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A membrane model of the human oral mucosa as derived from buccal absorption performance and physicochemical properties of the β-blocking drugs atenolol and propranolol

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The buccal absorption characteristics and physicochemical properties of the β-adrenoceptor blocking agents propranolol and atenolol have been investigated to evaluate their permeation properties across biological lipid membranes. The dissociation constants, solubilities of free base, and n-heptane partition coefficients show that propranolol in its unionized form is much more lipophilic than atenolol, both drugs being bases with a similar pKa. Buccal absorption was studied under conditions of varying drug concentration, contact time, and pH, and controlled through the use of a non-absorbable marker. The absorption findings are in general agreement with the pH-partition theory. A new compartmental diffusional model that includes membrane storage and a hypothetical 'aqueous pH-buffering surface system' allowed a more exhaustive interpretation to be made. A method for the estimation of the intrinsic pH and buffer capacity of the postulated surface system from drug pH-absorption data and partition coefficients alone is described. With human oral mucosa the intrinsic pH was near 6·7, and the buffering capacity of the system (expressed as the ratio ΔpH/ΔpH eff) about 2·86. The method was validated using published absorption data from the rat small intestine. Absorption of unionized drug through pores is shown to be negligible in the buccal absorption situation. The time course of absorption suggests membrane storage of lipophilic compounds; the *in vivo* partition coefficient of unionized propranolol relative to the mucous membrane could be calculated for the peusdo-steady state of absorption, i.e. the partition equilibrium between mouth content and membrane, to be approximately 776; this value is of the same order as the *in vitro* partition coefficient for the erythrocyte/plasma system. The lipid biophase of the buccal membrane is estimated semiquantitatively to be of intermediate polarity ($\epsilon = 3$–4).

Beckett & Triggs (1967) introducing the buccal absorption test as an '*in vivo* model of passive drug transfer through lipid membranes', postulated that the absorption of drugs from the oral cavity under varying experimental conditions might be of predictive value for their transfer across other physiological membrane systems.

The value of buccal absorption for renotubular absorption was confirmed as a model (Meyer, Kaye & Turner, 1974; Ankier & Kaye, 1976; Kaye & Long, 1976; Kiddie & Kaye, 1976). The purpose of this paper is to derive a physical diffusional model for buccal absorption itself and to introduce methods that allow the quantification of some of the model parameters. For this purpose two β-adrenoceptor blocking drugs, one much more lipophilic than the other were used.

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MATERIALS AND METHODS

Materials

The buffers used for buccal absorption test solutions were McIlvaine's citric acid/phosphate for pH 5 and 6, Sörensen's phosphate for pH 7, 7·4 and 8, and Sörensen's glycine for pH 9, 10 and 10·45 (Documenta Geigy, 1959). Buffer solutions were made isotonic (300 mosm) by titratim with NaCl solution (20% w/v). n-Heptane and amyl alcohol (Hopkin & Williams, Chadwell Heath), and benzene, chloroform, ethyl acetate and toluene (BDH Chemicals, Poole) were analytical reagent grade, octan-2-ol (BDH) was laboratory grade. As a marker a 100 μg ml^{-1} stock solution of phenol red (May & Baker, Dagenham) in double distilled water was prepared. Atenolol and propranolol hydrochloride were a gift from ICI (Macclesfield). For filtering, Whatman 42 ashless paper was used. An Advanced 3 L osmometer was used for osmotic titrations; pH readings were

taken on an Orion Research 701 pH meter against standard buffers pH 4, 7 and 9·2 (Intek Ltd, London). Spectrofluorometric drug concentrations were read on a Baird Atomic FP 100 fluorometer. The bi-exponential curve was computer fitted using a program to minimize the sum of squares of deviation.

Methods

The pKa was taken as the midpoint of the buffering plateau of the titration curve. The titration curves were established at room temperature (21°) from the measurement of the pH of a series of test tubes containing a constant volume of drug solution to which increasing volumes of titrant had been added (2 ml 0·01 M propranolol hydrochloride titrated with 0·01 N NaOH in steps of 0·1 ml, 1 ml 0·01 M atenolol with 0·005 N HCl in steps of 0·1 ml). The pH-independent water solubility of free base, S_0, was determined after Martin, Swarbrick & Cammarata (1975), from the pH and the final concentration of a drug solution that had been titrated with NaOH until precipitation became visible, according to:

$$S_0 = S/(1 + 10^{pK_a - pH_{lim}}) \quad .. \quad .. \quad (1)$$

where S is the drug concentration, and pH_{lim} the pH at the titration point when solute crystallization is imminent. Temperatures were measured individually for each estimation.

The partition coefficients of propranolol and atenolol were established in eight organic solvents (n-heptane, benzene, toluene, chloroform, amyl alcohol, diethyl ether, octan-2-ol, ethyl acetate) versus 0·1 N NaOH as the aqueous phase, those of quinine and salicylic acid in n-heptane and octanol with 0·1 N NaOH and 0·1 N HCl as watery phases. The aqueous phases were chosen such as to have the drugs quantitatively unionized and to obtain the partition coefficients of unionized drug, P_u. Before partition experiments the organic solvents were equilibrated with the respective aqueous solvents. All solutions were at room temperature (24 ± 1°); individual temperature measurements were not made.

For propranolol and atenolol in n-heptane, 8 mg of either drug was partitioned between 40 ml 0·1 N NaOH and 40 ml n-heptane in a 250 ml separating funnel, shaken for 30 min and allowed to separate overnight. Drug concentrations in the watery phase (cw) were measured fluorometrically, and the partition coefficients (P_u) calculated as:

$$P_u = \frac{8 - 40\,cw}{40\,cw} \quad .. \quad .. \quad (2)$$

The method for propranolol and atenolol in all other solvents, and for quinine and salicylic acid was as follows: a standard solution of each drug was made up containing a concentration from the upper limit of linearity of the assay in either 0·1 N NaOH (for bases) or 0·1 N HCl (for salicylic acid). A 5 ml aliquot of the standard solution was added to 5 ml organic solvent in a stoppered centrifuge tube, shaken for 30 min, and centrifuged for 5 min (3000 rev min^{-1}, r = 12 cm). The drug concentrations of standard solution and aqueous phase of each partition system were assayed to give the readings c_i and c_e, respectively. The partition coefficient then was calculated according to:

$$P_u = \frac{c_i - c_e}{c_e} \quad .. \quad .. \quad (3).$$

Quinine in 0·1 N NaOH was measured in direct fluorometric assay at λ exc/em = 350/450 nm, 3 ml aliquots of the lower aqueous layer or the standard solution having been acidified with 0·5 ml 2 N H_2SO_4. Similarly, the aqueous phases of propranolol and atenolol partition sytems were assayed directly, after acidification of 3 ml aliquots with 0·5 ml 1 N HCl, at the wavelengths λ exc/em = 295/340 nm for propranolol and λ exc/em = 270/305 nm for atenolol. Salicylic acid was measured spectrophotometrically (at 525 nm) as a coloured derivative obtained through adding 1 ml of Trindner's reagent (Trindner, 1954) to 3 ml aqueous layer.

Buccal absorption experiments were carried out in one subject (W.S) according to Beckett & Triggs (1967). Phenol red (phenolsulphonphthalein, 5 μg ml^{-1}) was included in the drug solutions as an internal marker to control drug loss through swallowing. It has been widely used as a marker for absorption studies in the lower alimentary tract (Brodie & Hogben, 1957; Hogben, Schanker & others, 1957; Hobsley & Silen, 1969). The pH and volume of the expelled solutions were recorded, a nominal 0·3 ml being added to the reading to account for froth. Then the samples, as well as a 20 ml aliquot of the original solution, were made up to 50 ml with distilled water, shaken vigorously, and filtered.

Propranolol and atenolol were measured spectrofluorometrically using a method after Shand, Nuckolls & Oates (1970) and Kaye (1974): a 3 ml sample in a 30 ml stoppered centrifuge tube was made alkaline with 0·2 ml 10 M NaOH, shaken mechanically for 10 min with 12 ml n-heptane containing 30% v/v amyl alcohol, and centrifuged for 5 min at 3000 rev min^{-1} (r = 12 cm). 10 ml of the

organic supernatant was transferred to 3 ml 0·01 N HCl in another centrifuge tube, shaken and spun again. The fluorescence of the lower aqueous layer was read at the specific wavelength λ exc/em = 295/340 nm for propranolol and λ exc/em = 270/305 nm for atenolol. The fluorescence was shown to be linear up to concentration limit of 10 μg ml^{-1} propranolol hydrochloride and 60 μg ml^{-1} atenolol. The two drugs and phenol red did not interfere with each other in the assay.

For phenol red, 3 ml of the filtrate was made alkaline with 0·1 ml 10 M NaOH and read spectrophotometrically at 560 nm, with a second reading at 625 nm to subtract background extinction.

With the values obtained for end volume (V_e), and the initial end concentrations of drug and marker ($c_{i,d}$, $c_{e,d}$, $c_{i,m}$, $c_{e,m}$), it was possible to calculate the percentage of absorption (% abs), the volume of swallowing (V_{sw}), and the volume of salivation (V_{sal}) according to:

$$\% \text{ abs} = \left(\frac{c_{e,m}}{c_{i,m}} - \frac{c_{e,d}}{c_{i,d}}\right) \times 100 \quad .. \quad .. \quad (4)$$

$$V_{sw} \text{ (ml)} = \left(1 - \frac{c_{e,m}}{c_{i,m}}\right) \times V_i \quad .. \quad .. \quad (5)$$

$$V_{sal} \text{ (ml)} = V_e - V_i \frac{c_{e,m}}{c_{i,m}} \quad .. \quad .. \quad (6).$$

The equations are derived on the assumption that drug is swallowed, or absorbed, or alternatively expelled, and that salivary secretions are free of drug. V_i in equations 5 and 6 stands for the initial volume (in ml) and was 20 ml in our experiments.

In the pH-absorption curve, every bulk pH was calculated as the mean of the test solution pHs at mid-contact time to account for the occurring pH shifts:

$$pH = pH_i + \tfrac{1}{2} \sum \frac{pH_e - pH_i}{n} \quad .. \quad (7).$$

Here n is the number of trials (4), and the subscripts indicate initial and end pH. The pH-absorption curve was also established in 15 volunteers to estimate swallowing and salivation, and to compare intra- and inter-subject variance.

RESULTS

The values for pKa and S_0 are given in Table 1, the partition coefficients of propranolol and atenolol in Table 2. In Table 2 the organic solvents are arranged in the order of increasing polarity (dielectric constant). The partition coefficients of quinine were 357 in 0·1 N NaOH: octanol and 0·30 in 0·1 N NaOH: n-heptane. Those of salicylic acid were 216 in 0·1 N HCl: octanol and 0·10 in 0·1 N HCl: n-heptane. While both propranolol and atenolol are bases with a comparable dissociation constant, the unionized form of propranolol is much more lipophilic than that of atenolol. The ionized species is assumed to be completely in solution in water, and not at all in the organic phases.

Table 1. *pKa and S_0 of propranolol and atenolol.* The pKa values were determined from the titration curves (established at room temperature, about 24°) as the midpoints of the buffering plateaux. The 'solubilities of free base' S_0, were measured according to Martin & others (1975) at 24°.

	Propranolol	Atenolol
pKa	9·45	9·60
S_0	0·07 mg ml^{-1}	9·5 mg ml (24°)

Table 2. *Partition coefficients (P_u) of propranolol and atenolol between various organic solvents, and 0·1 N NaOH.* The value for propranolol in n-heptane, and all atenolol values were in duplicate. The other values for propranolol were established in quadruplicate. [1] from Harrison (1972); [2] from Weast, Selby & Hodgman (1965); [3] value of 2-methylbutane-1-ol; [4] value not found in the literature.

Dielectric constant ε	Organic solvent	Propranolol P_u	Propranolol Log P_u	Atenolol P_u	Atenolol Log P_u
1·92[1]	n-Heptane	29·85	1·48	0·053	−1·28
2·28[2]	Benzene	309·6	2·49	0·014	−1·85
2·38[2]	Toluene	169·8	2·23	0·007	−2·16
4·34[2]	Diethyl ether	290·0	2·46	0·010	−2·00
4·81[2]	Chloroform	932·0	2·97	0·743	−0·13
5·82[2,3]	Amyl alcohol	391·0	2·59	3·28	0·52
—[4]	Octan-2-ol	110·9	2·05	0·937	0·03
6·02[2]	Ethyl acetate	2·98	0·48	0·852	−0·07

Swallowing and salivation, as calculated from loss of the dye marker are found to be strongly pH-dependent (Fig. 1), swallowing being minimal near the physiological pH.

The concentration-absorption relation at pH 7·4 shows a constant percentage of absorption over a 32-fold concentration range (0·5 — 16 μg ml^{-1} propranolol, 10–320 μg ml^{-1} atenolol): about 42% propranolol and <2% atenolol are absorbed in 5 min independently of the initial concentration (Fig. 2). The 160 μg ml^{-1} value of atenolol was not established). The dependence of absorption on pH in one subject (Fig. 3) demonstrates a marked difference

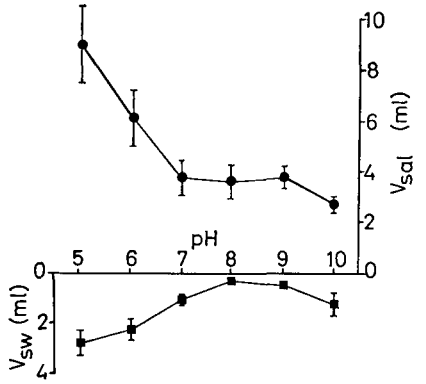


FIG. 1. Salivation (●) and swallowing (■) in the buccal absorption test during 5 min contact time. Means ± s.e.m., n = 8.

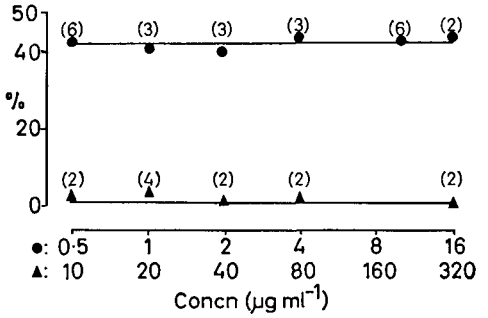


FIG. 2. Concentration (μg ml^{-1}) dependence of the buccal absorption (%) of propranolol (●) and atenolol (▲) over a 32-fold concentration range. Number of estimations in brackets.

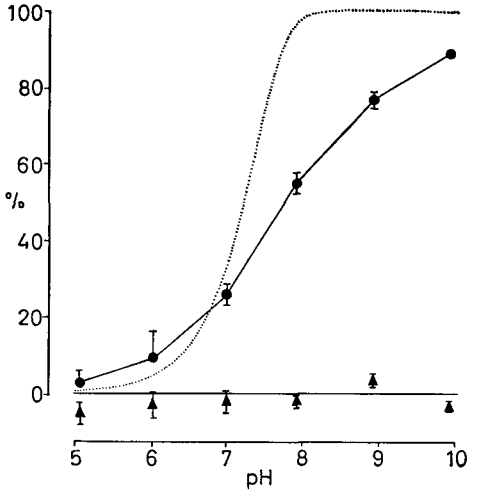


FIG. 3. pH dependence of buccal absorption (%) of propranolol (●) and atenolol (▲); means ± s.e.m. of four observations in one subject. The dotted line represents the theoretically expected absorption curve of propranolol—if the buffering system did not exist.

between the two compounds: whereas atenolol is not absorbed over the whole pH-range, the absorption of propranolol increases with increasing pH from 3·2% at pH 5·08, 9·3% (pH 6·02), 26·0% (pH 7·00), 55·8% (pH 7·93), 76·9% (pH 8·94) to 89·1% at pH 9·93. A similar curve from 15 untrained volunteers (4 at each pH-point) showed wider scatter and lower mean readings at points of high absorption.

The time course of propranolol absorption at pH 10·45—when it is 90% unionized—is given as curve A in Fig. 4. Atenolol was not absorbed to a measurable extent.

DISCUSSION

Buccal absorption findings have been interpreted qualitatively in terms of the pH-partition theory as passive diffusion of the non-ionized, non-protein bound drug species across a lipid boundary (Beckett & Triggs, 1967). The independence of absorption on the initial concentration confirms that the absorption process occurs principally as passive diffusion. As to the influence of ionization, the pH-partition theory (as e.g. summarized by Brodie, 1964), can be expressed by a set of mathematical equations that allow for the quantitative treatment of empirical data; the fraction of unionized drug at a given pH which can be calculated from the Henderson-Hasselbalch equation to be

$$f_u = 1/(1 + 10^{(pKa-pH)}) \text{ for a base} \quad .. \quad (8)$$

$$f_u = 1/(1 + 10^{(pH-pKa)}) \text{ for an acid} \quad .. \quad (9)$$

and

$$c_e = c_i\, e^{-\kappa_{app}} \quad .. \quad .. \quad .. \quad (10)$$

describes the decrease of drug concentration through diffusion from the initial concentration c_i to the end concentration c_e over a test interval t, which is characterized by the 'apparent rate constant' for the disappearance of total drug, κ_{app}. Since, however, only unionized drug is capable of permeating the membrane, the rate of transfer of a compound is described more specifically by the rate of transfer of the unionized species across the membrane, hence the 'true rate constant' κ, as derived from the apparent rate constant and the degree of ionization is:

$$\kappa = \kappa_{app}/f_u \text{ (Khalil \& Martin, 1967)} \quad (11)$$

Drug binding to salivary proteins is assumed to be negligible in the buccal absorption situation.

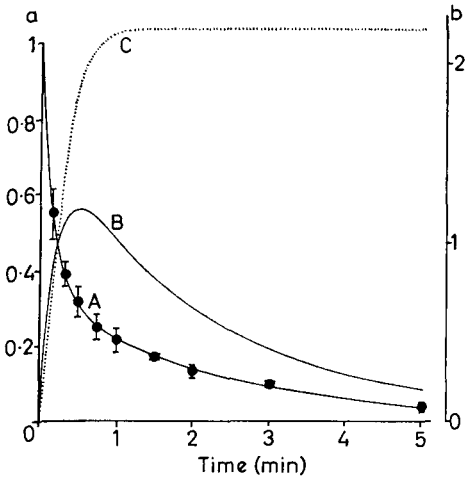


FIG. 4. Curve A: time course of buccal absorption of propranolol; means ± s.e.m. of four observations in one subject. Curve B: membrane storage. Curve C: the ratio of drug in membrane and mouth (D_B/D_A) reaches a constant value. Ordinates: a. Propranolol amount in mouth (D_A) or in membrane (D_B). b. Ratio D_B/D_A.

The true rate constant of absorption, κ, can be calculated for each pH from the empirical absorption at that pH. If the pH partition theory in its presented form (after Brodie, 1964) is adequate, the values of κ obtained would be expected to be identical, since the pH in the aqueous media is not expected to exert an influence on the permeation of the unionized drug molecules within the lipid membrane.

κ is obtained through taking the natural logarithm in equation 10.

$$\kappa_{app} = (1/t) \times \ln (c_i/c_e) \quad .. \quad .. \quad .. \quad (12)$$

and inserting equation 12 as well as equations 8 or 9 respectively into equation 11:

$$\kappa = ((1 + 10^{(pKa-pH)})/t) \times \ln(c_i/c_e) \text{ for a base} \quad (13)$$

$$\kappa = ((1 + 10^{(pH-pKa)})/t) \times \ln (c_i/c_e) \text{ for an acid} \quad (14)$$

With

$$c_i/c_e = 100/(100 - \% \text{ abs}) \quad .. \quad .. \quad (15)$$

and equation 13 the true rate constants of propranolol were calculated for the experimental pH points from the respective percentages of absorption. The values obtained are listed in Table 3 and plotted semilogarithmically against the bulk fluid pH in Fig. 5. It is evident that the values of κ are not identical at the different pH points.

Table 3. *The spurious effect of the bulk fluid pH on the rate constants of absorption of unionized propranolol (top) and p-n-hexylphenylacetic acid (bottom) is suggestive of a buffering surface system.* The pKa of *p*-n-hexylphenylacetic acid is 4·36 (Beckett & Moffat, 1969b). The buccal absorption values c_i and c_e of *p*-n-hexylphenylacetic acid were taken from Beckett & Moffat (1969a), Fig. 1A. The parameter values were calculated with equations 8, 9, and 13–17 given in the text and $\kappa_{app} = \kappa f_u$.

pH	f_u	$\frac{c_i}{c_e}$	κ (min^{-1})	pH_{eff}	κ_{app} (min^{-1})
5·08	$4·27 \times 10^{-5}$	1·033	149·9	5·90	$6·49 \times 10^{-3}$
6·02	$3·71 \times 10^{-4}$	1·103	52·3	6·38	$1·96 \times 10^{-2}$
7·00	$3·54 \times 10^{-3}$	1·351	17·0	6·87	$6·02 \times 10^{-2}$
7·93	$2·93 \times 10^{-2}$	2·262	5·6	7·31	$1·63 \times 10^{-1}$
8·94	$2·36 \times 10^{-1}$	4·329	1·2	7·56	$2·93 \times 10^{-1}$
9·93	$7·51 \times 10^{-1}$	9·174	0·6	7·74	$4·43 \times 10^{-1}$
4·0	$6·96 \times 10^{-1}$	5·625	0·5	6·17	$3·45 \times 10^{-1}$
5·0	$1·86 \times 10^{-1}$	4·272	1·6	6·25	$2·90 \times 10^{-1}$
6·0	$2·24 \times 10^{-2}$	2·567	8·4	6·44	$1·89 \times 10^{-1}$
7·0	$2·29 \times 10^{-3}$	1·534	37·4	6·79	$8·56 \times 10^{-2}$
8·0	$2·29 \times 10^{-4}$	1·199	158·5	7·16	$3·63 \times 10^{-2}$
9·0	$2·29 \times 10^{-5}$	1·087	728·2	7·50	$1·67 \times 10^{-2}$

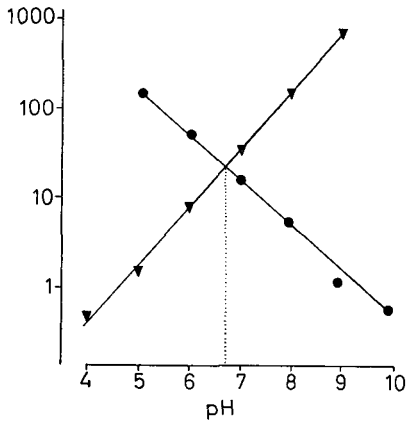


FIG. 5. Semilogarithmic plot of the rate constants (κ) of propranolol (●) and *p*-n-hexylphenylacetic acid (▼) to determine the intrinsic pH of the buffering surface system.

This is not a solitary finding: In Table 3 and Fig. 5 the rate constants of buccal absorption of an acid (*p*-n-hexylphenylacetic acid) under similar experimental conditions are included as calculated with equation 14 from the data given in Beckett & Moffat (1969a). The spurious effect of the pH on the true rate constants, showing as an angular deviation of the graphs of κ from the expected parallels to the abscissa, is found to be diametrical in the acid and the base, yet comparable in magnitude.

Hogben, Tocco & others (1959) postulated that in the rat small intestine there exists an effective or 'virtual' pH at the membrane surface that differs from the pH in the bulk fluid of the lumen. In equilibrium studies with simultaneous perfusion of intestinal lumen and mesenteric artery these authors obtained concentration gradients across the absorptive membrane that reflected a more acidic pH at the intestinal surface than was measured in the bulk gut content: the effective equilibrium pH values ranged from 5·3 to 6·1, whereas the bulk pH was kept near the physiological, 6·6.

The concept of an effective pH can be adopted in the present situation. It can be imagined that the true rate constants are pH-independent, but the experimental bulk pH has not been at the right value to calculate them. It appears to be appropriate to postulate the existence not only of a single effective pH, but also of a buffering surface system that mitigates any luminal pH changes towards its own intrinsic pH, thus giving rise to different effective pH values under different experimental conditions. An attempt to estimate the intrinsic pH of the buffering surface system is based upon the following consideration.

For the absorption of an unionized molecule it is insignificant whether it is of acidic or basic nature; the unionized species of both an acid and a base are expected to be absorbed with equal ease, i.e. with identical rate constants, if their partition properties into the membrane are the same. In other words, it may be assumed that at that point on the pH scale at which an acid and a base with identical partition coefficients are absorbed with equal true rate constants, a possible buffer system should exert no influence: this pH represents the system's own intrinsic pH. *p*-n-Hexylphenylacetic acid was chosen for comparison with propranolol because its partition coefficient in an n-heptane 20·1 N HCl system is 29·8 (Beckett & Moffat, 1969b), which is practically identical with that of propranolol (Table 2). In Fig. 5 the rate constant graphs of both compounds are superimposed; the intrinsic pH of the buffering surface system is read to be 6·7.

With the true rate constant at pH 6·7 (22·9 min^{-1}) it is possible to calculate the corresponding effective pH for every bulk pH by isolating pH in equations 13 and 14:

$$pH_{eff} = pKa - \log (\kappa t/1n\ (c_i/c_e) - 1) \text{ for a base} \quad (16)$$

$$pH_{eff} = pKa + \log (\kappa t/1n\ (c_i/c_e) - 1) \text{ for an acid} \quad (17)$$

The values are included in Table 3. A plot of the effective pH against the bulk pH gives an idea of the capacity of the buffering system, which is found to be practically identical in the two subjects studied so far (Fig. 6) Within limits, a change in 2·86 units of the bulk pH is required to change the effective pH by one unit ($\Delta pH/\Delta pH_{eff} = 2{\cdot}86$).

Another way to demonstrate the effect of the buffering surface system visually is to compare the empirical pH-absorption curve with that which would have been expected if the buffering layer did not exist. For this purpose κ_{app} for propranolol was calculated from the true κ (22·9 min^{-1}) for several pH values, and with it the percentage of absorption in a 5 min period.

From equations 10, 11 and 15 it follows that:

$$\% \text{ abs} = (1 - e^{-\kappa\, fu\, t}) \times 100 \quad .. \quad (18)$$

or, after also inserting equation 8 and the values for κ, t and pKa,

$$\% \text{ abs (pH)} = \left(1 - \frac{e - 114{\cdot}5}{1 + 10^{(9{\cdot}45 - pH)}}\right) \times 100 \quad (19)$$

A graph of this function is included as a dotted line in Fig. 3 and is self-explanatory.

The method of estimating intrinsic pH and buffer capacity of a buffering surface system by means of an acid-base pair, if it is valid, should not be confined to the buccal absorption site alone. Another potential acid-base pair is quinine and salicylic acid,

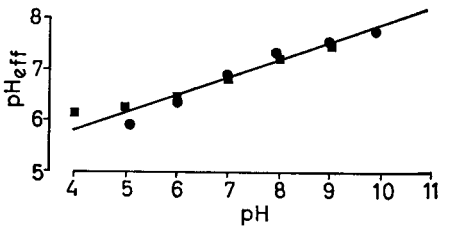


FIG. 6. Buffering effect of the surface system in two subjects (A.C.M. ■ and W.S. ●): within limits, $\Delta pH/\Delta pH_{eff} = 2{\cdot}86$.

which exhibit similar partition properties in a polar and an apolar solvent. A set of data for the pH-dependent absorption of salicylic acid and quinine from the rat small intestine is given by Brodie (1964). By applying the calculations and plots described above to these data, the values given in Table 4 are obtained. The intrinsic pH and buffer capacity of the rat small intestinal surface system are thus found to be pH 5·6 and $\Delta pH/\Delta pHeff = 4{\cdot}17$, respectively. The intrinsic pH of the buffer surface system as well as the effective pH pertinent to a bulk pH 6·6 (namely 5·8) fall well within the range 5·3–6·1 obtained by Hogben & others (1959) in equilibrium studies.

The concept of a buffering surface system differs from the views of Beckett & Moffat (1969b), who claimed that an effective surface pH does not exist but that the pH at the membrane surface is that of the experimental bulk pH. The experimental evidence for this was the finding that the absorption of amphetamine (pKa 9·7) at pH 7·7 was comparable to that of *p*-n-propylphenylacetic acid (pKa 4·36) at pH 6·36, when both drugs are 1% unionized, and that the absorption of methylamphetamine (pKa 10·0) at pH 9·0 was the same as that of *p*-cyclopentylphenylacetic acid (pKa 4·36) and pH 5·36 when both drugs are 90% unionized. But even if it is assumed that the compounds when compared with each other have similar partition coefficients (which are, in fact, given as 3·4, 0·62 17·3 and 2·6 in the above order; Beckett & Moffat, 1969b) so that absorption exclusively reflects the degree of ionization and not lipophilicity, the experimental data do not preclude the existence of a buffering surface system. They only suggest that if such a system should exist its intrinsic pH would not grossly differ from pH 7·0 or 7·2 (calculated means of the test pHs 7·7 and 6·36, and 9·0 and 5·36, respectively), and shifts in experimental bulk pH towards either side of the intrinsic pH would be followed by shifts in effective pH in a symmetrical manner. This is in line with our findings. Crouthamel, Tan & others (1971) in suggesting an alternative concept to the effective pH of Hogben & others (1959), interpreted absorption findings from stomach and intestine through partial transfer of ionized drug through pores and postulated that the κ_{app} is a linear function of the unionized fraction:

$$\kappa_{app} = \kappa_i + f_u(\kappa_u - \kappa_i)$$ (Crouthamel & others, 1971, eqn 3)

where κ_i and κ_u represent the pH independent rate constants of absorption for the ionized and unionized species, respectively. The values of κ_{app} and f_u for propranolol and *p*-n-hexylphenylacetic acid were calculated with equations 8, 9, 12 and 15 for each experimental pH, and are included in Table 3. A plot of κ_{app} over f_u (Fig. 7) clearly shows non-linearity for both compounds. Further evidence against pores in the buccal membrane is that the hydrophilic compound atenolol is not absorbed, and that absorption generally reaches zero values at pH values of high ionization (Beckett & Triggs, 1967; Beckett & Moffat, 1968, 1969a).

The presence of a buffering surface system supports the concept of an 'aqueous diffusion layer' of Suzuki, Higuchi & Ho (1970) and Ho & Higuchi

Table 4. *The buffering surface system in the rat small intestine as evaluated from the pH-dependent absorption of quinine (top) and salicylic acid (bottom).* The pH-absorption data and pKa values (8·4 for quinine, 3·0 for salicylic acid) were taken from Brodie (1964). The parameter values were calculated with eqns 13–17 in the text. Plots of κ and pH_{eff} *vs* pH similar to those in Figs 5 and 6, respectively, give the intrinsic pH as pH 5·55, and a buffer capacity $\Delta pH/\Delta pH\ eff = 4{\cdot}17$.

pH	%abs	κ (min^{-1})	pH_{eff}
4	9	39·5	5·18
5	11	4·9	5·27
7	41	0·23	5·93
8	54	0·046	6·09
4	64	0·19	5·19
5	35	0·73	5·56
7	30	59·5	5·64
8	10	174·4	6·18

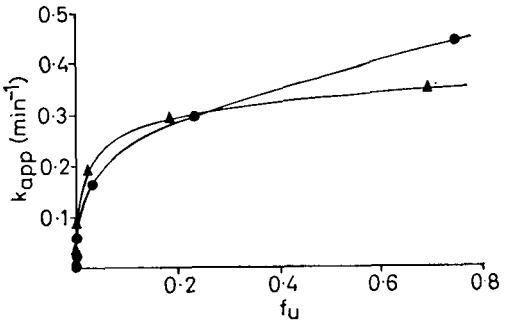


FIG. 7. The non-linear relation between κ_{app} (min^{-1}) (ordinate) and f_u for both propranolol (●) and *p*-n-hexylphenylacetic acid (▲) suggests that the concept of partial transfer of ionized drug through pores (Crouthamel & others, 1971) does not account for the empirical pH-dependence of buccal absorption.

(1971). The anatomical equivalent of such a layer may be sought in the apical glycocalix of the epithelial cells (the 'fuzzy coat'; Ito, 1969), or mucin of salivary origin adherent to the membrane surface. In either case a layer of mucopolysaccharide macromolecules rich in polar functional groups would be able to hold water in an organized, quasi crystalline state (Lehninger, 1975), acting as an aqueous surface system.

So far, the diffusion of various compounds into and across the buccal membrane has been compared using monoexponential rate constants; this was possible because constant time intervals were considered and no inferences about other time points were made. That this limitation is reasonable becomes evident on examination of the time course of absorption.

The time course of simple diffusion from one compartment to another is described by a monoexponential term of the form:

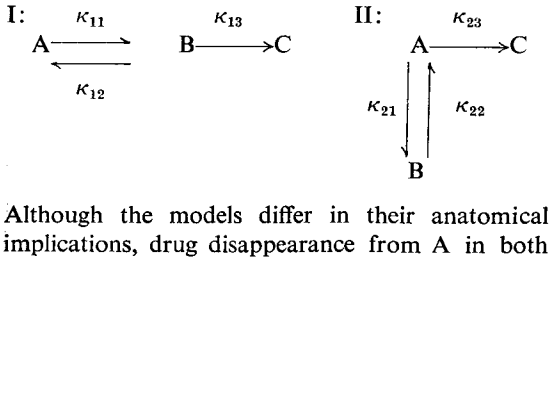
$$D(t) = Xe^{-\kappa t}, \qquad (20)$$

and serial diffusions in multicompartment systems by a sum of exponentials for each compartment, thus

$$D(t) = \sum_{i=1}^{n} Xi\, e^{-\kappa i t} \text{ (Loo \& Riegelman, 1970)} \qquad (21)$$

Compartmental models may be regarded as visualizations of such additive exponential terms, where typically the number of compartments is n + 1.

Of models proposed for the time course of buccal absorption, two attract closer attention. These are that of Dearden & Tomlinson (1971) and that of Beckett & Pickup (1975). Both are three compartmental models comprising the oral cavity, one compartment capable of drug storage, and the general circulation. The difference between them is that in one case (I: membrane storage; Beckett & Pickup, 1975), the storing compartment is connected in series, whereas in the other (II: protein binding; Dearden & Tomlinson, 1971) it lies in parallel with the stream of absorption.



Although the models differ in their anatomical implications, drug disappearance from A in both models is describable in terms of a biexponential function

$$D_A(t) = X_1 e^{-at} + X_2 e^{-bt} \qquad (22)$$

and the individual rate constants in the models I and II can be expressed in either case by the parameters X_1, a, X_2, and b of equation 22 [The equations for the rate constants in model I are given in Doluisio, Crouthamel & others 1970, those for model II are $\kappa_{22} = (aX_2 + bX_1)/(X_1 + X_2)$: $\kappa_{23} = ab/\kappa_{22}$; $\kappa_{21} = a + b - \kappa_{22} - \kappa_{23}$, obtained through coefficient comparison].

The choice between the models has to be made on anatomical grounds and model I appears to be the more adequate. In model II it is not possible to localize the binding proteins since if they are of salivary origin and dissolved in the test fluid, they would be expelled and their drug charge not count as disappeared from A, and if they were membrane bound, they would be in series as in model I.

The empirical time course of absorption of propranolol was submitted to a computer fitting procedure for the biexponential function equation 22. Optimal fit was achieved with the parameter values $X_1 = 0{\cdot}659$, $a = 0{\cdot}0985\ s^{-1}$, $X_2 = 0{\cdot}340$ and $b = 0{\cdot}0074\ s^{-1}$. The close agreement between empirical points and theoretical function (curve A in Fig. 4) confirms that a three compartmental model is basically valid. The rate constants in model I were calculated from the parameter values using the equations given by Doluisio & others (1970): $\kappa_{11} = (aX_1 + bX_2)/(X_1 + X_2) = 0{\cdot}068\ s^{-1}$, $\kappa_{13} = ab/\kappa_{11} = 0{\cdot}011\ s^{-1}$, and $\kappa_{12} = a \pm b - \kappa_{11} - \kappa_{13} = 0{\cdot}028\ s^{-1}$. With them, the drug content in B, D_B, was established from

$$\frac{dD_A}{dt} = \kappa_{12} D_B - \kappa_{11} D_A, \qquad (23)$$

which after isolating D_B and inserting the first derivative of equation 22 for dD_A/dt yields

$$D_B(t) = \frac{1}{\kappa_{12}}(\kappa_{11} D_A(t) - aX_1 e^{-at} - bX_2 e^{-bt}) \qquad (24)$$

The time course of drug in B is included in Fig. 4 as curve B. Equally the time course of the ratio D_B/D_A was calculated and is included in Fig. 4 as curve C. The ratio D_B/D_A rises from zero at t = 0 to a value of about 2·18 within 90 s, and remains constant for the rest of the absorption experiment irrespective of declining absolute concentrations. This suggests that in the plateau phase D_B/D_A can be looked at as a concentration-independent characteristic magnitude,

and since by definition it gives the ratio of drug contents of membrane and oral cavity, its equilibrium value describes a distribution equilibrium between membrane and oral cavity in the way of a distribution coefficient. (The equilibrium state of distribution considered here is identical with the pseudo-steady state of absorption of Suzuki & others (1970); the wording only emphasises the different aspects of it.)

In the pseudo-steady state of absorption, about 2·18 times more drug is stored within the membrane than remains in the oral cavity. This value allows the establishment of an *in vivo* partition coefficient relative to the buccal membrane, if the following assumptions are made concerning the volume of distribution: the surface of the oral cavity is about 100 cm^2 (Ho & Higuchi, 1971), out of which the buccal part—a third—is lined by an epithelium of 0·5 mm thickness, and the rest by one of 0·25 mm thickness (Landay & Schroeder, 1977). Since under sink conditions with a linear distribution of concentration within the membrane, the effective volume is half the available (see later), the lipid volume of partitioning is calculated to be 1·66 cm^3 with the anatomical mucous membrane occupying about 3·3 cm^3. With the dose ratio 2·18/1, and the respective volumes of the oral cavity and membrane, the concentration ratio, i.e. the partition coefficient between membrane and test buffer pH 10·45, is calculated to be

$$P = \frac{c_i}{c_w} = \frac{2{\cdot}18}{1{\cdot}66} \times \frac{20}{1} = 26{\cdot}3 \quad .. \quad .. \quad (25)$$

Since ionized drug is confined to the aqueous phase and only the unionized species equilibrates between aqueous and lipid phases, the partition coefficient of the unionized species (P_u) such as also established in the *in vitro* systems, appears to be a more appropriate measure of the partition properties of a compound. From Fig. 6, the effective pH for a bulk pH 10·45 is approximately 8·0 and the degree of ionization at that pH can be calculated by equation 8. This allows calculation of the *in vivo* partition coefficient of unionized propranolol relative to the buccal mucous membrane, and is found to be:

$$P_u = \frac{c_i}{c_w f_u} = \frac{P}{f_u} = \frac{26{\cdot}3}{0{\cdot}034} = 774 \quad .. \quad (26)$$

The high value of this partition coefficient is not surprising if the partitioning of propranolol in other biological systems is considered, e.g. between plasma and erythrocyte. Cotham & Shand (1975) report that at room temperature and physiological pH the degree of protein binding of propranolol in plasma is 94%, and that about 30% of the total blood content is accumulated in the erythrocytes. McDevitt, Frisk-Holmberg & others (1976) found the percentage of free drug to be 6·8% in normal subjects. Assuming that on an average 6·4% of the plasma propranolol content is unbound, and that the plasma : erythrocyte volume ratio is 55:45, the *in vitro* partition coefficient of unionized propranolol relative to the erythrocyte phase can be calculated to be:

$$P_u = \frac{c_i}{c_w \times f_u \times f_f}$$

$$= \frac{30 \times 55}{70 \times 45 \times 8{\cdot}834 \times 10^{-3} \times 0{\cdot}064} = 926 \quad (\ 27$$

The partitioning of propranolol and atenolol into the buccal membrane does not allow a definite answer to be given about the adequate model lipid for this membrane. Two criteria can be derived: in its unionized form, propranolol is readily taken up into the membrane, whereas atenolol apparently is not taken up; hence an organic model solvent should show a marked difference between the partitioning of propranolol and that of atenolol. Then, the partition coefficient of propranolol in biological material is at least 20–30 times higher than in n-heptane; this should be reflected in the model lipid.

An inspection of the partition coefficients of propranolol and atenolol in various organic solvents (Table 2) shows that both criteria together are not fulfilled optimally by the very high and very low polarity solvents, which include the model lipids for the buccal membrane suggested by Beckett & Moffatt (1969b; n-heptane, $\epsilon = 1{\cdot}92$) and Vora, Higuchi & Ho (1972; isobutanol, $\epsilon = 17{\cdot}7$). The highest difference in log P_u between propranolol and atenolol, and the highest absolute propranolol values are found rather in an intermediate polarity range. It appears that organic solvents from this range (like vinyl ether $\epsilon = 3{\cdot}94$, isopropyl ether $\epsilon = 3{\cdot}88$, phenyl ether $\epsilon = 3{\cdot}65$, dibenzylamine $\epsilon = 3{\cdot}6$, ethyl oleate $\epsilon = 3{\cdot}17$, or ethyl palmitate $\epsilon = 3{\cdot}20$; Weast, 1972) deserve further attention as model lipids.

To summarize our findings, the following physical model of the oral mucous membrane is proposed (Fig. 8):

Three serial compartments are involved in the absorptive process. The first compartment, the oral cavity (A), contains the bulk fluid, which is thought

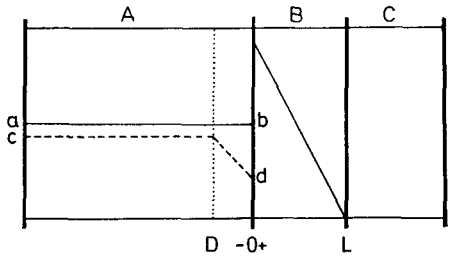


FIG. 8. Unidimensional scheme of the three compartmental diffusional model of the human oral mucous membrane. A, B and C designate the compartments oral cavity (with aqueous surface layer of the membrane), lipoidal membrane, and blood plasma, respectively. The line a——b represents schematically the concentration of total drug, the line c– – –d that of its unionized fraction in the aqueous phase. OD is the thickness of the buffering surface system, OL that of the pharmacological lipid membrane. It is assumed that within the membrane no ionized drug occurs, and that the concentration decreases linearly from a value at +0 determined by the partition coefficient, to zero at L.

to be well stirred and has an equal drug concentration and pH throughout. The same overall drug concentration is also found in the aqueous buffering layer at the membrane surface, which therefore belongs to A in compartmental terms. The concentration of unionized drug within the buffering layer, however, depends on the locally prevailing pH, which itself is influenced by (a) the rate of diffusion of buffer ions from the oral cavity, (b) a possible H^+ ion secretion, postulated for the intestinal wall (Hogben & others, 1959) but not yet reported for the buccal membrane, and (c) the thickness D of the layer of bound water, largely subject to the effect of mechanical stirring (Stehle & Higuchi, 1972) and probably a main source of between subject variance. It is the concentration of unionized drug at −0, that equilibrates with that in the lipid membrane at +0 according to the *in vivo* partition coefficient of the lipid biophase. Compartment B, the membrane, is thought to be homogeneous, of intermediate polarity, and devoid of pores. Compartment C, representing the general circulation, is thought to act as a sink, i.e. it has an infinite volume of distribution in comparison with the amount of drug dissolved, so that the effective concentration is zero for all practical purposes and no back diffusion occurs. Zero concentration at point L implies that in the pseudo-steady state of absorption, when a partition equilibrium between compartments A and B is attained and the distribution of concentration within the membrane is linear, the effective volume of partitioning in B is exactly half the available volume. Although it is likely that there exists a second aqueous diffusion layer attached to the capillary wall, this has not been included in the model because its effect could not be shown empirically; to do so, more than the classical buccal absorption test would be required.

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REFERENCES

ANKIER, S. I. & KAYE, C. M. (1976). *Br. J. clin. Pharmac.*, **3**, 672.

BECKETT, A. H. & TRIGGS, E. J. (1967). *J. Pharm. Pharmac.*, **19**, *Suppl.*, 31S–41S.

BECKETT, A. H. & MOFFAT, A. C. (1968). *Ibid.*, **20**, *Suppl.*, 239S–247S.

BECKETT, A. H. & MOFFAT, A. C. (1969a). *Ibid.*, **21**, *Suppl.*, 139S–143S.

BECKETT, A. H. & MOFFAT, A. C. (1969b). *Ibid.*, **21**, *Suppl.*, 144S–150S.

BECKETT, A. H. & PICKUP, M. E. (1975). *Ibid.*, **27**, 226–234.

BRODIE, B. B. & HOGBEN, C. A. M. (1957). *Ibid.*, **9**, 345–380.

BRODIE, B. B. (1964). In: *Absorption and Distribution of Drugs*, pp. 16–48 Editor: Binns, T. B., Edinburgh: Livingstone.

COTHAM, R. & SHAND, D. G. (1975). *Clin. Pharmac. Ther.*, **18**, 535–538.

CROUTHAMEL, W. G., TAN, G. H., DITTERT, L. W. & DOLUISIO, J. T. (1971). *J. pharm. Sci.*, **60**, 1160–1163.

DEARDEN, J. C. & TOMLINSON, E. (1971). *J. Pharm. Pharmac.*, **23**, *Suppl.*, 68S–72S.

Documenta Geigy (1959). *Scientific Tables*, 5th Ed., p. 105. Basle: Geigy.

DOLUISIO, J. T., CROUTHAMEL, W. G., TAN, G. H., SWITOSKY, J. V. & DITTERT, L. W. (1970). *J. pharm. Sci.*, **59**, 72–76.

HARRISON, R. D. (1972). *Book of data*, p. 89. Harmondsworth: Penguin.

HO, N. F. H. & HIGUCHI, W. I. (1971). *J. pharm. Sci.*, **60**, 537–541.

HOBSLEY, M. & SILEN, W. (1969). *Gut*, **10**, 787–795.

HOGBEN, C. A. M., SCHANKER, L. S., TOCCO, D. J. & BRODIE, B. B. (1957). *J. Pharmac. exp. Ther.*, **120**, 540–545.

HOGBEN, C. A. M., TOCCO, D. J., BRODIE, B. B. & SCHANKER, L. S. (1959). *Ibid.*, **125**, 275–282.
ITO, S. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **28**, 12.
KAYE, C. M. (1974). *Br. J. clin. Pharmac.*, **1**, 84–86.
KAYE, C. M. & LONG, A. D. (1976). *Ibid.*, **3**, 196–197.
KHALIL, S. A. & MARTIN, A. N. (1967). *J. pharm. Sci.*, **56**, 1225–1233.
KIDDIE, M. A. & KAYE, C. M. (1976). *Br. J. clin. Pharmac.*, **3**, 350P–351P.
LANDAY, M. A. & SCHROEDER, H. E. (1977). *Cell Tiss. Res.*, **177**, 383–407.
LEHNINGER, A. L. (1975). *Biochemistry*. 2nd Edn., pp. 271–274. New York: Worth.
LOO, J. C. K. & RIEGELMAN, S. (1970). *J. pharm. Sci.*, **59**, 53–55.
MARTIN, A. N., SWARBRICK, J. & CAMMARATA, A. (1975). *Physikalische Pharmazie*, Stuttgart: Wissenschaftliche Verlagsgesellschaft.
MCDEVITT, D. G., FRISK-HOLMBERG, M., HOLLIFIELD, J. W. & SHAND, D. G. (1976). *Clin. Pharm. Ther.*, **20**, 152–157.
MEYER, W., KAYE, C. M. & TURNER, P. (1974). *Eur. J. clin. Pharmac.*, **7**, 287–289.
SHAND, D. G., NUCKOLLS, E. M. & OATES, J. A. (1970). *Clin. Pharmac. Ther.*, **11**, 112–120.
STEHLE, R. G. & HIGUCHI, W. I. (1972). *J. pharm. Sci.*, **61**, 1931–1935.
SUZUKI, A., HIGUCHI, W. I. & HO, N. F. H. (1970). *Ibid.*, **59**, 644–651.
TRINDNER, P. (1954). *Biochem. J.*, **57**, 301–303.
VORA, K. R. M., HIGUCHI, W. I. & HO, N. F. H. (1972). *J. pharm. Sci.*, **61**, 1785–1791.
WEAST, R. C. (Ed). (1972). *Handbook of Chemistry and Physics*, 53rd Ed., p. E43. Cleveland: Chemical Rubber Co.
WEAST, R. C., SELBY, S. M. & HODGMAN, C. D. (Eds). (1965). *Handbook of Chemistry and Physics*, 46th Ed. p. E49. Cleveland: Chemical Rubber Co.