with distilled water to 100 ml. All chemicals and solvents should be of reagent grade quality. An aqueous solution containing 10 mg.% SA, when subjected to the above procedure, yields an absorbance in the ferric nitrate reagent phase of about 0.14 in cells of 1-cm. path length.

Procedure B—Salicyluric acid is determined by essentially the same procedure as that described for SA, except that reagent grade ethylene dichloride is used instead of carbon tetrachloride. To account for the contribution of SA to the absorbance of the ferric nitrate reagent phase, 1.06 times the absorbance value obtained in Procedure A is subtracted. Multiplication by 1.06 corrects for the small difference in the partitioning of SA when using carbon tetrachloride and ethylene dichloride, respectively, for the extractions. This difference has been constant and reproducible during the several years that the procedure has been used in this laboratory. A solution containing 10 mg.% SU yields an absorbance of about 0.059 in the ferric nitrate reagent phase (1-cm. cell path length) while a 10-mg.% solution of SA produces an absorbance of about 0.15 in the procedure described here. Table II shows the results of assays of solutions containing known concentrations of both SA and SU.

Procedure C—Total salicylate in the urine is determined by hydrolyzing SU and SAG to SA. Three milliliters of the urine sample and 3 ml. of concentrated hydrochloric acid are heated in a sealed 20-ml. ampul at 100° for 16 hr. This length of time is necessary to bring about an essentially complete hydrolysis of SU to SA. Two milliliters of the hydrolyzed solution is then analyzed for SA by Procedure A. The concentration of SAG in the sample is determined by subtracting the sum of the SA and SU concentrations (the latter corrected for molecular weight by multiplying by 0.708) from the total SA concentration of the hydrolyzed sample.[1] Thus, $(SAG) = (SA_{total}) - [(SA_{free}) + 0.708 (SU)]$ where the terms in parentheses denote concentrations in metric (rather than molar) units, and (SAG) is expressed in terms of (SA). Urine blanks are usually somewhat less than 1 mg./hr. apparent total SA. The recovery of total salicylate following an oral dose of SA or aspirin has been consistently between 95 and 100% under properly controlled experimental conditions (*i.e.*, when complete urine collections could be monitored in the laboratory or in a clinical research center). When the dose of SA exceeds 1 g. in adults (and proportionately less in infants and children), gentisic acid (GA) determinations are necessary to account adequately for the administered dose.

TABLE I—PARTITION COEFFICIENT OF SALICYLURIC ACID IN SEVERAL ORGANIC SOLVENTS[a]

Solvent	Partition Coefficient (Organic/Aqueous)
Carbon tetrachloride	<0.002
Chloroform	0.05
Ethylene dichloride	0.15
Ether	4.0

[a] Solutions of 100-mg. % salicyluric acid in 0.1 *N* HCl were equilibrated with equal volumes of organic solvent at room temperature.

TABLE II—DETERMINATION OF SALICYLURIC ACID IN THE PRESENCE OF SALICYLIC ACID

Composition of Solutions, mg. %		Found, mg. %[a]	
Salicylic Acid	Salicyluric Acid	Salicylic Acid	Salicyluric Acid
0	25	0	24.5
5	25	5.07	24.5
10	25	10.0	24.6
25	25	25.1	24.1
50	25	50.7	24.5

[a] Average of two determinations.

[1] Determination of SAG by enzymatic hydrolysis for 16 hr. with β-glucuronidase yields results which are about 20% lower than those obtained by acid and heat hydrolysis. This appears to be due to the rather slow rate of the enzymic reaction.

TABLE III—VERIFICATION OF ASSAY METHOD FOR SALICYLATE AND ITS METABOLITES AND SALICYLAMIDE AND ITS METABOLITES IN URINE SAMPLES CONTAINING BOTH GROUPS OF SUBSTANCES

		Concentrations, as mg. % SA or SAM Equivalent			
		A	B		
Metabolite[a]	Sample[b]	Urine After Salicylate Admin.	Urine After Salicylamide Admin.	Equal parts, A + B	
				Assay	Theoretical
SAMG	I	0.2	6.9	3.5	3.6
	II	0.3	12.7	6.5	6.5
SAMS	I	0.1	9.1	4.4	4.6
	II	0.2	8.7	4.4	4.4
SA	I	0.6	0.3	0.4	0.4
	III	1.5	0.7	1.1	1.1
	II	2.1	0.8	1.5	1.5
SAG	I	2.7	0.0	0.9	1.4
	III	6.1	0.0	2.5	3.0
	II	8.1	0.0	3.5	4.0
SU	II	17.8	0.6	9.8	9.2
	I	21.3	0.3	11.0	10.8
	III	34.1	0.5	16.8	17.3

[a] SAMG, salicylamide glucuronide; SAMS, salicylamide sulfate; SA, salicylic acid; SAG, salicylic glucuronides; SU, salicyluric acid. [b] Roman numerals refer to a specific urine sample obtained after either SA or SAM administration.

This is due to saturation effects with respect to SU formation, resulting in an increase in the fraction of the dose metabolized to other products. GA is determined by the method of Becher (9). Experience has shown that the concurrent assay of reference solutions containing known concentrations of GA is desirable to achieve satisfactory results.

Assay Methods for Salicylamide and Its Metabolites—The procedures were those of Levy and Matsuzawa (5) with minor modifications. In the determination of total salicylamide (SAM), carbon tetrachloride was substituted for ethylene dichloride. In the assays of SAM glucuronide and SAM sulfate, the pH after enzymic hydrolysis was adjusted to 6.5 with 0.1 *N* sodium hydroxide solution instead of adding phosphate buffer. Gentisamide (GAM) was determined as such (rather than as GA). In this procedure, 3 ml. of urine sample, 2 ml. of pH 4.5 acetate buffer (0.4 *M*), and 2 ml. of beef liver beta-glucuronidase[2] are incubated at 37° for 48 hr. After adjusting the pH to 6.0 with 0.1 *N* NaOH solution, enough water is added to yield a total volume of 10 ml. Twenty milliliters reagent grade ether is added and the mixture is shaken for 5 min. Fifteen milliliters of the ether phase is extracted with 6 ml. of a 5% solution of sodium bicarbonate. To 4 ml. of the aqueous phase are added 1 ml. of concentrated HCl and 1 ml. of Folin-Ciocalteu reagent and the absorbance of this solution is determined 20 min. later at 750 mμ. Reagent blanks (using water instead of a urine sample) and controls are run concurrently.

Determination of Salicylic Acid, Salicylamide, and Their Metabolites in the Presence of One Another—The difference in the pKa of SA, SU, and SAM affords selective extraction of the latter at pH 6.5 from aqueous solution. Total SAM is determined by extraction of free SAM following enzymic hydrolysis with beta-glucuronidase and sulfatase. Absorbance values in the determination of SA and SU (Procedures A and B) are corrected for the contribution by SAM. These corrections are negligible and essentially equal to urine blank values since practically no free SAM appears in the urine. Total SA from salicylate and its metabolites is determined in the presence of SAM metabolites by subtracting the SA equivalent of the total SAM obtained by beta-glucuronidase and sulfatase hydrolysis from the total SA obtained by hydrolysis of both SA and and SAM metabolites to SA. The analytical procedures were checked by separately assaying urine samples obtained following SA administration and samples obtained following SAM administration, and then assaying mixtures of equal parts of these urines. Table III shows results obtained at low concentrations of SA and SAM metabolites, *i.e.*, under the most difficult conditions.

[2] Ketodase, Warner-Chilcott, Morris Plains, N. J.

RESULTS AND DISCUSSION

Salicylic acid is eliminated in man mainly by formation of salicyluric acid, by renal excretion of free drug, and by glucuronide conjugation (10). Salicylamide is metabolized to the sulfate, the glucuronide, and to gentisamide; excretion of free salicylamide is negligible (5). Both drugs are subject to capacity-limited effects in their biotransformation in man even in the usual therapeutic dose range. This involves the conjugation of salicylate with glycine to form salicylurate (3, 11) and the formation of salicylamide sulfate from salicylamide (5). It has been reported that at high levels of salicylamide, saturation of the glucuronide formation process also becomes evident (12). The elimination of salicylate in man is considerably slower than that of salicylamide and the experimental design of the study reported here was based on this consideration. Salicylate was administered 2 hr. before salicylamide; body levels of the former declined by less than 25% in the period of time when salicylamide and its metabolites were present in the body. The effect of salicylamide on the formation of salicylic glucuronides is shown in Figs. 1 and 2 for two subjects. Similar results were obtained in the third subject.[3] The excretion of salicylic glucuronides decreased precipitously shortly after salicylamide administration and returned to normal levels within about 2 hr. The short duration of the salicylamide effect is due to the rapid elimination of this drug. Figure 2 shows also the time course of salicylurate excretion following salicylate administration. The excretion rate of this metabolite was essentially constant during the first 12 hr., reflecting the limited capacity of man to form salicylurate

[3] Graphs of all the individual data are not shown due to space limitations but are available from the authors upon request.

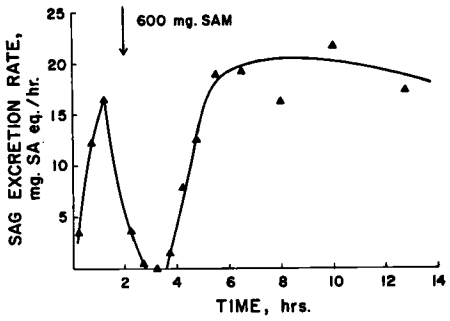

Fig. 1—Excretion rate of salicylic glucuronides following oral administration of 2.32 g. sodium salicylate. Arrow indicates time when 0.6 g. salicylamide was taken. Subject A.

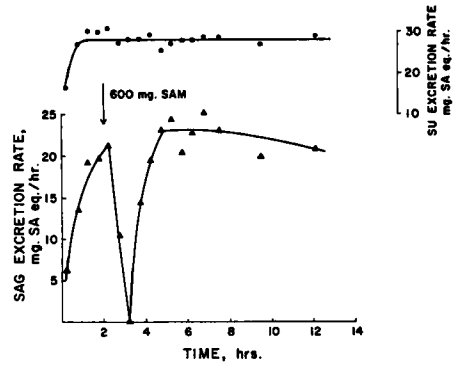

Fig. 2—Excretion rate of salicylic glucuronides following oral administration of 2.32 g. sodium salicylate. Arrow indicates time when 0.6 g. salicylamide was taken. Subject B. Upper curve shows urinary excretion rate of salicyluric acid. Note lack of effect of salicylamide on the latter.

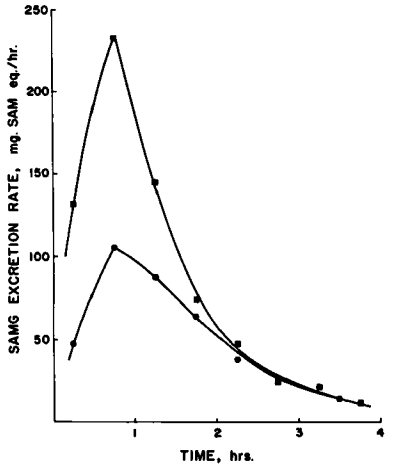

Fig. 3—Excretion rate of salicylamide glucuronide following oral administration of 0.6 g. salicylamide alone (■) and 2 hr. after 2.32 g. sodium salicylate (●). Subject A.

(3, 11). Salicylamide, which is not conjugated with glycine, had no effect on this process in any of the subjects.

The effect of salicylate on the excretion of salicylamide glucuronide is shown in Fig. 3 for one of the subjects and is representative of the data obtained in all three subjects.[3] There was a pronounced decrease in the excretion of salicylamide glucuronide in the presence of salicylate. Since the excretion of salicylamide conjugates is excretion rather than formation rate limited (5, 13), the terminal phase in the excretion rate plot reflects the excretion kinetics of the respective metabolite. Figure 4 is a semilogarithmic plot of salicylamide glucuronide excretion rate in the terminal excretion phase as a function of time. Each of the curves in the figure is displaced on the time axis relative to the others to facilitate comparison of slopes. It is evident that the excretion half-life of salicylamide glucuronide is about 0.8 hr. and that salicylate has no effect on this process. Therefore, the pronounced decrease in the excretion of salicylamide in the presence of salicylate is due to decreased formation of salicylamide glucuronide. This conclusion is substantiated by the lower total recovery of salicylamide glucuronide in the presence of salicylate (Table IV).

Figure 5 shows the excretion rate of salicylamide sulfate in one of the subjects following administration of salicylamide alone or in the presence of salicylate. Similar data were obtained with the other subjects.[3] Figure 6 is a semilogarithmic plot of salicylamide sulfate excretion rate as a function of time in the terminal phase of the process, representing an excretion half-life of about 1 hr. While salicylate has no effect on the process of excretion of salicylamide sulfate, there may be an inhibition of sulfate formation since the maximum excretion rate of salicylamide sulfate was consistently decreased in the presence of salicylate. This inhibition is also suggested by the data in Table IV, which shows the total composition of the urinary excretion products of salicylamide when given alone and with salicylate. While the output of salicylamide glucuronide decreased by about 100-mg. salicylamide equivalent in the presence of salicylate, the output of salicylamide sulfate increased by only about 20-mg. salicylamide equivalent. The major increase was in gentisamide output, which is usually very small when this dose of salicylamide is administered alone. Unfortunately, gentisamide output was determined only when salicylamide and salicylate were given together, but an estimate of

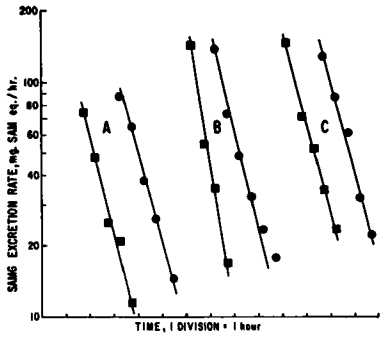

Fig. 4—Semilogarithmic plot of the terminal phase of salicylamide glucuronide excretion following oral administration of salicylamide alone (■) and 2 hr. after salicylate (●). Letters identify data from subjects A, B, and C.

TABLE IV—EFFECT OF SALICYLATE ON THE METABOLIC FATE OF SALICYLAMIDE IN MAN[a]

Subject	Sex	Age, yr.	Weight, kg.	Urinary Excretion Products[b], mg. SAM Equivalent: Total Recovery Control[c]	Total Recovery With SA[d]	SAMG Control	SAMG With SA	SAMS Control	SAMS With SA	GAM Control[e]	GAM With SA
A	M	39	81	569	548	374	226	196	268	<31	54
B	M	31	57	588	577	394	325	195	174	<12	78
C	M	27	86	550	590	326	234	224	233	<50	122
Mean				569	572	365	262	205	225	<31	85

[a] 0.6 g. salicylamide administered orally alone or 2 hr. following 2.32 g. sodium salicylate. [b] SA, salicylate; SAM, salicylamide; SAMG, salicylamide glucuronide; SAMS, salicylamide sulfate; GAM, gentisamide. [c] Excluding GAM. [d] Including GAM. [e] Estimated maximum value, dose − (SAMG + SAMS).

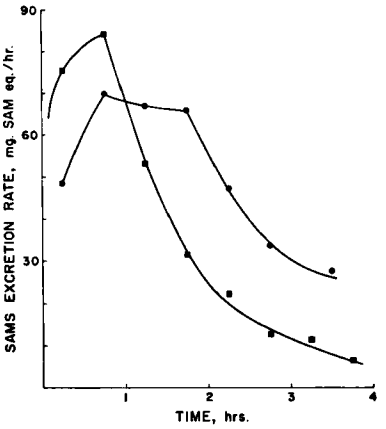

Fig. 5—Excretion rate of salicylamide sulfate following oral administration of 0.6 g. salicylamide alone (■) and 2 hr. after 2.32 g. sodium salicylate (●), Subject A.

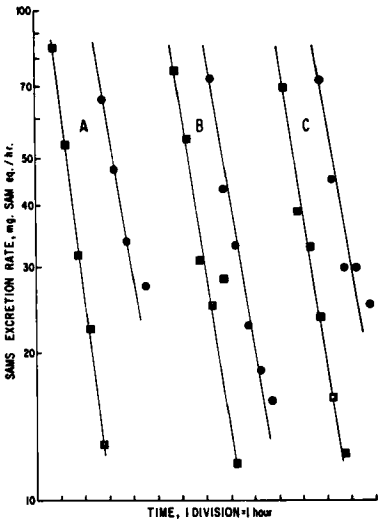

Fig. 6—Semilogarithmic plot of the terminal phase of salicylamide sulfate excretion following oral administration of salicylamide alone (■) and 2 hr. after salicylate (●). Letters identify data from subjects A, B, and C.

the maximum possible gentisamide output from salicylamide in the absence of salicylate has been obtained by subtracting the output of salicylamide glucuronide and sulfate from the total dose. While the lack of a significant increase in salicylamide sulfate formation may be due to the limited capacity of the body to form this metabolite (5), the decreased maximum excretion rate in the presence of salicylate is suggestive of salicylate-induced inhibition of sulfate formation. This problem is presently being studied in more detail.

The most likely reason for the mutual inhibition by salicylate and salicylamide of the formation of their respective glucuronides appears to be competition for uridine diphosphoglucuronic acid (UDPGA) or for UDP glucuronyl transferase, reflecting a limited capacity in man for glucuronide formation. Alternatively, or in addition, there may be a noncompetitive inhibition by salicylate of the glucuronide formation process. It has been suggested that high levels of salicylate inhibit the formation of salicylic glucuronides in mice due to an uncoupling effect of salicylate on oxidative phosphorylation (14); preliminary observations in this laboratory suggest that high levels of salicylate inhibit salicylic glucuronide formation also in man. Aspirin in doses as low as 0.6 g. decreases appreciably the urinary excretion of endogenous corticosteroid glucuronide in man, but not the excretion of free corticosteroid (15). The uncoupling effect of salicylate is thought to be responsible also for the decreased biosynthesis of sulfate esters following salicylate administration to rats (16); it is not unreasonable to suspect that the same effect could result in an inhibition of salicylamide sulfate formation in man. However, the results of the present study are only suggestive of salicylate-induced inhibition of salicylamide sulfate formation; the data can be explained also by assuming salicylate-induced delayed absorption of salicylamide although this latter possibility seems unlikely. The most important finding of this study is the pronounced mutual inhibition in the formation of glucuronides of and by two widely used and commonly available drugs given in usual therapeutic doses. One or both of these drugs is likely to have similar inhibiting effects on the glucuronide conjugation of other drugs; this is now being investigated in this laboratory. There is thus the possibility for frequent occurrences of interactions in drug biotransformation since many individuals taking prescribed drugs are also frequent users of mild analgesics, the latter being taken usually without knowledge of the attending physician. It is interesting also to speculate to what extent the many medicinal preparations consisting of combinations of salicylates with other drugs owe their enhanced effectiveness to a salicylate-induced inhibition of the elimination of one of these other drugs, rather than to the direct pharmacologic effects of salicylate itself.

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Keyphrases

Biotransformation—salicylamide, sodium salicylate
Salicylamide effect—salicylic glucuronide formation
Salicylate, sodium, effect—salicylamide glucuronide formation
Salicylurate excretion—salicylate administration
UV spectrophotometry—analysis

Effect of Complex Formation on Drug Absorption VII

Effect of Complexation and Self-Association on the Absorption of Caffeine

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The effect of the formation of caffeine complexes having a higher apparent lipoid–aqueous partition coefficient than caffeine itself on the absorption of this drug from the rat stomach was investigated. In one such system, caffeine–salicylic acid, the salicylic acid actually decreased the absorption of caffeine due to an effect of the former on the gastric mucosa. In another such system, caffeine–*p*-hydroxybenzoic acid, the *p*-hydroxybenzoic acid did not increase the absorption of caffeine. The intestinal transfer of caffeine alone was studied at low concentrations (where caffeine exists mainly in monomeric form) and at high concentrations (where significant self-association occurs). The intestinal transfer rate constant of caffeine at high concentrations was significantly lower than at low concentrations. The mechanism of this effect was explored.

In the initial studies in this laboratory of the effect of complex formation on drug absorption, it was found that complexation with caffeine decreased significantly the overall[1] absorption of salicylic acid from the stomach of the rat (1). This effect was qualitatively consistent with the apparent lipoid–aqueous partition coefficient (PC) of salicylic acid and of the caffeine–salicylic acid complex; the latter showed an appreciably lower apparent PC than the former. It appeared reasonable to assume that the absorption of caffeine might be enhanced by complexation with salicylic acid, since this complex has a higher apparent PC than caffeine itself (1). Studies were therefore initiated to test this assumption. The investigation was then extended to include a study of the caffeine–salicylate ion complex and the caffeine–*p*-hydroxybenzoic acid complex. The latter was of considerable interest since it has a higher apparent PC than caffeine as well as an appreciably higher stability constant than the salicylic acid–caffeine complex (2). In the course of these studies it became apparent that the self-association of caffeine at high concentrations (3) can affect the absorption of this drug. Therefore, this effect was studied also.

Received April 3, 1968, from the Biopharmaceutics Laboratory, Department of Pharmaceutics, School of Pharmacy State University of New York at Buffalo, Buffalo, NY 14214
Accepted for publication May 21, 1968.
This investigation was supported in part by grant R01-AM 08753-03 PET from the U. S. Public Health Service, Bethesda, Md., and by Public Health Service Predoctoral Fellowship No. F1-GM-24005 (for RHR) from the National Institute of General Medical Sciences.
Previous paper: Levy, G., and Mroszczak, E. J., *J. Pharm. Sci.*, **57**, 235(1968).
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[1] In the context used in this paper, "overall" refers to the combined effect or behavior of free and complexed drug.

EXPERIMENTAL

In Situ Gastric Absorption Rate Measurements—The procedure described previously by the authors (1) was followed with a few modifications. The