# Application of Salivary Concentration Data to Pharmacokinetic Studies with Antipyrine

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**Abstract** □ The concentrations of antipyrine in plasma and saliva were equivalent in normal volunteers and in patients with acute viral hepatitis following oral doses of antipyrine. Estimates of clearance, volume of distribution, and half-life made from either plasma or saliva samples were not statistically different in these subjects. Differences in the disposition kinetics of antipyrine during the acute phase of viral hepatitis and on recovery were detected using either plasma or saliva samples.

**Keyphrases** □ Antipyrine—pharmacokinetics, normal subjects and hepatitis patients, plasma and saliva concentration data □ Pharmacokinetics—antipyrine, normal subjects and hepatitis patients, plasma and saliva concentration data □ Analgesics—antipyrine, pharmacokinetics, normal subjects and hepatitis patients, plasma and saliva concentration data

Antipyrine, used as an investigational marker of drug metabolism in humans, is thought to be metabolized in the liver; when given orally, less than 0.1% of the dose is excreted unchanged in the stool and less than 5% is excreted unchanged in the urine (1). The plasma half-life of antipyrine has small intrasubject variability (2–5) and has been correlated with genetic (2, 5, 6) and environmental (2, 3) factors, sex, and age (7). The antipyrine half-life or clearance also correlates with the half-life or clearance of phenylbutazone (4), phenytoin (8), and warfarin (9). However, no significant correlation was found between the antipyrine half-life and the half-life of amobarbital, glutethimide, or sulfinpyrazone (10). Because the many studies with antipyrine may eventuate in its use as a marker of drug metabolism, we decided to investigate the possibility that the disposition kinetics of antipyrine could be determined by studying the concentrations of antipyrine in saliva.

The concentrations of drugs in saliva have been used to determine the disposition kinetics of many drugs [*e.g.*, salicylate (11), theophylline (12), acetaminophen (13), digoxin (14), sulfonamides (15, 16), and tolbutamide (17)]. In humans, the concentration of antipyrine in saliva correlates with that in plasma (18).

Recently, two papers reported the equivalence of antipyrine concentrations in plasma and saliva and demonstrated the utility of salivary antipyrine measurements in estimating pharmacokinetic parameters in normal subjects and epileptic patients (19, 20). The present study confirmed these findings and extends the use of antipyrine salivary concentrations by demonstrating their validity in patients with acute viral hepatitis.

## EXPERIMENTAL

**Subjects and Treatment**—Subjects 1 and 2 were normal, healthy, male volunteers who weighed 70.1 and 75 kg, respectively. Subjects 3 and 4 were studied during the acute phase of viral hepatitis (Studies 3A and 4A) and after recovery (Studies 3B and 4B); these latter studies took place 2–6 weeks after the initial study and after biochemical indexes of hepatic function had returned to normal. Subjects 3 and 4 weighed 51.0 and 60.5 kg, respectively.

Each subject fasted for approximately 12 hr before and 1 hr after ingesting 250 ml of an aqueous solution containing 1.0 g of antipyrine. During the first 8 hr, blood was drawn from a peripheral vein *via* an indwelling catheter; venipuncture was used thereafter. Heparin was used to prevent coagulation; plasma was obtained by centrifugation. Plasma samples were obtained prior to each dose for use as an analytical blank.

Saliva was collected at the same time that blood was drawn. If saliva flow was insufficient, the subjects chewed on a piece of plastic film[1] for 3 min; they then expectorated into a sampling container. Each saliva sample collected from Subjects 1 and 2 was weighed to determine the effect of saliva flow on antipyrine concentration. After each dose, the patients rinsed their mouths thoroughly before providing the first saliva sample. All plasma and saliva samples were frozen until they were analyzed.

**Procedure**—The following were added to a 15-ml tube fitted with a lined screw cap[1]: 0.5–2 ml of plasma or 0.5–2 g of saliva, both containing an estimated 1.0–30 μg of antipyrine; internal standard solution (10 μg of 4-bromoantipyrine[2] in 0.1 ml of water); and 7 ml of dichloromethane. The tube was then gently shaken by hand for 10 min and centrifuged. The organic phase was transferred carefully to a second 15-ml tube, which had a conical tip at its base, and the solvent was evaporated to dryness on a water bath at 45°. Immediately before injection into the gas chromatograph, 15 μl of distilled water was added to the tube and mixed gently.

Approximately 5 μl of water was injected into the gas chromatograph with a flame-ionization detector[3]. A glass column (1.8 m long and 0.32 cm o.d.), packed with a 3% OV-17 on 100–120-mesh Gas Chrom Q[4], was used. The injector port, fitted with a glass liner, was maintained at 150°; the oven was kept at 220°, and the detector was kept at 250°. The injector port liner was changed and the column was treated with a silylating agent[5] prior to each day's analyses.

Under these conditions, antipyrine and the internal standard had retention times of 1.6 and 3.5 min, respectively. Calibration curves, obtained by adding known amounts of antipyrine to samples of plasma and saliva and taking them through the analytical procedure, had an average coefficient of variation of 5.6% in the 1–30-μg range.

**Data Analysis**—A log-linear least-squares method was used to fit the data obtained in the postabsorptive period (usually those data obtained at times greater than 1 hr after dosing) to a straight line and thus to estimate the plasma and saliva half-life ($t_{1/2}$) values. Clearance was determined by dividing the dose by the area under the saliva and plasma concentration–time data sets ($AUC$) from zero to infinite time. The oral dose was assumed to be completely absorbed (21). The trapezoidal rule was used to determine the $AUC$. The area beyond the last data point was estimated by dividing the least-squares estimate of the concentration at that time by the elimination rate constant. The volume of distribution of antipyrine was determined using (22):

$$\text{volume of distribution} = \frac{\text{clearance} \times t_{1/2}}{0.693} \qquad \text{(Eq. 1)}$$

The correlation between plasma and saliva antipyrine concentrations was determined by standard regression analysis techniques. A paired $t$-test was used to compare the pharmacokinetic parameters in plasma to those in saliva for each individual.

## RESULTS AND DISCUSSION

**Comparison of Salivary and Plasma Concentrations**—The results

[1] Teflon (du Pont).
[2] Melting point 110–113°.
[3] Model 1200, Varian, Palo Alto, Calif.
[4] Applied Science Laboratories, State College, Pa.
[5] Silyl-8, Pierce Chemical Co., Rockford, Ill.

**Table I—Regression Analysis of Antipyrine Concentrations Measured in Saliva against Those Found in Plasma**

| Study | $r$ | $p$ |
|---|---|---|
| All data | 0.958 | <0.01 |
| Study A data | 0.967 | <0.01 |
| Nonstudy A data[a] | 0.926 | <0.01 |
| Subject 1 | 0.909 | <0.01 |
| Subject 2 | 0.984 | <0.01 |
| Subject 3 (Studies A and B) | 0.966 | <0.01 |
| Subject 4 (Studies A and B) | 0.992 | <0.01 |

[a] All data minus Study A data.

of the regression analysis of the plasma and saliva antipyrine concentrations are shown in Table I. As indicated, the correlation coefficients were all statistically significant.

Although the overall ratio between saliva and plasma concentrations was very close to 1, the correlation coefficients in Table I show that there was considerable scatter in the data. The major contribution to this scatter was probably analytical error. Because of the scatter, the ability to estimate plasma concentration from a single saliva antipyrine determination may err in the order of 20% at low antipyrine concentrations where analytical error is greatest. However, this limitation may not affect the use of saliva antipyrine concentrations in pharmacokinetic investigations involving multiple determinations.

These studies are in agreement with two recent reports which found no significant differences between saliva and plasma antipyrine concentrations in normal subjects (19, 20) and epileptic patients (19). When data from individuals with acute viral hepatitis were analyzed separately (Study A data, Table I), no apparent change in the correlations was observed, either for data obtained during disease or for the remaining analyses. This particular disease state apparently did not alter the ratio of saliva to plasma concentrations of antipyrine.

The influence of saliva flow on the ratio of antipyrine concentrations found in saliva to those found in plasma was examined by plotting this ratio *versus* the weight of saliva collected during 3 min (Fig. 1). The lack of correlation ($r$ = 0.034) suggests that partitioning of antipyrine between saliva and plasma is independent of saliva flow.

**Estimation of Pharmacokinetic Parameters**—Table II shows the estimates of clearance, half-life, and volume of distribution for each study as calculated from saliva and plasma concentration–time data sets. No significant differences were noted between these estimates. Similar findings were reported recently (19, 20). The agreement in Table II is better than might have been anticipated, primarily because the multiple concentration data tend to average out analytical error. Salivary data could have been used to detect the difference in the pharmacokinetic parameters of Subject 4 during the acute phase of hepatitis (Study 4A, Fig. 2) and after the subject recovered (Study 4B, Fig. 2). It is in comparison studies of this sort that the use of salivary concentration will be most applicable. Saliva antipyrine concentration measurements have also been used to detect differences in antipyrine pharmacokinetic parameters in epileptic patients treated with anticonvulsants compared to those found in normal volunteers (19).

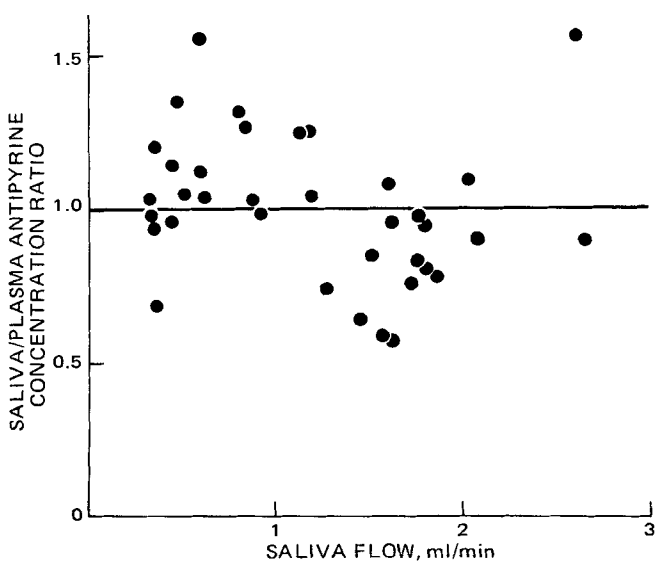


**Figure 1**—*Ratios of the concentrations of antipyrine in saliva to those in plasma* versus *the saliva flow during the 3-min collection period. The line would be obtained if the ratio were independent of plasma flow.*

**Table II—Pharmacokinetic Parameters for Antipyrine Calculated from Saliva and Plasma Concentration–Time Data**

| | Clearance, ml/hr/kg | | Volume of Distribution, liter/kg | | Half-Life, hr | |
|---|---|---|---|---|---|---|
| Subject | Plasma | Saliva | Plasma | Saliva | Plasma | Saliva |
| 1 | 38.8 | 38.2 | 0.71 | 0.64 | 12.7 | 11.6 |
| 2 | 34.1 | 40.2 | 0.60 | 0.60 | 12.1 | 10.3 |
| 3A | 8.9 | 10.9 | 0.58 | 0.66 | 45.0 | 42.3 |
| 3B | 58.0 | 50.8 | 0.80 | 0.63 | 9.4 | 8.6 |
| 4A | 30.0 | 29.5 | 0.53 | 0.50 | 12.2 | 11.8 |
| 4B | 45.9 | 48.5 | 0.49 | 0.54 | 7.5 | 7.8 |
| Mean | 36.0 | 36.6[a] | 0.62 | 0.60[a] | 16.5 | 15.4[a] |

[a] These pairs of values were not significantly different.

Estimates of antipyrine clearance may effectively serve as a standard for evaluating the clearance of other drugs metabolized in the liver, although caution must be used in interpreting the results (23). The potential widespread clinical use of antipyrine clearance as an index of the capacity of the liver to metabolize drugs is enhanced because concentrations of antipyrine are equal in plasma and in saliva, because multiple saliva samples are easily obtained, and because determinations can be made by a simple GLC assay. If useful correlations between antipyrine clearance and the clearance of other drugs could be established, then the complete oral availability of antipyrine (21) and its low toxicity would make antipyrine a suitable test drug for individualizing doses of drugs that exhibit large intersubject variability in clearance. Data presented in this paper and elsewhere (19) indicate that it may prove possible to use saliva antipyrine concentration measurements to detect changes in pharmacokinetic parameters resulting from disease and coadministration of other drugs.

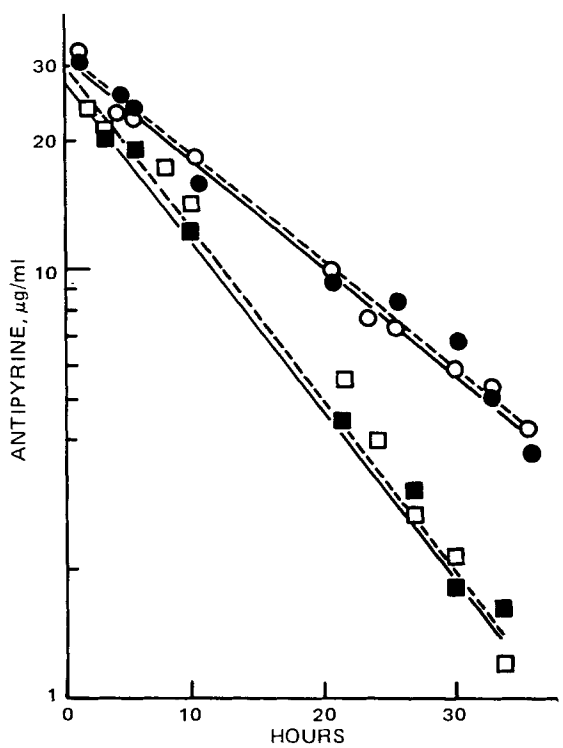


**Figure 2**—*Antipyrine concentrations in plasma (solid symbols, micrograms per milliliter) and in saliva (open symbols, micrograms per milliliter) at various times after a 1-g oral dose of antipyrine in Subject 4 during the acute phase of viral hepatitis (Study 4A, ○, ●) and after recovery (Study 4B, □, ■).*

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# Application of a Convective Diffusion Model to Membrane Transport


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**Abstract** □ Studies were carried out on the permeation rate of butamben through a dimethicone membrane. Under conditions of "aqueous diffusion layer control," the permeation rate was accurately described by a mathematical model based on convective diffusion theory. In accordance with the model, the rate of permeation from a saturated donor phase was shown to be equal to the rate of dissolution from a pure solid.



**Keyphrases** □ Diffusion, convective—model applied to permeation rate of butamben through dimethicone membrane □ Membrane transport—convective diffusion model applied to permeation rate of butamben through dimethicone membrane □ Permeation rate—butamben through dimethicone membrane, convective diffusion model □ Butamben—permeation rate through dimethicone membrane, convective diffusion model


One fundamental process that occurs during drug absorption is the transport of the active ingredient across various biological membranes. The study of membrane transport is thus of considerable interest, and the theoretical aspects of permeation models were recently reviewed (1).

In the model most frequently used to describe membrane transport, the membrane is considered to be in series with a stagnant or unstirred liquid diffusion layer on each side (2–5). For a liquid flowing past a surface, however, fluid flow occurs even at very small distances from the solid. Therefore, convective diffusion theory that accounts for fluid flow as well as diffusion should be applied (6). The differential equation of convective diffusion theory requires a mathematical description of the liquid flow past the dissolving surface. Although flow profiles are generally nonlinear, a constant velocity gradient can be assumed if the dissolving surface is relatively short (7, 8). Such a model recently was applied to describe drug dissolution into a moving liquid (9, 10). The purpose of this study was to evaluate the application of convective diffusion theory to membrane transport under conditions where transport across the dynamic liquid layer adjacent to the membrane is the rate-limiting step.

## EXPERIMENTAL

The laminar flowcell was a modification of the dissolution cell described previously (10). The dissolution cell was adapted for membrane transport studies by constructing a donor compartment in the die such that the membrane would be positioned coplanar with the die surface. As shown in Fig. 1, the donor compartment consists of a cylindrical metal cup over which the membrane is positioned. When placed in the die as shown, the membrane made a watertight seal over the donor compartment. The donor compartment was stirred with a magnetic stirring bar.

The permeation rates were determined as follows using butamben as the permeating species. The donor cup was filled with an aqueous suspension of butamben and was mounted in the die with the membrane (thickness 0.025 cm) positioned as described. The membrane was prepared as described by Roseman (11). The die was placed in the flowcell, which was leveled and mounted on a vibration-free platform. Distilled