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Application of Salivary Salicylate Data to Biopharmaceutical Studies of Salicylates

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Abstract □ Concentrations of salicylic acid in saliva and plasma were measured in three subjects following the administration of 650 mg. of aspirin. Concentrations of salicylic acid in saliva were proportional to concentrations in plasma, saliva–plasma ratios in the three subjects being 0.0293 ± 0.0013, 0.0303 ± 0.0033, and 0.0394 ± 0.0043. The saliva–plasma ratios were independent of the plasma concentrations of salicylic acid observed in this study, the maximal concentration being 50 mcg./ml. Measurement of salivary concentrations of salicylic acid may be a useful technique in the evaluation of different formulations of aspirin or other salicylates. In the present study, delayed release of aspirin from enteric-coated tablets was demonstrated from the time course of the concentration of salicylic acid in saliva.

Keyphrases □ Salivary excretion—relationship between salicylic acid concentration in saliva and plasma after aspirin administration, man □ Salicylic acid levels after aspirin administration—relationship between saliva and plasma concentrations, man □ Absorption, aspirin—relationship between salicylic acid concentration in saliva and plasma, man □ Aspirin absorption—relationship between salicylic acid concentration in saliva and plasma, man

The secretion of drugs and other foreign compounds in saliva is well known. The salivary excretion of weak acids such as salicylate (1), sulfonamides (2, 3), and barbiturates (3, 4) has been investigated in some detail. Killman and Thaysen (2) found that the concentrations of several sulfonamides in human saliva were proportional to the concentration of the unbound drug in plasma. These observations indicated that measurement of concentrations of drugs in consecutive samples of saliva may be a useful technique in investigations of the kinetics of absorption and elimination of drugs, although difficulties may arise if the plasma binding characteristics of the drugs varied significantly during the time course of the experiments.

In the present study, the relationship between the concentrations of salicylic acid in saliva and plasma was investigated at various times after aspirin administration. Furthermore, the measurement of salicylic acid levels in serial samples of saliva was examined as a technique in the evaluation of aspirin preparations, although the different preparations were not evaluated thoroughly. The aim of the present work was to develop a technique that would obviate the need for collecting blood samples in some biopharmaceutical studies with salicylates. All studies were conducted using mixed saliva. It was considered that methods of collecting saliva should be as simple as possible if measurements of salivary levels of salicylic acid were to have practical value in the evaluation of different pharmaceutical formulations.

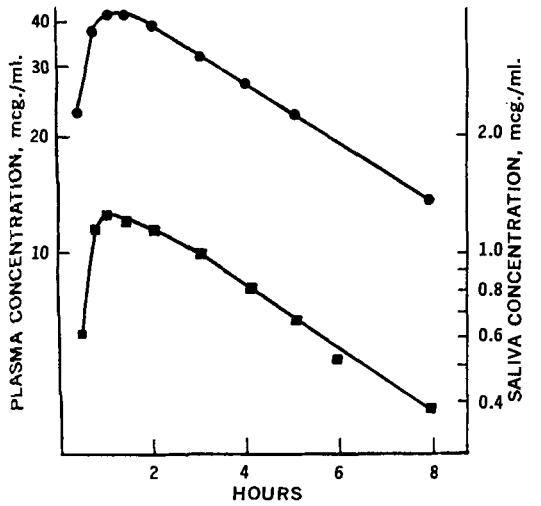

Figure 1—*Concentrations of salicylic acid in saliva and plasma following the administration of 650 mg. aspirin in hard gelatin capsules, Subject 1. Key: ●, plasma; and ■, saliva.*

EXPERIMENTAL

Three healthy male subjects were used in the study. Aspirin was administered in two dosage forms. Hard gelatin capsules containing 650 mg. aspirin[1] were prepared extemporaneously. Enteric-coated tablets[2], each containing 325 mg. aspirin, were also administered. The capsules or tablets were taken with approximately 200 ml. of water, 1–3 hr. after breakfast. Food was withheld for at least 2 hr. after dosage. The subjects were instructed to cleanse their mouths after any meal or drink by rinsing with water. Toothpaste could not be used, because small amounts of a compound which interfered with the salicylic acid assay were left in the mouth.

Mixed saliva was collected at approximately 1-min. intervals for a total of 3 min. at the times listed below. Saliva production was stimulated by chewing on a small piece of Teflon. The mixed saliva, produced at a rate of 3–8 ml./3 min., was allowed to drain into 20-ml. screw-capped vials and frozen until assayed. When taken, blood samples were withdrawn at the midpoint time of the saliva collection. Simultaneous blood and saliva samples were collected following the administration of aspirin capsules in one test on each of the three subjects. In three further studies on each subject, saliva only was collected. Aspirin capsules were administered in two of the latter tests, while enteric-coated tablets were administered in the third. At least 1 week elapsed between the different studies. Following dosage with aspirin capsules, the blood and saliva samples were usually taken at 0, 15, 30, 45, 60, 90, and 120 min. and thereafter at approximately hourly intervals for a further 6–8 hr. After the administration of the enteric-coated aspirin tablets, saliva was collected at 0.5-hr. intervals for 5 hr. and then hourly for 7–9 hr.

Salicylate in plasma was extracted by the method of Rowland and Riegelman (5). This method was modified slightly in the assay of salicylate in saliva. Aliquots of saliva (2–5 g.) were weighed into screw-capped tubes and diluted to 5 ml. with water. The samples were acidified with 25% potassium hydrogen sulfate solution (0.5 ml.) and extracted with ether (6 ml.) by mixing on a tilt-action shaker for 15 min. The resulting coarse emulsion was easily broken by centrifugation. Aliquots of the ether extract (4 ml.) were then shaken with 0.1 *M* phosphate buffer, pH 7.0 (5 ml.). Traces of ether were removed from the phosphate buffer by blowing air through the solutions for 30 sec. Aqueous standard solutions of salicylic acid were extracted in a similar fashion.

Fluorescence of the final solutions was measured on a spectrofluorometer[3]. The excitation wavelength was 305 nm., while fluorescence was measured at 400 nm. for plasma extracts and at 350 and 400 nm. for saliva extracts. The emission spectrum of salicylic acid at pH 7.0 is maximal at 400 nm. and minimal at 350 nm. The fluorescence intensity of control saliva was usually low and relatively constant; fluorescence intensity at 400 nm. of control saliva was subtracted from the intensity of unknowns to give the fluorescence intensity due to salicylate. In one subject the fluorescence intensity of control saliva was variable, although quite low. In this case the fluorescence intensity due to salicylic acid in saliva was calculated from the relationship:

$$X = \frac{R}{R - 0.02}\left(A - \frac{B}{R}\right) \qquad \text{(Eq. 1)}$$

where:

X = fluorescence intensity at 400 nm. due to salicylate
A = total fluorescence at 400 nm.
B = total fluorescence at 350 nm.
R = ratio of fluorescence intensity of control saliva at 350 nm. to intensity at 400 nm. and is assumed to remain constant throughout the study

The ratio of fluorescence intensity of salicylic acid at 350 nm. to intensity at 400 nm. was 0.02.

Standard curves were linear in the range 0.4–6 mcg./5 ml. and 2–12 mcg./0.2 ml. in the assay of salicylic acid in saliva and plasma, respectively. The recovery of salicylic acid from saliva was not significantly different from recovery from aqueous standard solutions. The concentration of salicylic acid in saliva was expressed as micrograms per milliliter, the weight per milliliter of saliva being 1.00 g. Replicate assays of salicylic acid in saliva indicated that the procedure was accurate to within ±0.02 mcg./ml. In the reported experiments, the concentrations are from single assays of samples of plasma and saliva.

In preliminary studies, no significant quantities of hydrolyzable salicylate (aspirin) were detected in saliva by the method of Rowland and Riegelman (5). Consequently, no attempt was made to prevent the hydrolysis of aspirin in the saliva samples.

RESULTS

After simultaneous collection of saliva and blood, time course plots of salicylic acid levels in saliva and plasma showed good parallelism in each of the three subjects (Fig. 1). Ratios of the concentration of salicylic acid in saliva to the concentration in plasma are shown in Table I. The coefficient of variation in these ratios in each subject was very small. No significant trends in the saliva–plasma ratios were detected, either with time or with the plasma concentration of salicylic acid. No delay was detected between the appearance of salicylic acid in plasma and saliva. As anticipated from these results, the correlations between levels of salicylic acid in saliva and plasma were highly significant, $p < 0.001$

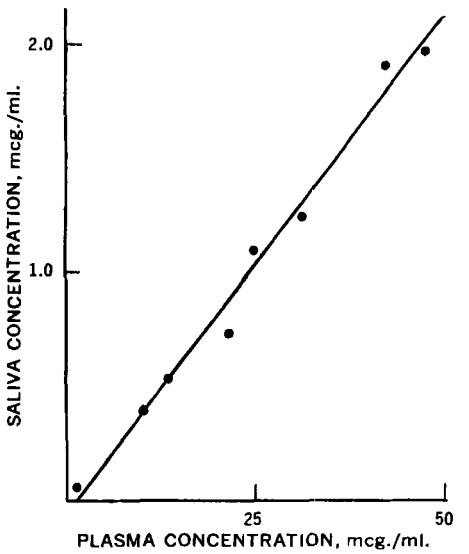

Figure 2—*Relationship between salivary and plasma levels of salicylic acid, Subject 3.*

[1] Merck & Co., Rahway, N. J.
[2] Ecotrin, batch code 860h24, Smith Kline & French, Philadelphia, Pa.
[3] Perkin-Elmer Hitachi, model 203.

Table I—Relationships between Salivary and Plasma Concentrations of Salicylic Acid

	Subject 1	Subject 2	Subject 3
Saliva–plasma ratio (±coefficient of variance)	0.0293 (±4.4%)	0.0307 (±10.8%)	0.0394 (±10.9)
Regression of salivary concentration *versus* plasma concentration:			
Slope	0.0316	0.0302	0.0370
(95% confidence limits)	(0.0292 to 0.0340)	(0.0266 to 0.0338)	(0.0313 to 0.0427)
Intercept	−0.062	+0.013	−0.071
(95% confidence limits)	(−0.139 to +0.015)	(−0.071 to +0.098)	(−0.230 to +0.090)
Correlation coefficient between salivary and plasma concentrations	0.997	0.988	0.992

in the three subjects, with correlation coefficients ranging from 0.988 to 0.997 (Table I and Fig. 2). Consistent with the parallelism between the salivary and plasma levels of salicylic acid, the slope of the regression line of salivary concentration was very similar to the mean saliva–plasma ratio. Furthermore, the intercept of the regression line was not significantly different from zero (Table I).

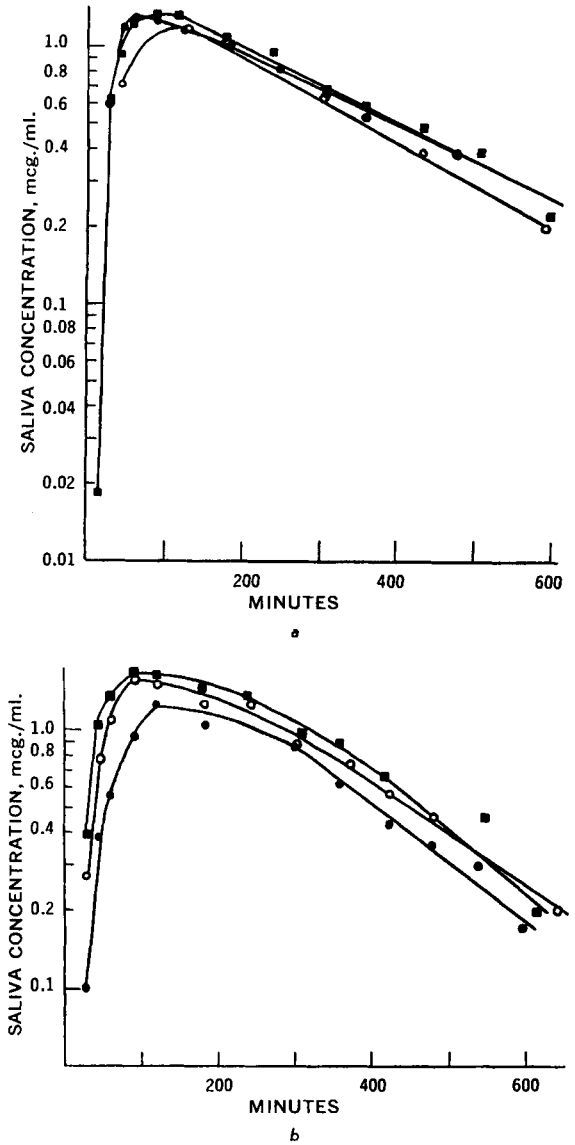

Figure 3—*Concentrations of salicylic acid in saliva following the administration of 650 mg. aspirin in hard gelatin capsules on three different occasions. Blood samples were also taken in one experiment* (●). *Key:* a, *Subject 1; and* b, *Subject 2.*

Saliva–plasma ratios of salicylic acid were not affected by venipuncture, although the flow rate of saliva was clearly reduced during venipuncture. In studies where both saliva and blood were collected, some saliva samples were taken without simultaneous venipuncture. In these studies, the concentrations of salicylic acid collected in the presence or absence of venipuncture fell on the same smooth concentration–time curves. Further evidence for a lack of effect of venipuncture on the salivary levels of salicylic acid was obtained from the two studies where saliva only was collected following dosage with aspirin capsules. The salivary concentration–time curves were very similar in the three studies on Subjects 1 and 3 (Fig. 3). Salivary levels of salicylic acid differed at any given time in the three studies on Subject 2, although the forms of the concentration–time curves were very similar (Fig. 3).

Parallelism between the concentrations of salicylic acid in saliva and plasma was also demonstrated by the similar half-lives in these fluids. Half-life values shown in Table II were calculated from the concentrations of salicylic acid in saliva and plasma from 3 to 11 hr. after administration of aspirin. Elimination of salicylic acid appeared monoexponential within this period.

Area analysis was consistent with the parallelism between the concentrations of salicylic acid in saliva and plasma. In each subject, the ratio between the areas under the salivary and plasma concentration–time curves was similar to the mean saliva–plasma ratio (Table II). The areas under the salivary concentration–time curves differed in repeated studies on the same subject. The greatest differences were noted in Subject 2, where the salivary levels of salicylic acid varied significantly, although the forms of the salivary concentration–time curves were very similar.

The salivary levels of salicylic acid were also determined in each of the three subjects following the administration of enteric-coated tablets. The studies were not continued for a sufficient time to evaluate the products fully. However, differences between the absorption of aspirin from capsules and enteric-coated tablets were quite evi-

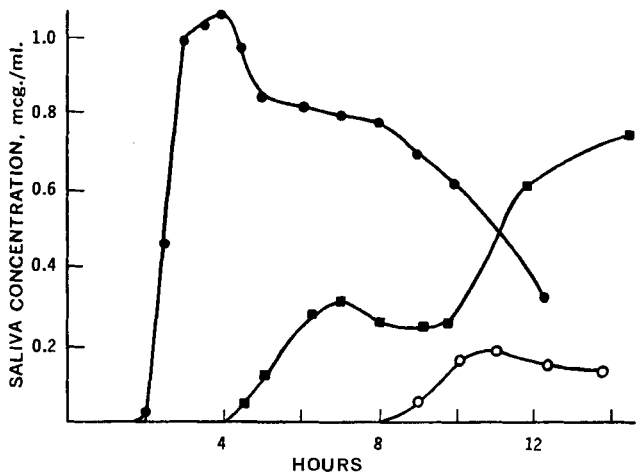

Figure 4—*Concentrations of salicylic acid in saliva of three subjects following the administration of 650 mg. aspirin in enteric-coated tablets.*

Table II—Half-Lives and Areas under Salicylic Acid Concentration–Time Curves

	Subject 1	Subject 2	Subject 3
Half-life, min.:			
Saliva	211[a], 170, 191	159[a], 169, 164	160[a], 187, 242
Plasma	233[a], 193	169[a]	221[a]
Area, mcg. min. ml.$^{-1}$:			
Saliva	486[a], 402, 481	424[a], 531, 594	729[a], 866, 882
Plasma	17,200[a], 13,900	14,000[a]	19,700[a]
Area ratio, saliva–plasma	0.0283	0.0302	0.0370

[a] Indicates results of experiments where both saliva and blood samples were collected.

dent. The absorption of aspirin was delayed for up to 8 hr. after dosage with the enteric-coated tablets, the delay being quite different in the three subjects (Fig. 4). By comparison, significant levels of salicylic acid were found in saliva 30 min. after the administration of aspirin capsules (Figs. 1 and 3).

DISCUSSION

Saliva–plasma ratios of salicylic acid reported in this paper differ markedly from those found in a previous study. Leulier *et al.* (6) found parotid saliva–plasma ratios of 0.31–0.76 when plasma levels ranged from 290 to 480 mcg./ml. after a dose of 6 g. sodium salicylate. These plasma levels and saliva–plasma ratios were considerably higher than those attained in the present study. However, lower saliva–plasma ratios of salicylic acid were anticipated at the lower plasma concentrations observed in the present study, since the fraction of protein-free salicylic acid in plasma decreases as the plasma concentration decreases (7) and a diffusional equilibrium is established between the concentration of lipid-soluble drugs in saliva and the concentration of the protein-free, unionized forms of the drugs in plasma (2, 3). Accordingly, the saliva–plasma ratio of salicylic acid might change slightly in serial samples of saliva following a single dose of aspirin or salicylic acid. However, in the present study, no significant correlation was observed between the saliva–plasma ratios and the concentrations of salicylic acid in plasma ranging up to 50 mcg./ml. Proportionality between salivary and plasma levels of salicylic acid cannot be assumed outside this range of plasma concentrations.

The parallelism between the salivary and plasma concentration–time curves is also in contrast to previous work. Borzelleca and Putney (1) measured the levels of salicylic acid in parotid saliva and plasma of dogs anesthetized with pentobarbitone (pentobarbital), saliva flow being stimulated by pilocarpine. Salivary levels of salicylic acid were dose related. However, in time-course studies, the saliva–plasma ratios of salicylic acid increased throughout the experiments, in marked contrast to the constant ratios observed in this study. The experimental conditions are markedly different in these two studies and no hypothesis is suggested for the different findings without further experimentation.

The proportionality between salicylic acid levels in saliva and plasma indicated that measurement of salicylic acid levels in saliva may be a useful technique in the evaluation of different salicylate preparations. Differences in the release of aspirin from enteric-coated tablets and capsules were clearly shown in the present study. Although the enteric-coated tablets were not fully evaluated, the time of release of aspirin from these tablets appeared very variable. Some variation was also noted in the availability of aspirin capsules. The dissolution of aspirin is influenced by formulation in hard gelatin capsules (8) and may lead to erratic absorption. Aqueous solutions of aspirin are completely absorbed (8) and have been used as reference dosage forms in studies on the availability of aspirin from formulations. However, some aspirin was retained in the mouth after ingestion of solutions of aspirin (650 mg. in 250 ml.), and capsules were used as the reference dosage form in this study.

Measurement of the excretion of salicylates in urine has been used as an alternative technique for the estimation of plasma levels in the evaluation of different formulations of aspirin. The total recovery of salicylates in urine is a good index of the absorption of different preparations of aspirin, since over 95% of orally administered doses can be recovered in urine (9). However, the rate of elimination of free salicylate in urine fluctuates considerably (9); and the rate of excretion of the major metabolite, salicyluric acid, does not parallel plasma levels of salicylic acid (10). While only a limited number of subjects were examined in the present study, measurement of salicylic acid levels in saliva should give a more accurate estimate of changes in the plasma concentration of salicylic acid than can be obtained from urinary studies.

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