Effect of Glucosamine on Urinary Excretion of Salicylate

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Significant increases in the excretion rate of sodium salicylate were observed upon the addition of an equal amount of glucosamine hydrochloride to an orally administered solution dosage form. It is suggested that the enhanced excretion rate is the consequential result of consecutive reaction kinetics dictated by an increased absorption rate. A possible mechanism for this potentiation is explored.

THE EFFECT OF adjuvants on drug absorption has attracted considerable attention (1). An adjuvant, in this context, is considered an inert material which will modify the rate or extent of absorption of a drug. In many instances, the adjuvant is the solvent or a component of the vehicle (1).

Numerous attempts have been made to potentiate the absorption of drugs for a more rapid attainment of therapeutic blood levels. Soluble citrates have been used in attempts to potentiate the absorption of tetracycline antibiotics. Similar claims have been made for glucosamine (1). Nelson's carefully controlled experiments, however, have indicated no significant differences in the absorption of tetracycline hydrochloride when administered with various adjuvants, including citric acid and glucosamine hydrochloride (2).

Glucosamine potentiation of salicylate absorption has been claimed (3), but the reported data are limited to mean values; no statistical justification was presented to substantiate the published results. Furthermore, no attempts were made to elucidate the mechanism by which glucosamine operates to increase the absorption rate. Wagner has pointed out that although adjuvants have been shown to alter absorption, usually insufficient control of variables and inadequate description of the experiments cast considerable doubt on the conclusions drawn (1).

Therefore, it was decided to re-evaluate the effect of glucosamine hydrochloride on salicylate absorption under stringent experimental conditions utilizing urinary excretion kinetics.

EXPERIMENTAL

Materials.—Crystalline sodium salicylate U.S.P. was employed for each experiment in doses of 0.3 Gm. This amount of drug was dissolved prior to administration in 20 ml. of distilled water, alone or in combination with an equal amount (0.3 Gm.) of glucosamine hydrochloride.[1]

Absorption Studies.—Healthy male adults were selected to serve as a test group. Each subject fasted for at least 9 hr. before and for 2 hr. after the start of each experiment. Subsequent to the 2-hr., postingestion fasting period, the subject was permitted to return to his accustomed diet. No attempt was made to restrict the usual diet or activity of the individual after the first 2 hr. of the experimental period. The only specific exception to this routine was the exclusion of dairy products, such as cheese and milk, from the diet. This restriction was necessitated by the urinary alkalinization produced by such foods, and was enforced during the entire experimental period.

To minimize the effect of hunger created by the overnight fasting period, all experiments were started between 8 and 10 a.m. Preliminary experiments initiated considerably later than this time period consistently showed a tendency toward urinary alkalinization.

The subjects were instructed to void their bladder of overnight urine about 2 hr. prior to the absorption experiment. A urine sample was collected immediately prior to the administration of the drug to obtain blank values. The drug solutions were administered together with 180 ml. of water. The solution dosage form was chosen to obviate the multiplicity of factors influencing the absorption rate of drugs from solid dosage forms. Replicate experiments were performed with each subject, and the individual served as his own control.

Urine samples were generally collected at 1, 2, 3, 4, 6, 8, and 12-hr. intervals after ingestion of the test solution. The time of urine collection and the volume were noted carefully. The interval between successive experiments in the same individual was 2 days or more. The test solutions were administered in a random fashion.

Urine pH.—In view of the fact that relative excretion rates were being measured, it was critical that the elimination rate constant of the drug remain independent of extraneous factors. In consideration of the strong dependency of salicylate excretion on urine pH (4–7), the pH of all urine samples was determined upon collection. A Beckman Zeromatic pH meter was used to obtain the pH values.

The urine pH of the subjects generally ranged from values of 5.1 to 6.0. On occasion, elevations of a magnitude of 1 to 2 pH units were observed without apparent reason, other than normal physiologic variations. Such elevation in pH results in an increase in the ratio of excreted salicylic acid to excreted metabolites (SA_e/SG_e+SU_e),[2] as well as an

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[1] Glucosamine hydrochloride was supplied through the courtesy of Pfizer Laboratories, Brooklyn, N. Y.

[2] SA = salicylic acid, SG = salicylglucuronide, SU = salicyluric acid.

TABLE I.—URINARY EXCRETION OF SALICYLATE[a] AFTER ORAL ADMINISTRATION OF SODIUM SALICYLATE WITH AND WITHOUT GLUCOSAMINE HYDROCHLORIDE

	1 hr.				2 hr.					
	Control[b]		Glucosamine[c]		Control		Glucosamine		K[d]	
Subject	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Control	Glucosamine
A	27.0	21.5	30.7	26.5	58.9	51.3	64.0	58.2	0.050	0.055
B	26.2	25.7	28.6	28.1	59.4	56.9	66.3	64.6	0.122	0.133
C	18.3	19.6	21.4	18.8	52.8	48.7	52.5	49.5	0.170	0.127
D	15.0	16.0	19.1	18.2	43.7	49.0	64.4	56.5	0.099	0.117
E	25.5	24.9	30.1	36.1	60.6	57.0	68.3	76.5	0.126	0.114
Mean	22.3		25.8		54.4		62.1		0.113	0.109

[a] Expressed as milligrams of salicylic acid. [b] Sodium salicylate (0.3 Gm.) in 20 ml. of water. [c] Sodium salicylate (0.3 Gm.) and 0.3 Gm. of glucosamine hydrochloride in 20 ml. of water. [d] Elimination rate constant (hr.$^{-1}$).

over-all increase in the elimination rate constant. An unusually high urine pH during one test period and not during another could distort significantly the comparative data and result in erroneous conclusions. Therefore, it was decided to discontinue any experiment in which a urine sample with a pH value greater than 6 was obtained

Analytical Methods.—Urine salicylate concentration was determined colorimetrically according to Trinder (8) with a Bausch & Lomb Spectronic 20 spectrophotometer at 525 mμ. All readings were corrected for blank values.

The use of ferric salts in the determination of salicylate in urine is nonspecific, since the development of a purple color will occur in the presence of any free phenolic group. Thus, both salicylic acid and its metabolite, salicyluric acid, will react to varying degrees with ferric ion and produce a purple color. Estimation of urine salicylate results in the determination of apparent salicylate, *i.e.*, a combination of salicylic and salicyluric acids. The second principal metabolite of salicylic acid, the glucuronide, does not have a free phenolic group and will not be detected by this procedure.

To insure that glucosamine hydrochloride was not affecting the metabolism of salicylate, a differential assay was employed selectively to determine individually both salicylic and salicyluric acid in the urine. The assay utilized was first suggested by Smith *et al.* (5) and is based on the differential extractability of salicylate and salicylurate by ethylene dichloride and carbon tetrachloride. Smith and his colleagues derived equations which permit the separate estimation of these materials.

Excretion Rates and Elimination Rate Constants.—Individual cumulative plots of the amounts of salicylate excreted *versus* time were constructed. The method used for estimating the excretion rates was the linear approximation of an arc, where the slope of this line approximates the instantaneous slope at the midpoint of the arc. The logs of individual excretion rates then were plotted against time, enabling the calculation of the individual elimination rate constant (9). The latter was estimated by linear regression

Salicylate–Glucosamine Interaction.—To determine the existence of a salicylate–glucosamine interaction, a diffusion study was conducted. The test solutions were placed within a dialysis tube formed by a 7-cm. length of Visking membrane. The tube was sealed at both ends, then suspended in citrate buffer (pH 5.2) maintained at 37°. The outer solution was stirred, and samples were withdrawn at 5-min. intervals. In one experiment, a solution containing 0.002 mole of sodium salicylate and 0.002 mole of sodium chloride was studied. In a second experiment, the sodium chloride was replaced with 0.002 mole of glucosamine hydrochloride. In each case, citrate buffer at pH 5.2 was used as the solvent.

The pH of 5.2 was chosen since it is intermediate to the pKa of salicylic acid (pKa 3) and the pKb of glucosamine (pKb 7.5). At this pH, the total amount of undissociated electrolyte is maximal, and the conditions for ionic interaction are optimal.

RESULTS AND DISCUSSION

Results of the comparative study of urinary excretion of salicylate metabolite(s) in the presence and absence of glucosamine hydrochloride are shown in Table I. The mean data for the first 4 hr. of study are plotted in Fig. 1. The results reveal a small but definite increase in the initial excretion rate of salicylate after coadministration of glucosamine hydrochloride. The difference in amounts of excreted salicylate at the 1- and 2-hr. level is statistically significant. Analysis of variance yielded an F value >13 ($p \cong 0.002$) for the 1-hr. values of Table I. The difference between the 2-hr. and 1-hr. values (*i.e.*, amounts excreted between 1 and 2 hr.) gives $F = 9.8$ ($p \cong 0.008$).

It is of interest that a rather high degree of uniformity within each group was observed. Levy (10) had stated that large variation in salicylate excretion within a group is apparently related to differences in gastric emptying. It is believed that the administration of the drug in solution with a large volume of water induces gastric emptying, and transport of the drug from the stomach to the intestines will therefore be rapid. This may account, in part, for the more uniform results obtained in this study.

As noted, the Trinder assay is nonspecific and gives a measure of both free salicylate and salicyluric acid. The two species, however, at equimolar concentration do not show equal absorbances. Solutions of salicyluric acid[3] were analyzed and found to have 0.81 as much color as an equimolar quantity of salicylic acid. Smith *et al.* (5) reported a value of 0.82. To determine if the glucosamine-induced increase in urinary excretion of apparent salicylate was due to a decrease in the SU_e/SA_e ratio, differential assay of samples was performed. A decrease in this ratio would result in a higher amount of apparent salicylate, even though the sum, SA_e+SU_e, may remain constant.

The results indicated that where the urine pH remained below the chosen experimental level of 6, no measurable excretion of salicylic acid was ob-

[3] Salicyluric acid was supplied through the courtesy of Dr. Kenneth C. Blanchard, Johns Hopkins School of Medicine, Baltimore, Md.

served. In all cases only salicyluric acid was being detected by the Trinder assay.

Further evidence of the exclusivity of the glycine conjugate in the ultimate fate of the dose of sodium salicylate (300 mg.) was provided by experiments with four subjects from whom urine was collected over a 24-hr. period. In these studies, assays were performed according to Smith *et al.* (5) to determine quantitatively the glucuronide metabolite (SG_e) as well as SA_e and SU_e. In nine such experiments (three of which were conducted with glucosamine HCl), salicyluric acid excreted in the urine accounted for 92 to 98% of the given dose. The glucuronide accounted for 0 to 2% of the administered dose. No trace of SA_e was detected.

It was concluded that glucosamine does not interfere with the metabolism of salicylate to salicylurate. The glycine conjugate was the sole phenolic salicylate derivative present in the urine, regardless of whether glucosamine was administered. In addition, the enhanced excretion rate of salicylurate does not appear to be manifested at the expense of the glucuronide.

The metabolism of numerous drugs and foreign substances has been described as a first-order process (11–16). In view of the findings of Bray *et al.* (17), in their studies of the formation of hippuric acid, and in consideration of the low doses of sodium salicylate employed in this study, it is reasonable to assume that the formation of SU involves a first-order process, *i.e.*,

$$d\mathrm{SU}/dt = k_m\mathrm{SA} \qquad \text{(Eq. 1)}$$

where k_m is the metabolic rate constant, and SA is the amount of salicylic acid in the body.

In a number of other studies where sulfates, glucuronide ethers, and glycine conjugates have been formed as drug metabolites, it has been found that the rate of their elimination was greater than the rate of their formation (11–14). If one assumes the following sequence

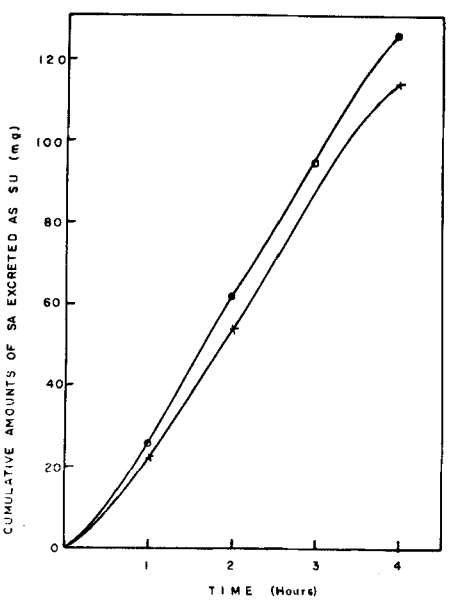

Fig. 1.—Mean cumulative excretion plot of orally administered sodium salicylate + glucosamine HCl (○), contrasted with orally administered solution of an equivalent dose of sodium salicylate alone (×).

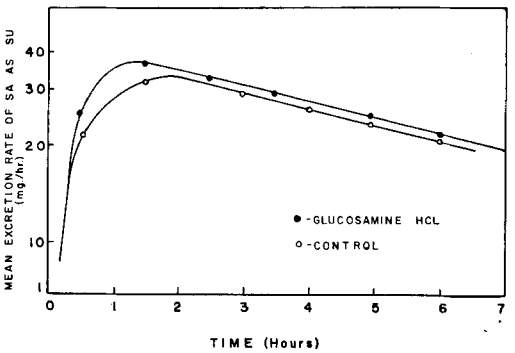

Fig. 2.—Mean excretion rate of salicylic acid as its glycine conjugate, salicyluric acid, after administration of sodium salicylate in a 0.3-Gm. dose.

$$\mathrm{SA} \xrightarrow{k_m} \mathrm{SU} \xrightarrow{k_e} \mathrm{SU}_e \qquad \text{(Eq. 2)}$$

then a rate-limiting process, such as that suggested by Eq. 2, necessitates that $k_e \gg k_m$.

Although this assumption must be employed cautiously, it appears to have validity in this particular study. It has been observed repeatedly that the concentration of salicyluric acid in the human plasma is zero, or so close to zero to be within the limits of error of the determination (5–7). On the other hand, as noted above, the glycine conjugate fully accounts for the salicylate in the urine. If the plasma concentration of salicyluric acid is truly zero, then the renal clearance of this conjugate is infinitely high. Even if traces of the conjugate are present in the plasma, the resultant clearance would be within the range of that of iodopyracet or *p*-aminohippurate (7). In either case, it is reasonable to as sumethat salicyluric acid is excreted predominantly by the tubules and perhaps is even produced there.

In view of these considerations, the two-step formation and excretion process of salicyluric acid would be rate-limited by the metabolic transformation. Thus, the rate of excretion of salicyluric acid would involve pseudo first-order kinetics rather than consecutive reaction kinetics. Mathematically, the above relationships are expressed as

$$d\mathrm{SU}_e/dt \cong d\mathrm{SU}/dt = k_m\mathrm{SA} \qquad \text{(Eq. 3)}$$

Cummings and Martin (18) have questioned the validity of Eq. 3. These authors note that "when . . . a drug is slowly metabolized and the metabolite is rapidly excreted, that is when $k_f < k_e$, the drug and metabolite would always be excreted together and the observed rate of metabolite excretion is a function of k_f and k_e."[4]

This criticism does not appear to apply to the use of Eq. 3 in this investigation, since free salicylate was not excreted concurrently with salicylurate. It is of interest that these findings seem to indicate that at the dosage level employed there was an almost total tubular reabsorption of salicylic acid.

Therefore, assuming Eq. 3 is valid, one may predict that the rate of excretion of the metabolite will approximate a first-order process and will be proportional to the amount of free salicylate in the body. Log excretion rate *versus* time plots were

[4] k_f refers to the formation rate constant (SA → SU). In this paper k_m is employed.

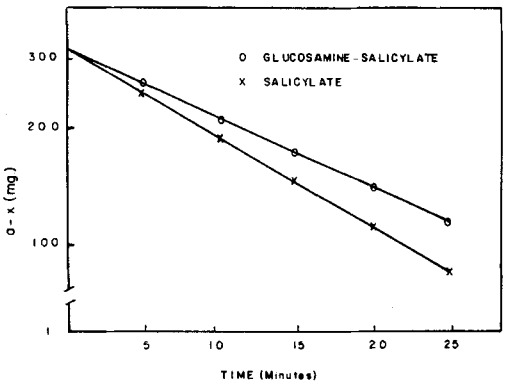

Fig. 3.—Semilogarithmic plot illustrating the diffusion of sodium salicylate, alone and in the presence of glucosamine hydrochloride at pH 5.2. (a = milligrams of sodium salicylate in dialysis membrane at time zero; x = milligrams of sodium salicylate which has diffused at time *t*.)

constructed and found to be linear in the post-absorption phase. This is indicative of an over-all first-order excretion of the metabolite. A semi-log plot of mean excretion rate *versus* time is shown in Fig. 2. The linear portion of the individual curves provided the elimination rate constants shown in Table I. According to the model of salicylate metabolism and excretion proposed to explain the expermental findings, these elimination rate constants should approximate closely the transformation rate constants for the metabolism of SA to SU.

The biological half-life of salicylic acid calculated in this study was about 6.2 hr. This is in excellent agreement with the 6-hr. half-life reported by Levy and Sahli (19) and the 6.1-hr. half-life reported by Brodie and Burns (20). The latter value was calculated on the basis of plasma level studies.

If dSU_e/dt is proportional to the amount of salicylic acid in the body, then providing k_m is invariant, comparison of excretion rate should provide a comparison of absorption rates under different experimental conditions. It is quite doubtful that glucosamine affects the formation or excretion of salicylurate. This contention is supported by the ubiquity of the glycine conjugate and the fact that no significant difference in the elimination rate constants was observed between the glucosamine experiments and the controls (Table I). Since glucosamine is excreted solely by glomerular filtration and is not significantly reabsorbed (21), any complexation with salicylate which may inhibit metabolism would most certainly result in the excretion of free salicylate. In addition, glucosamine does not appear to affect transport processes in the tubules. For example, it does not interfere with glucose reabsorption or with the tubular secretion of *n'*-methylnicotinamide (22).

Before attributing the enhanced metabolite excretion rate observed in the presence of glucosamine to a potentiation of the absorption rate, one further consideration must be examined. It is conceivable that the difference in excretion rate is due to an effect of glucosamine on the protein binding of salicylate. For example, sulfinpyrazone, a uricosuric agent, appears to function—at least partially—by displacing uric acid from its association with plasma protein and thereby promoting urate excretion (23). It is unlikely that glucosamine functions in a manner similar to sulfinpyrazone, since equilibrium dialysis experiments have shown that glucosamine is not bound to plasma protein (22).

Thus, it appears that the increased salicylurate excretion rate obtained after coadministration of glucosamine HCl is a result of an increase in the amount of salicylate in the body and, in turn, of an increased absorption rate of the drug in the presence of glucosamine.

The mechanism of this potentiation does not appear to be attributable to simple physical-chemical principles. In an attempt to obtain a better understanding of this phenomenon, diffusion studies were undertaken to ascertain the existence of a possible interaction between salicylate and glucosamine. The results are depicted in Fig. 3. The diffusion of salicylate is considerably delayed in the presence of glucosamine. A 25% difference exists between the half-lives of the two diffusion experiments. These results offer support for the presence of a glucosamine–salicylate interaction. This is further substantiated by a recent patent which claims the preparation of glucosamine acetylsalicylate. This compound is prepared by conversion of glucosamine hydrochloride to the free base and the subsequent addition of the base to an aqueous slurry of aspirin (24).

Consideration of the above data suggests the possibility of the *in vivo* existence of such an interaction, particularly when the effective pH of the intestine has been reported as 5.3 (25). Evidence of the active transport of glucosamine has been reported (26). Although not experimentally justified, the possibility of membrane penetration of ionic salicylate combined with glucosamine provides an interesting consideration. Such a mechanism may provide an explanation to the rather unusual effect of glucosamine on salicylate absorption.

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