Dialysis of Ephedrine and Pentobarbital from Whole Human Saliva and Simulated Saliva

ROBERT E. DAVIS*, CHARLES W. HARTMAN†, and JULIAN H. FINCHER

Abstract □ It was shown *in vitro* that whole human saliva inhibits the passage of ephedrine through a dialysis membrane, whereas the passage of pentobarbital is unaffected. The binding of ephedrine in whole human saliva was found to be independent of pH. A simulated saliva was prepared which afforded the same results as whole human saliva.

Keyphrases □ Saliva, human, simulated—drug dialysis rates □ Dialysis rates—drug in simulated and human saliva □ Ephedrine, sodium pentobarbital—dialysis rates, saliva □ UV spectrophotometry—analysis

The sublingual and buccal routes for the administration of drugs have certain advantages over other routes (1). Drugs absorbed here are not exposed to gastrointestinal secretions which may cause decomposition. Moreover, they bypass the liver in the first circulatory pass (2) and are not exposed to microsomal enzymes before entering the general circulation, as is usually the case in gastrointestinal absorption. Other routes of administration may have these advantages but are generally less desirable than the sublingual and buccal routes for esthetic reasons.

A number of drugs are reported to readily enter the circulation by absorption from the oral cavity, but the physicochemical aspects of the process have not been extensively investigated. The present study, using a dynamic dialysis system, was undertaken to investigate the interactions that might occur between drug molecules and the constituents of salivary secretions. Such interactions, if they occur, might influence drug absorption through the oral mucosa. Ephedrine and pentobarbital were chosen for this research on the basis that pentobarbital reportedly is absorbed sublingually and bucally in therapeutic doses, whereas ephedrine is not (3).

EXPERIMENTAL

Materials—A 3% w/v aqueous solution of ephedrine and a 5% w/v aqueous solution of sodium pentobarbital[1] were used for the dialysis experiments.

Standard dialysis tubing[2] was used in the experimentation. This tubing had an average pore diameter of 4.8 nm. and was expected to retain materials having molecular weights greater than 12,000.

Dialysis Media—Three dialysis media were employed: a control, a simulated saliva, and whole human saliva.

The control medium was 0.165% w/v solution of sodium chloride, which possesses approximately the same osmotic characteristics as whole human saliva.

A formula for simulated saliva was not found in the literature. Even though the USP (4) contains formulas for simulated gastric and intestinal fluids, one major component of human gastrointestinal secretions, mucin, was omitted. The simulated saliva prepared for these studies was composed of substances found in appreciable quantities in whole human saliva. The quantities used were based on a resolution of the literature findings (5–26). A supplier for salivary mucin could not be found. However, salivary mucin consists of two principal types: those rich in *N*-acetyl galactosamine and neuraminic acids and those containing L-fucose (5). Gastric mucins also consist of the same general mucoproteins and mucopolysaccharides (27). Thus, gastric mucin was used in the place of salivary mucin. The formula for the simulated saliva is given in Table I. The physical properties of the simulated and the whole human saliva are compared in Table II.

Table I—Formula for Simulated Saliva

Material	meq./l.	Wt./l.
Mucin, gastric		1.0 g.
α-Amylase		2.0 g.
Sodium chloride	2.0	0.117 g.
Potassium chloride	20.0	0.149 g.
Sodium bicarbonate	25.0	2.100 g.
Distilled water		q.s. 1000 ml.

The whole human saliva used in the studies was collected from volunteers without external aid. For the large quantities needed, the subjects collected saliva for 4–5 hr. to minimize the nonuniformity among samples that is often encountered with forced flow. Loss of carbon dioxide and deterioration of the saliva were minimized by storage in tightly stoppered containers and by refrigeration.

The drugs were dialyzed into a 0.9% w/v solution of sodium chloride. This solution was selected to simulate the osmotic qualities of the blood and other body fluids.

Dialysis Procedure—For the dialysis of the drugs, a single dialysis tube was suspended by a string into 500 ml. of a 0.9% w/v solution of sodium chloride in an 8-fl. oz. conical graduate situated in a 2000-ml. beaker The temperature was maintained at 37° by pumping water to and from the beaker through a heat exchanger. Agitation and stirring were maintained within the conical graduate by a magnetic stirrer with a 1.27-cm. (0.5-in.) Teflon-coated stirring bar.

Each dialysis tube was sealed by tying one end, and 5.0 ml. of the dialysis medium was added to the tube. A volume of stock solution of sodium pentobarbital or ephedrine was added to effect a desired

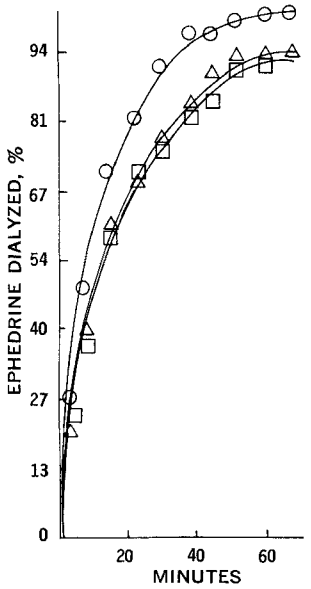

Figure 1—*Dialysis of ephedrine from the control* (○), *simulated saliva* (△), *and whole human saliva* (□) *media at a pH of 10.0.*

[1] Nembutal sodium, Abbott Laboratories, North Chicago, Ill.
[2] Oxford Laboratories, San Mateo, Calif.

Table II—Physical Comparison of Simulated Saliva to Whole Human Saliva

	Whole Human Saliva	Simulated Saliva
Color	Egg-shell white	Orange tint
Clarity	Opalescent	Clear
Specific gravity[a]	1.015	1.007
pH		
Initial	7.3	7.2
Final[b]	7.8–8.1	7.8–8.0

[a] Average values. [b] Rise of pH due to loss of carbon dioxide.

concentration. The volume of stock solution used was in each case insignificant in terms of the total volume in the bag. Ephedrine was tested in the control medium and both salivary media at three pH values, and pentobarbital was tested at one pH.

The pH range of the three media when the ephedrine was added without any buffers was 9.7–10.0. Sodium dihydrogen phosphate and citric acid, 10 g./l., were added to the media to give pH ranges of 7.3–7.4 and 4.5–4.8, respectively. The pH of the three media from which pentobarbital was dialyzed was 9.4.

Assay Procedures—Spectrophotometric assays for ephedrine and pentobarbital were carried out using a Beckman DU spectrophotometer[3], model 2400. Ephedrine was measured at 257 nm. after the method of Wheeler and Kaplan (28); pentobarbital was measured at 260 nm. as described by Conners (29).

RESULTS AND DISCUSSION

The effects of salivary constituents on the dialytic behavior of ephedrine at pH 10.0 are illustrated in Fig. 1. Data obtained at other pH values gave profiles that were qualitatively similar to Fig. 1. It is readily seen that ephedrine dialyzes more rapidly from the control medium than from the salivary media. Such difference might be explained by an interaction between the mucoproteins of salivary mucin and the ephedrine species present.

Interactions between mucin and amines were previously reported by Levine *et al.* (30). Also, organic molecular complex formation of the polymer type (31–33) involving mucin appears feasible, since the mucoproteins of mucin possess multiple carbonyl functions.

In the absence of interacting species in the dialysis medium, it would be expected that the dialytic rate of ephedrine would follow

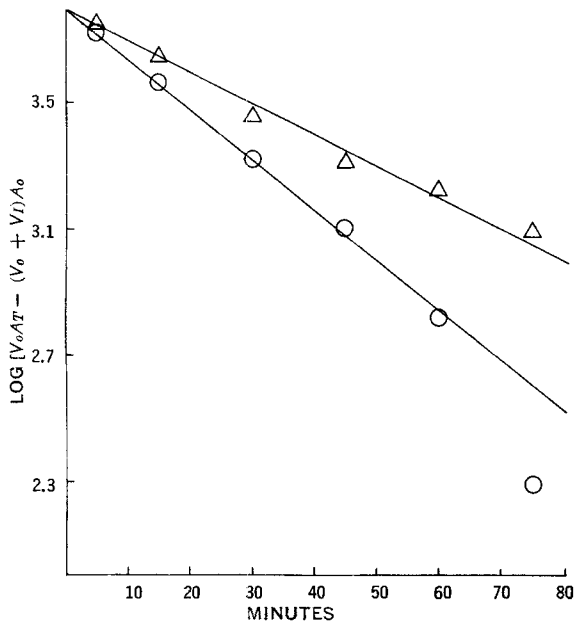

Figure 2—*Plot of the data of the dialysis of ephedrine at pH 10.0 using an expression of Fick's law. Key:* ○, *control medium; and* △, *whole human saliva medium.*

[3] Beckman Instruments, Inc., Fullerton, Calif.

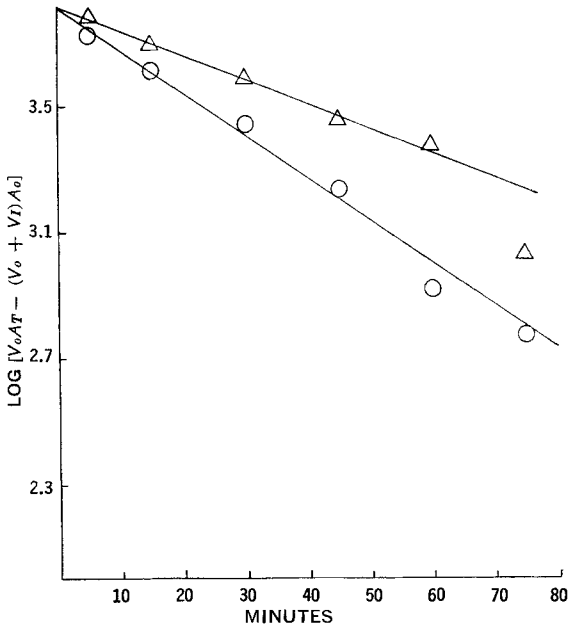

Figure 3—*Plot of the data of the dialysis of ephedrine at pH 4.5 using an expression of Fick's law. Key:* ○, *control medium; and* △, *whole human saliva medium.*

Fick's law (34). Where the pH's of the internal and external media are equal, Fick's law can be written as

$$\frac{dA_o}{dt} = k\left(\frac{A_I}{V_I} - \frac{A_o}{V_o}\right) \qquad \text{(Eq. 1)}$$

where:

A_o = amount of drug dialyzed into outside medium
A_I = amount of drug inside dialysis bag
V_o = volume of outside reservoir
V_I = volume inside dialysis bag
t = time
k = dialysis rate constant

A solution to this differential equation has the following form:

$$\log [V_oA_T - (V_o + V_I)A_o] = -\frac{V_o + V_I}{2.3V_oV_I}kt + \log (V_oA_T) \qquad \text{(Eq. 2)}$$

where $A_T = A_o + A_I$.

The data taken from the dialysis of ephedrine from the pH 10.0 control medium are plotted according to this equation as Curve B of Fig. 2. The linearity over 2–3 half-lives suggests that the model is appropriate.

Curve A of Fig. 2 shows the data collected on the dialysis from the whole human saliva medium plotted in the same manner. The drug dialyzed slower from the saliva, and this is taken to indicate a complexation of the ephedrine by saliva. The excellent linearity of this plot may be explained by assuming complex formation between

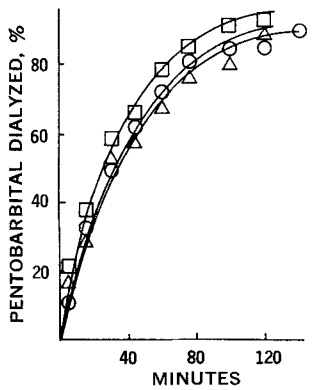

Figure 4—*Dialysis of pentobarbital from control* (○), *simulated saliva* (△), *and whole human saliva* (□) *media at a pH of 9.4.*

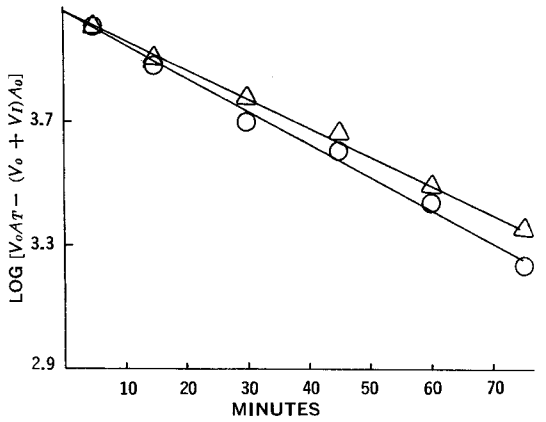

Figure 5—*Plot of the data of the dialysis of pentobarbital at pH 9.4 using an expression of Fick's law. Key: ○, control medium; and △, whole human saliva medium.*

ephedrine species and binding sites of the mucoproteins if the number of bound sites is negligible compared to the total available sites in this system. By assuming that the binding sites on the mucoproteins are comparable, the complexation of ephedrine by individual sites may be written:

$$E + S \overset{K}{=} C \qquad \text{(Eq. 3)}$$

where E represents free ephedrine species, S represents a binding site, C represents the complex, and K is the binding constant.

It can be shown that the concentration of unbound ephedrine in the dialysis bag can be expressed as

$$[E] = A_I/\{V_I(K[S] + 1)\} \qquad \text{(Eq. 4)}$$

If the bound ephedrine cannot be dialyzed, then Fick's law can be written:

$$\frac{dA_o}{dt} = k\left([E] - \frac{A_o}{V_o}\right) \qquad \text{(Eq. 5)}$$

Substituting Eq. 4 into Eq. 5 and utilizing the fact that $A_T = A_o + A_I$, this differential equation may be written:

$$\frac{dA_o}{dt} = k\left(\frac{V_oA_T - (V_o + V_I + K[S]V_I)A_o}{V_IV_o(K[S] + 1)}\right) \qquad \text{(Eq. 6)}$$

Under the conditions of the experimentation, V_I is much less than V_o. If $K[S]$ is of the order of unity, then $K[S]V_I$ is negligible compared to $(V_I + V_o)$, and the solution to the equation becomes

$$\log[V_oA_T - (V_o + V_I)A_o] = -\frac{V_I + V_o}{2.3V_IV_o(K[S] + 1)}kt + \log(V_oA_T) \qquad \text{(Eq. 7)}$$

That the slope of Curve A is less than the slope of Curve B is in keeping with the assumed model. From Eq. 2:

$$\text{slope of Curve B} = \frac{V_o + V_I}{2.3V_oV_I}k \qquad \text{(Eq. 8)}$$

while from Eq. 7:

$$\text{slope of Curve A} = \frac{V_o + V_I}{2.3V_oV_I(K[S] + 1)}k \qquad \text{(Eq. 9)}$$

The ratio of the slopes, under identical conditions of pH, allows estimation of the extent of binding:

$$\frac{\text{slope B}}{\text{slope A}} = K[S] + 1 \qquad \text{(Eq. 10)}$$

Since the number of binding sites on the mucoproteins has not been characterized in this research, the value of K cannot be known from these data. It can be shown, however, from the data collected at pH 10.0 that $K[S]$ has a value of 0.8. When the data collected at pH 4.5 were treated in the same manner, $K[S]$ was also found to be 0.8. It is concluded, therefore, that pH has no overall effect on the binding of ephedrine species by mucoproteins of saliva. The data collected do, however, show that pH has a marked effect on the rates of dialyses. For example, the slope of Curve A in Fig. 3 for the dialysis of ephedrine from a pH 4.5 buffered solution is 0.0134; the slope of Curve A in Fig. 2 for the dialysis of ephedrine from a pH 10.0 solution is 0.0180.

It is clear from the data at pH 4.5, where for practical purposes the ephedrine is 100% protonated, that the protonated form dialyzes at a significant rate. The rate of dialysis at pH 10.0, where 81% of the drug is unprotonated, is greater than that at pH 4.5, indicating that the free base passes the membrane at a significantly greater rate than the protonated form. The fact that the complex formation was pH independent does not appear to be consistent with the findings of Levine *et al.* (30), who postulated that the ammonium ion was necessary for complexation of quaternary amines by mucin.

A similar experiment was performed using pentobarbital. The data, as plotted in Fig. 4, show no significant differences in the rate of pentobarbital being dialyzed from the control, whole human saliva, or synthetic saliva media. When the data were plotted according to Eq. 2, the linearity obtained again demonstrated the appropriateness of the model (Fig. 5).

Studies of oral mucosal drug absorption, *in vitro*, should ideally be conducted using whole human saliva. Collection of adequate quantities of whole human saliva for experimentation in this area is difficult. To circumvent these difficulties, a synthetic saliva was prepared. The synthetic saliva afforded similar results in dialysis experiments.

These studies with ephedrine suggest that the constituents of salivary secretions could significantly affect the release of other amine drugs from drug systems. Since the passage of ephedrine through dialytic membranes was affected by saliva constituents, it is possible that the absorption of other amine drugs from the oral cavity would be influenced similarly. Additional experiments using other amine drugs are necessary to substantiate these possibilities. It is concluded, however, from these data that salivary secretion does slow the rate of passage of ephedrine through dialytic membranes, and that this decrease in rate is due to a binding of the drug to saliva constituents. This binding is pH independent.

A simulated saliva was prepared which afforded the same results as whole human saliva. It is suggested that such a solution would be an appropriate substitute for whole human saliva in future *in vitro* studies.

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Solubility of Sodium Salicylate in Mixed Solvent Systems

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Abstract □ The solubility of sodium salicylate was determined in aqueous mixtures of dioxane, acetone, 1-propanol, ethanol, and methanol. The solubility had fair parallelism to dielectric constants over a wide range of concentration, indicating adherence to the coulomb relationship. The solubility curves for sodium salicylate are examined from a suppressive dissociative point of view, insofar as ionization may be taking place without complete dissociation. Comparison of the solubility curves for an acid and its sodium salt is considered. The possibility that uni-univalent electrolytes in solutions below a dielectric constant of 30 are completely associated is shown for several systems in plots of various concentration notations. Adherence of the predicted linearity from the Born equation for these systems is discussed. However, the theoretical ionic radius is found to be about twice the expected value.

Keyphrases □ Salicylate, sodium, solubility—mixed solvent systems □ Alcohols, acetone, dioxane–water mixtures—sodium salicylate solubility □ Solubility, sodium salicylate—dielectric constant relationship □ Ion, radii—sodium, salicylate

The solubility of chemical compounds described as salts are normally thought of as having maximum solubility in water relative to other less polar solvents such as alcohols. The direction of decreasing solubility with decreasing polarity is generally based on the charge-separating ability of the solvent or the dielectric constant.

It is of interest to consider the solubility changes that occur as a function of the dielectric constant for a typical, useful pharmaceutical such as sodium salicylate. This pharmaceutical was also chosen because data are available (1) on salicylic acid in the same solvent systems and a comparison could be made for an acid and its sodium salt.

The Born equation (2), which deals with the relationship between solubility and the reciprocal of the dielectric constant, was also tested in this study.

The diminution of solubility of a salt with a concomitant decrease in dielectric constant obviously would be related to the degree of dissociation of the particular salt and might be related to the water content of these binary mixtures.

EXPERIMENTAL

Reagents—The reagents used were methanol[1], 1-propanol[1], 1-pentanol[2], 1-octanol[2], 1-butanol[3], 1-decanol[4], ethanol[5], and acetone[6]. Deionized water was used for all binary mixtures prepared. The sodium salicylate[7] was USP grade. Dioxane[8] (*p*-dioxane), stabilized, was also used in these studies.

The purity of the solvents was tested by the following procedure. The refractive indexes of each solvent were determined initially and throughout their use with a Bausch and Lomb Abbe-3 refractometer at 25°. The results are given in Table I.

The instrument was checked against 99 mole % free benzene (Fisher certified reagent), and the experimental value of 1.4983 was obtained at 25° *versus* the literature value of 1.498 at 25°.

[1] Fisher Certified, Fisher Scientific Co.
[2] Baker Analyzed, J. T. Baker Co., Phillipsburg, N. J.
[3] Eastman Kodak No. 50, Eastman Kodak, Rochester, N. Y.
[4] No. 5189, Matheson, Coleman & Bell, East Rutherford, N. J.
[5] U. S. Industrial Chemical Corp.
[6] Analytical Reagent, No. 2240, Mallinckrodt.
[7] USP, J. T. Baker Co., Phillipsburg, N. J.
[8] No. C 608258, Amend Drug and Chemical Co., Inc.