

## **Quantitation of Rate of Gastrointestinal and Buccal Absorption of Acidic and Basic Drugs Based on Extraction Theory**

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*Equations have been derived which quantitatively describe the rate of gastrointestinal and buccal absorption of acidic and basic drugs as a function of pH of aqueous luminal contents and time. The equations have been used to fit observed data in the literature, and the estimated parameters are reported. An equation which describes the renal clearance of an acidic or basic drug as a function of urinary pH is also derived. In essence, the equations quantitate the pH-partition hypothesis and explain most, if not all, related observed data in the literature. The results suggest that the aqueous diffusion layer may not rate-limit absorption of monomeric drug molecules but that absorption is rate-limited by transfer of drug out of the membrane in vivo.*

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**KEY WORDS:** rate of absorption; rate of renal reabsorption; extraction theory; partition coefficient *in vivo*; pH of luminal contents.

### **INTRODUCTION**

Several authors (1–7) have developed equations in an attempt to explain the change in rate of absorption of acidic and basic drugs with change in pH of the aqueous luminal contents of the gastrointestinal tract of animals. Analogously, several authors (8–14) have developed equations in an attempt to explain the rate of passive reabsorption of acidic, basic, and neutral drugs from aqueous fluids in the kidney tubules to the renal interstitial fluid and the change in renal clearance of acidic and basic drugs with change in urinary pH.

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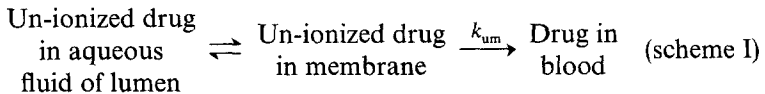
For purposes of discussion,  $k_{app}$  is defined as the apparent first-order rate constant for disappearance of total drug from the aqueous fluids of the gastrointestinal lumen, or buccal cavity, or for reabsorption of drug in the kidney tubule. The theory to be presented disregards the aqueous diffusion layer on the lumen side of the membrane and is based on simple extraction theory. The equations derived account for *all* of the following in quantitative terms: (a) the observed rates of gastrointestinal or buccal absorption, (b) the "pH shifts" that occur, and (c) the limiting  $k_{app}$  for buccal or gastrointestinal absorption that occurs in a homologous series as the series is ascended. By "pH shift" is meant that a plot of  $k_{app}$  vs. pH is shifted to higher pH values than a plot of fraction of drug which is un-ionized vs. pH for an acidic drug, and that a plot of  $k_{app}$  vs. pH is shifted to lower pH values than a plot of fraction of drug which is un-ionized vs. pH for a basic drug. It is shown that the equations derived in this report are capable of fitting  $k_{app}$  vs. pH data which were available in the literature and capable of quantitatively explaining all of the above phenomena. To our knowledge, this is the first time that the parameters of a mathematical model have been *directly* estimated by fitting  $k_{app}$  vs. pH data, where the *observed* pH values in the luminal contents or buccal cavity are employed.

## THEORY

Equations for  $k_{app}$  are derived for two different models.

### Model A

Model A assumes that only undissociated molecules transfer from aqueous fluid in the gastrointestinal lumen or buccal cavity into the membrane and out of the membrane into the circulating blood as indicated in scheme I:



It is assumed that transfer of undissociated molecules through the aqueous diffusion layer on the lumen side of the membrane is much more rapid than transfer of undissociated molecules out of the membrane. Rapid equilibration of undissociated molecules in the aqueous fluids of the lumen with undissociated molecules in the membrane is then consistent with this assumption. There may be an initial lag period before equilibrium occurs, and the equations derived pertain to the condition subsequent to the end of this initial lag period.

Material balance gives

$$A_w + A_{um} + A_b = D \quad (1)$$

where  $A_w$  is the total amount of drug in aqueous fluid of the lumen at time  $t$ ,  $A_{um}$  is the amount of undissociated molecules in the membrane at time  $t$ ,  $A_b$  is the amount of drug in the blood at time  $t$  (which arose from, but does not necessarily still exist as, undissociated molecules), and  $D$  is the total dose of drug introduced into the lumen, and hence is a constant.

Differentiation of equation 1 with respect to time  $t$  gives

$$dA_w/dt + dA_{um}/dt + dA_b/dt = 0 \quad (2)$$

By definition,

$$K_u = \frac{C_{um}}{C_{uw}} = \frac{A_{um}/V_m}{A_{uw}/V_w} = \frac{A_{um}}{A_{uw}} \cdot \frac{V_w}{V_m} \quad (3)$$

where  $K_u$  is the intrinsic partition coefficient of undissociated molecules between the membrane and aqueous fluids of the lumen,  $C_{um}$  is the concentration of undissociated molecules in the membrane,  $C_{uw}$  is the concentration of undissociated molecules in aqueous luminal contents under intrinsic conditions (i.e.,  $pH \rightarrow 0$  for a monobasic acid and  $pH \rightarrow 14$  for a monacidic base when  $pK_w = 14$  at  $24^\circ\text{C}$ ),  $V_m$  is the effective volume of the membrane, and  $V_w$  is the effective volume of the aqueous fluids of the lumen.

Rearrangement of equation 3 gives equation 4:

$$A_{um} = (V_m/V_w) \cdot K_u \cdot A_{uw} \quad (4)$$

Equation 4 holds under conditions where model A holds, i.e., negligible back diffusion from blood into the membrane and essentially instantaneous distribution of un-ionized drug between the membrane and the lumen.

By definition,

$$P_u = (V_m/V_w) \cdot K_u = (V_m/V_w) \cdot (C_{um}/C_{uw}) \quad (5)$$

$$f_u = C_{uw}/(C_{uw} + C_{iw}) = C_{uw}/C_w \quad (6)$$

where  $P_u$  is the intrinsic partition coefficient which incorporates the phase volume ratio,  $f_u$  is the fraction of total drug in the aqueous fluid of the lumen which is undissociated,  $C_{iw}$  is the concentration of ionized drug in the aqueous fluid of the lumen, and  $C_w$  is the total concentration of drug in the aqueous fluid of the lumen.

From equation 6, one obtains equations 7 and 8:

$$C_{uw} = f_u \cdot C_w \quad (7)$$

$$A_{uw} = V_w C_{uw} = f_u V_w C_w = f_u A_w \quad (8)$$

Substituting from equations 5 and 8 into equation 4 gives

$$A_{um} = f_u P_u A_w \quad (9)$$

Differentiating equation 9 with respect to time yields

$$dA_{um}/dt = f_u P_u (dA_w/dt) \quad (10)$$

The rationale for equations 9 and 10 is as follows. The undissociated drug is assumed to partition between the aqueous fluid of the lumen and the membrane in much the same manner that a drug partitions between an aqueous buffer and an organic solvent *in vitro*. Equation 9 expresses the mass balance of this partitioning. It is also assumed that the rate of transfer of undissociated molecules through the bulk aqueous phase and the aqueous diffusion layer on the lumen side of the membrane is so rapid compared with the rate of transfer of undissociated molecules out of the membrane that the rate *into* the membrane may be ignored. Hence equation 10 may be written.

The  $dA_b/dt$  of equation 2 represents the rate of *appearance* of drug in the blood and is given by equation 11 when back diffusion from the blood to the membrane is assumed to be negligible.

$$dA_b/dt = k_{um} \cdot A_{um} \quad (11)$$

where  $k_{um}$  is the first-order rate constant for transport of the undissociated drug out of the membrane.

Substituting from equation 9 into equation 11 gives

$$dA_b/dt = k_{um} f_u P_u A_w \quad (12)$$

Substituting from equations 10 and 12 into equation 2 yields

$$dA_w/dt + f_u P_u (dA_w/dt) + k_{um} f_u P_u A_w = 0 \quad (13)$$

Rearrangement of equation 13 gives

$$-\frac{dA_w}{dt} = \left\{ \frac{k_{um} f_u P_u}{1 + f_u P_u} \right\} A_w \quad (14)$$

Since

$$A_w = V_w \cdot C_w \quad (15)$$

substituting for  $A_w$  in equation 14 from equation 15 and cancelling the  $V_w$ 's gives

$$-dC_w/dt = \{k_{um} f_u P_u / (1 + f_u P_u)\} C_w \quad (16)$$

where

$$k_{app} = k_{um} f_u P_u / (1 + f_u P_u) \quad (17)$$

It should be noted that from equations 2 and 10 one obtains equation 18:

$$\frac{dA_b}{dt} = - \left[ \left( \frac{1 + f_u P_u}{f_u P_u} \right) \frac{dA_{um}}{dt} \right] \quad (18)$$

Equation 18 indicates that in this theory the rate of appearance of drug in the blood is proportional to, but not equal to, the rate of change of amount of drug in the membrane.

Also, let  $f_E$  be the fraction of the total drug in the aqueous fluids of the lumen which is extracted by the membrane. This is analogous to the o/w partitioning of drug between an organic solvent and an aqueous phase *in vitro*. Then

$$f_E = \frac{V_w C_{um}}{V_m C_{um} + V_w C_{uw} + V_w C_{iw}} = \frac{V_m C_{um}}{V_m C_{um} + V_w C_w} = \frac{A_{um}}{A_{um} + A_w} \quad (19)$$

Substituting for  $A_{um}$  in equation 19 from equation 9 and simplification gives

$$f_E = f_u P_u / (1 + f_u P_u) \quad (20)$$

Substituting from equation 20 into equation 17 yields

$$k_{app} = k_{um} \cdot f_E \quad (21)$$

In the equations above,  $f_u$  is given by equation 22 for a monobasic acid and by equation 23 for a monoacidic base:

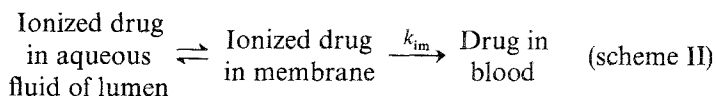
$$f_u = 1 / (1 + 10^{pH - pK_a}) \quad (22)$$

$$f_u = 1 / (1 + 10^{pK_a - pH}) \quad (23)$$

More complicated expressions giving  $f_u$  for dibasic acids, diacidic bases, amphoteric compounds, etc., are readily obtained.

## Model B

Model B assumes that undissociated molecules transfer from aqueous fluid in the gastrointestinal lumen or buccal cavity into the membrane and out of the membrane into the circulating blood as indicated in scheme I for model A. In addition, model B assumes that ionized drug transfers from aqueous fluid in the gastrointestinal lumen or buccal cavity into the membrane and out of the membrane into the circulating blood as indicated in scheme II:



Material balance gives

$$A_w + A_{um} + A_{im} + A_b = D \quad (24)$$

where  $A_w$ ,  $A_{um}$ , and  $D$  are as defined above,  $A_{im}$  is the amount of ionized drug in the membrane at time  $t$ , and  $A_b$  is the amount of drug in the blood at time  $t$  which arose from transport of both undissociated molecules and ions out of the membrane (but the same ratio of molecules to ions need not necessarily exist in blood as in the membrane).

Differentiation of equation 24 with respect to time gives

$$dA_w/dt + dA_{um}/dt + dA_{im}/dt + dA_b/dt = 0 \quad (25)$$

The same assumptions are made with respect to ions as made for undissociated molecules under scheme I above.

By definition,

$$K_i = \frac{C_{im}}{C_{iw}} = \frac{A_{im}/V_m}{A_{iw}/V_w} = \frac{A_{im}}{A_{iw}} \cdot \frac{V_w}{V_m} \quad (26)$$

where  $K_i$  is the intrinsic partition coefficient of ionized drug between the membrane and aqueous fluid of the lumen,  $C_{im}$  is the concentration of ionized drug in the membrane, and  $C_{iw}$  is the concentration of ionized drug in aqueous luminal contents under intrinsic conditions (i.e.,  $pH \rightarrow 14$  for a monobasic acid and  $pH \rightarrow 0$  for a monoacidic base when  $pK_w = 14$  at  $24^\circ\text{C}$ ).

Rearrangement of equation 26, and assumptions with respect to ions similar to those made for un-ionized drug above, gives

$$A_{im} = (V_m/V_w) \cdot K_i \cdot A_{iw} \quad (27)$$

By definition,

$$P_i = (V_m/V_w) \cdot K_i = (V_m/V_w) \cdot (C_{im}/C_{iw}) \quad (28)$$

From equation 6, one obtains

$$C_{iw} = (1 - f_u)C_w \quad (29)$$

Hence

$$A_{iw} = V_w C_{iw} = (1 - f_u)V_w C_w = (1 - f_u)A_w \quad (30)$$

Substituting from equations 28 and 30 into equation 27 gives

$$A_{im} = (1 - f_u)P_i A_w \quad (31)$$

Differentiating equation 31 with respect to time yields

$$dA_{im}/dt = (1 - f_u)P_i(dA_w/dt) \quad (32)$$

The rationale for equations 31 and 32 is analogous to the rationale for equations 9 and 10 discussed above under model A.

The  $dA_b/dt$  in equation 25 represents the rate of *appearance* of drug in the blood from both undissociated molecules and ions passing out of the membrane and hence is given by equation 33:

$$dA_b/dt = k_{um}A_{um} + k_{im}A_{im} \quad (33)$$

where  $k_{im}$  is the first-order rate constant for transport of ionized drug out of the membrane and the other symbols are as defined above.

Substituting from equations 9 and 31 into equation 33 gives

$$dA_b/dt = k_{um}f_uP_uA_w + k_{im}(1 - f_u)P_iA_w \quad (34)$$

Substituting from equations 10, 32, and 34 into equation 25 gives

$$\frac{dA_w}{dt} + f_uP_u \frac{dA_w}{dt} + (1 - f_u)P_i \frac{dA_w}{dt} + k_{um}f_uP_uA_w + k_{im}(1 - f_u)P_iA_w = 0 \quad (35)$$

Rearrangement of equation 35 gives

$$-\frac{dA_w}{dt} = \left\{ \frac{k_{um}f_uP_u + k_{im}(1 - f_u)P_i}{1 + f_uP_u + (1 - f_u)P_i} \right\} A_w \quad (36)$$

Substituting from equation 15 into equation 36 and cancelling the  $V_w$ 's gives

$$-\frac{dC_w}{dt} = \left\{ \frac{k_{um}f_uP_u + k_{im}(1 - f_u)P_i}{1 + f_uP_u + (1 - f_u)P_i} \right\} C_w \quad (37)$$

where

$$k_{app} = \frac{k_{um}f_uP_u + k_{im}(1 - f_u)P_i}{1 + f_uP_u + (1 - f_u)P_i} \quad (38)$$

In equation 38,  $f_u$  for a monobasic acid is given by equation 22 and for a monoacidic base by equation 23.

For model B, equation 39 gives the fraction of the total drug in the aqueous fluids of the lumen which is extracted by the membrane ( $f_E$ ); this equation is analogous to equation 19 for Model A:

$$f_E = (A_{um} + A_{im})/(A_{um} + A_{im} + A_w) \quad (39)$$

Substituting from equations 9 and 31 into equation 39, followed by simplification, gives

$$f_E = [f_uP_u + (1 - f_u)P_i]/[1 + f_uP_u + (1 - f_u)P_i] \quad (40)$$

The relationship between equations 38 and 40 is at once apparent and of interest.

## Explanation of Various Observed Phenomena by the Equations

### First-Order Absorption

Equations 17 and 38 indicate that at fixed pH of luminal or buccal contents,  $k_{app}$  is a constant. Equations 14, 16, 36, and 37 indicate that at fixed pH of luminal or buccal contents disappearance of total drug is apparent first order. Crouthamel *et al.* (7), Kakemi *et al.* (15-17), Shore *et al.* (1), and Hogben *et al.* (2) have all demonstrated first-order disappearance of total drug from the luminal contents of animal intestine, and Beckett *et al.* (18,19) have demonstrated first-order disappearance of total drug from the contents of the buccal cavity in man. Hence the above equations are in conformity with these observations.

### Asymptotic Nature of $k_{app}$ in a Homologous Series

For an *acidic drug*, as  $pH \rightarrow 0$ ,  $f_u \rightarrow 1$ , and from equations 17 and 38 one obtains equation 41:

$$k_{app} \rightarrow k_{um}P_u/(1 + P_u) \quad (41)$$

For an *acidic drug*, as  $pH \rightarrow 14$ ,  $f_u \rightarrow 0$ , and from equation 38 one obtains equation 42:

$$k_{app} \rightarrow k_{im}P_i/(1 + P_i) \quad (42)$$

In the absence of absorption of ions, then from equation 17, under the same conditions,

$$k_{app} \rightarrow 0 \quad (43)$$

For a *basic drug*, as  $pH \rightarrow 14$ ,  $f_u \rightarrow 1$ , and from equations 17 and 38 one obtains equation 41 under these conditions. As  $pH \rightarrow 0$ ,  $f_u \rightarrow 0$ , one obtains equation 42 from equation 38. In the absence of absorption of ions, one obtains equation 43 under these conditions.

In a homologous series, such as the *n*-alkanoic acids, as the series is ascended both the undissociated molecules and the ionized species become more and more lipophilic, hence  $K_u$ ,  $P_u$ ,  $K_i$ , and  $P_i$  become larger and larger. Hence, for higher members of such a series of *acidic* compounds, as  $pH \rightarrow 0$  equation 41 reduces to equation 44:

$$k_{app} \rightarrow k_{um} \quad (44)$$

Also, as  $pH \rightarrow 14$ , equation 42 reduces to equation 45:

$$k_{app} \rightarrow k_{im} \quad (45)$$

Equation 44 is an entirely different prediction than that made by the equations of Suzuki *et al.* (4). Those authors' equations predict that as  $K_u$  increases,



diffusion through the aqueous diffusion layer, or so-called stagnant water layer, becomes rate-limiting. That the observed  $k_{app}$  does become asymptotic at low pH values of contents of the buccal cavity as the homologous series of *n*-alkanoic acids is ascended is indicated by the data of Beckett and Moffat (20). Also, their data indicate that for low members of the series,  $k_{app} \rightarrow 0$  as the pH is progressively increased, but for higher members of the series  $k_{app}$  approaches a limiting value as  $pH \rightarrow 14$ . The latter is explained in this theory by equation 42.

The shape of  $k_{app}$  vs. pH plots, or plots of percent absorbed in a given time vs. pH, based on data reported by Beckett and Moffat (20) and Crouthamel *et al.* (7), is readily explained by equations 17 and 38. Equations 41 through 45 are also useful in obtaining preliminary estimates of parameters for digital computer fitting of  $k_{app}$ , pH data to either equation 17 or equation 38 as shown later under *Results*.

#### *Asymptotic Nature of $f_E$*

Equations 20 and 40 indicate that  $f_E$  becomes asymptotic as  $P_u$  is increased at any fixed pH. Under conditions used to obtain the intrinsic partition coefficient of the undissociated species *in vitro* (i.e., pH  $\rightarrow 0$  for an acidic drug and pH  $\rightarrow 14$  for a basic drug),  $f_u = 1$  and equation 20 reduces to equation 46:

$$(f_E)_I = P_u / (1 + P_u) \quad (46)$$

where  $(f_E)_I$  represents the fraction extracted under intrinsic partition coefficient conditions. Equation 46 indicates that  $(f_E)_I$  becomes asymptotic as  $P_u$  increases.

#### *The pH Shifts*

Equations 17 and 38 readily explain the so-called pH shift of the  $k_{app}$ , pH profile away from the  $f_u$ , pH profile. For an acidic drug, this may be most readily seen by rearranging equation 20 and substituting for  $f_u$  from equation 22 as follows:

$$f_E = \frac{f_u P_u}{1 + f_u P_u} = \frac{1}{1 + (1/f_u P_u)} = \frac{1}{1 + [(1 + 10^{pH - pK_a})/P_u]} \quad (47)$$

Equation 47 is readily rearranged to equation 48:

$$pH = pK_a + \log[P_u(1/f_E - 1) - 1] \quad (48)$$

Equation 48 indicates that the extraction curve of an acidic drug is shifted to higher pH values than the  $f_u$  vs. pH curve. When  $P_u \geq 2$  and  $f_E = 0.5$ , equation 48 becomes equation 49:

$$(pH)_{0.5E} = pK_a + \log(P_u - 1) \quad (49)$$

where  $(pH)_{0.5E}$  represents the  $pH$  at which there is 50% extraction. Equation 49 indicates that the midpoint of the extraction curve and the midpoint of the  $f_u$  vs.  $pH$  curve (namely, the  $pK_a$ ) are separated by  $\log(P_u - 1)$  units of  $pH$ .

For a basic drug, by similar manipulation, one obtains equations 50 and 51:

$$pH = pK_a - \log [P_u(1/f_E - 1) - 1] \quad (50)$$

When  $P_u \geq 2$  and  $f_E = 0.5$ , equation 50 becomes

$$(pH)_{0.5E} = pK_a - \log(P_u - 1) \quad (51)$$

The relationships expressed in equations 49 and 51 are illustrated in Fig. 1.

It is interesting that equations analogous to, but not the same as, equations 48 through 51 were published by Craig (21) and Golumbic *et al.*

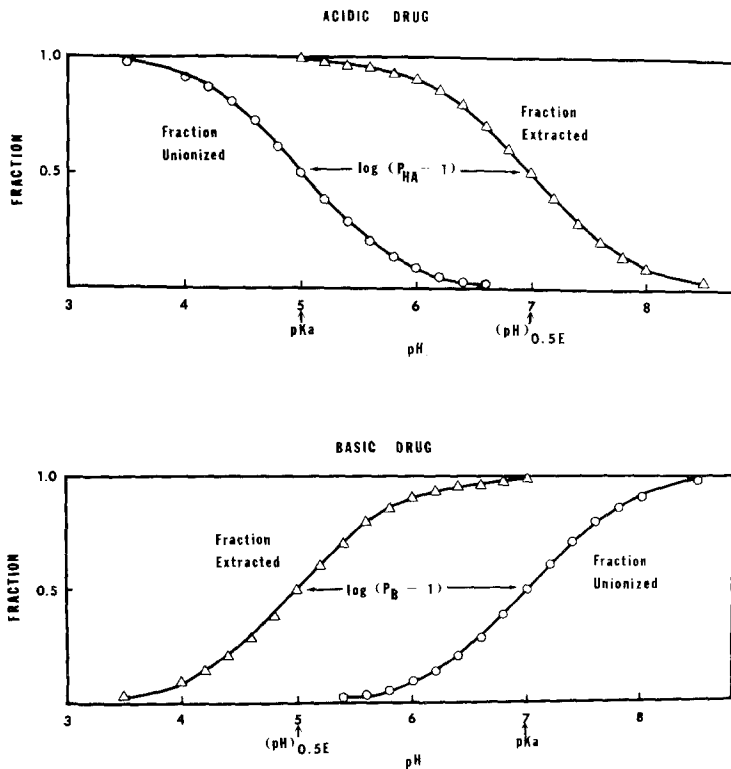


Fig. 1. Plots of fraction extracted and fraction un-ionized against  $pH$  for a monobasic acid and a monoacidic base. The  $pH$  shift shown is based on equations in the text.

(22,23) in the period 1943 to 1950 before Brodie and coworkers elaborated the  $pH$ -partition hypothesis (1,2), yet this extraction theory was never incorporated into the latter theory, but may be very pertinent.

Ion absorption also causes an additional shift in the  $k_{app}$ ,  $pH$  profile away from the  $f_u$ ,  $pH$  curve in the same direction as discussed above. Hence there are really two factors which contribute to the so-called  $pH$  shifts.

### Possible Modification of the Derived Equations

In special circumstances, equations 16 and 37 will require modification. For amphoteric compounds, dibasic acids, diacidic bases, etc., more than two species may transfer into and out of the membrane. This would lead to more terms in the expressions for  $k_{app}$  than shown in equations 17 and 38. However, the theory could be readily extended to such compounds.

Equations 16 and 37 assume no back diffusion of drug from blood. Since the volumes of distribution of drugs are much larger than the effective volumes of luminal contents,  $C_w \gg C_b$  during most of the absorption process, where  $C_b$  is the blood concentration of the drug. However, if the drug were infused intravenously, and at the same time perfused in the lumen of the intestine, as in some of the experiments of Brodie *et al.* (1,2), then  $C_b$  may approach or even equal  $C_w$ . Equations 16 and 37 could be modified to cover such conditions, but the modifications made would depend on the assumptions made.

Various other modifications of experimental conditions such as changing luminal contents to hypotonic or hypertonic states or changing buffer capacity of luminal contents may require modification of the equations. However, the authors also feel that such modification of experimental conditions also probably modifies the properties of the membrane and makes interpretation of data collected in such studies extremely complicated. As applied to data collected in normal animals and man to date under normal physiological conditions, the derived equations appear to explain the observations very well.

### Application of the Derived Equations to Reabsorption of Drug in Kidney Tubules

Equations 17 or 38 should also apply to reabsorption of drugs in the distal tubule of the kidney. Equation 52 is a reasonable expression for the excretion rate of a drug:

$$\frac{dA_U}{dt} = \sigma k_1 V_d C_p + \frac{T_m C_p}{K_m + C_p} - k_{app} V_T C_U \quad (52)$$

where  $dA_U/dt$  is the excretion rate of the drug (mass/time),  $\sigma$  is the fraction

of the drug in plasma at the total concentration ( $C_p$ ) which is free or non-protein-bound,  $k_1$  is a first-order rate constant for glomerular filtration ( $\text{time}^{-1}$ ),  $V_d$  is the appropriate volume of distribution for glomerular filtration,  $T_m$  is the transport maximum (mass/time),  $K_m$  is the "Michaelis constant" of the transport mechanism (mass/volume),  $k_{app}$  is given by either equation 17 or 38,  $V_T$  is the effective volume of tubule fluid from which reabsorption occurs, and  $C_U$  is the concentration of drug in the urine. The first term on the right-hand side of equation 52 is the glomerular filtration component, the second term is the transport component, and the third term is the reabsorption component.

The uncorrected renal clearance ( $R_c$ ) is the excretion rate divided by the total plasma concentration ( $C_p$ ) and is given by equation 53:

$$R_c = \frac{dA_U/dt}{C_p} = \sigma k_1 V_d + \frac{T_m}{K_m + C_p} - k_{app} V_T \cdot \frac{C_U}{C_p} \quad (53)$$

If the transport mechanism is in the first-order region (i.e.,  $K_m \gg C_p$ ), then equation 53 becomes equation 54:

$$R_c = V_d(\sigma k_1 + k_2) - k_{app} V_T \cdot (C_U/C_p) \quad (54)$$

where  $k_2 = T'_m/K_m$  and  $T'_m = T_m/V_d$ .

Equations 53 and 54 predict that a plot of  $R_c$  vs.  $pH$  for an acidic drug will have a skewed S-shape. At low urine  $pH$ ,  $k_{app}$  will be large, the reabsorption contribution will be large, and  $R_c$  will be small. As the  $pH$  is progressively raised,  $R_c$  will increase curvilinearly. When urine  $pH$  is high,  $k_{app}$  will be small, the reabsorption contribution will be small, and  $R_c$  will asymptotically approach the value  $V_d(\sigma k_1 + k_2)$ . Davis and Smith (24) and Levy *et al.* (25) published data giving the renal clearance of salicylate as a function of urine  $pH$ . The curves have a similar shape to that predicted above.

A plot of  $R_c$  vs. urine  $pH$  for a basic drug would be expected to have a skewed inverted S-shape based on equations 53 and 54. At low urine  $pH$ ,  $k_{app}$  will be small, the reabsorption component will be small, and  $R_c$  will be large and approach the asymptotic value of  $V_d(\sigma k_1 + k_2)$ . As the  $pH$  of urine is progressively raised,  $R_c$  will decrease curvilinearly. When the urine  $pH$  is high,  $k_{app}$  will be large, the reabsorption contribution will be large, and  $R_c$  will be small.

## EXPERIMENTAL

### Fitting of Observed $k_{app}$ , $pH$ Data to Model A

#### *Buccal Absorption of Ortho-, Meta-, and Paratoluic Acids in Man*

Beckett and Moffat (20) presented a graph of percent absorbed in 5 min against observed  $pH$  of buccal contents for the ortho-, meta-, and

paratoluic acids in man. The data resulted from application of their buccal absorption test. Beckett kindly supplied the senior author the numerical values which were plotted on their graph. The values of "percent absorbed in 5 min" were converted to  $k_{app}$  values by means of equation 55:

$$k_{app} = \frac{-[\ln 1 - (\% \text{ absorbed}/100)]}{5 \times 60} \times 10^3 \quad (55)$$

where  $k_{app}$  has dimensions of  $\text{sec}^{-1} \times 10^3$ . The  $k_{app}$ , pH values thus obtained for the three acids were simultaneously fitted to equations 17 and 22 by the method of least squares using the program NONLIN and an IBM 360/67 digital computer.

#### *Buccal Absorption of C<sub>4</sub> Through C<sub>8</sub> n-Alkanoic Acids in Man*

Beckett and Moffat (20) presented a graph of percent absorbed in 5 min against observed pH of buccal contents for the C<sub>4</sub> through C<sub>12</sub> n-alkanoic acids in man. Beckett kindly supplied the senior author the numerical values which were plotted on the graph. The values of "percent absorbed in 5 min" were converted to  $k_{app}$  values by means of equation 55. These data were divided into two groups: (a) one for the C<sub>4</sub> through C<sub>8</sub> acids and (b) the other for the C<sub>9</sub> through C<sub>12</sub> acids. The reasons for these groupings were as follows. First, it was desirable to test the fit of the data for the C<sub>4</sub> through C<sub>8</sub> acids to both models A and B, since, although ion absorption was suspected, the magnitude of the ion absorption relative to the absorption of the un-ionized molecules was relatively small. Second, the data for the C<sub>9</sub> through C<sub>12</sub> acids could not be fitted by electronic calculator at all well to model A, hence least-squares fitting was only attempted to model B. Third, a simultaneous least-squares fit of the data for all acids (C<sub>4</sub> through C<sub>12</sub>) to model B was not feasible with the program NONLIN, since there would be 21 parameters to estimate and the program allows only 16 parameters to be estimated.

The  $k_{app}$ , pH values for the five n-alkanoic acids, C<sub>4</sub> through C<sub>8</sub>, were simultaneously fitted to model A (equations 17 and 22) by the method of least squares using the program NONLIN and an IBM 360/67 digital computer.

#### **Fitting of Observed $k_{app}$ , pH Data to Model B**

##### *Buccal Absorption of C<sub>4</sub> Through C<sub>12</sub> n-Alkanoic Acids in Man*

As explained above, two simultaneous fittings of  $k_{app}$ , pH data were made to equations 22 and 38, one employing the data for the C<sub>4</sub> through C<sub>8</sub> acids and the other employing the data for the C<sub>9</sub> through C<sub>12</sub> acids. The method of fitting was as described above.

### Gastrointestinal Absorption of Barbitol and Sulfaethidole in Rat Intestine

The  $k_{app}$  and luminal pH values for absorption of barbitol and sulfaethidole in the rat small intestine, reported by Crouthamel *et al.* (7), were fitted to equations 22 and 38 individually by the method described above. Before fitting, the  $k_{app}$  values with dimensions  $\text{min}^{-1}$  were converted to  $\text{hr}^{-1}$  for scaling purposes.

## RESULTS

### Buccal Absorption of Ortho-, Meta-, and Paratoluic Acids in Man

Figure 2 shows the results of the simultaneous fitting of the three sets of data to model A (equations 17 and 22). The lines drawn through the points are the model-predicted  $k_{app}$  values, namely,  $k_{app}^{\wedge}$ , based on the estimated parameters shown in Table I. The standard deviations of the estimated parameters, shown in Table I, were calculated by means of equation 56:

$$SD = \sqrt{\Sigma \text{dev}^2 / (N - P_*) \cdot C_{ii}} \quad (56)$$

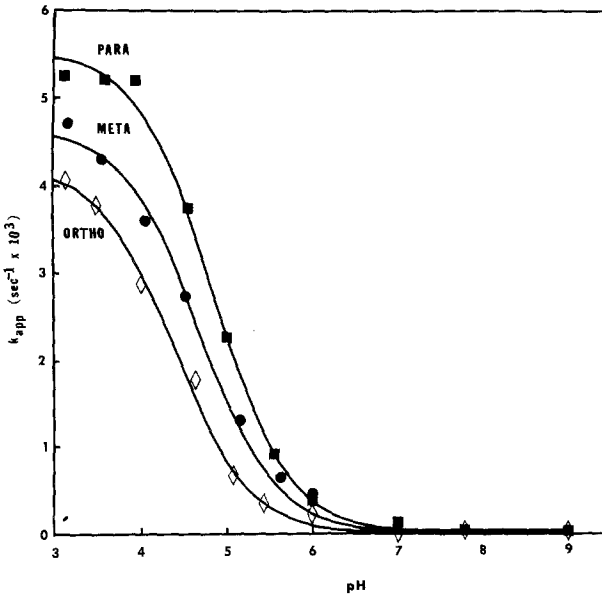


Fig. 2. Fit of the  $k_{app}$ , pH data of Beckett and Moffat (20) for buccal absorption of ortho-, meta-, and paratoluic acids to model A, based on the parameters shown in Table I.

**Table I.** Estimated Parameters and Measures of Fit for Simultaneous Nonlinear Least-Squares Fitting of  $k_{app}$ , pH Data<sup>a</sup> for Buccal Absorption of Ortho-, Meta-, and Paratoluic Acids in Man to Model A (Equations 17 and 22)

Parameter	Estimate	SD <sup>b</sup>
$k_{um}$ (sec <sup>-1</sup> × 10 <sup>3</sup> )	7.48	1.33
$P_u$ { ortho	1.30	0.519
meta	1.66	0.778
para	2.81	1.86
$pK_a$ { ortho	4.04	0.115
meta	4.26	0.123
para	4.27	0.224
Measures of fit		
$r_1^{2c}$	0.998	
$r_2^{2d}$	0.996	
Corr. <sup>e</sup>	0.998	

<sup>a</sup> $k_{app}$  values were calculated by means of equation 55 from values of percent absorbed in 5 min, kindly supplied by Beckett as data plotted in Fig. 5A of the paper of Beckett and Moffat (20).

<sup>b</sup>Standard deviation of estimated parameter. Since there were 27 data points and seven parameters were estimated, there were 20 degrees of freedom.

<sup>c</sup> $r_1^2 = [\Sigma k_{app}^2 - \Sigma(k_{app} - k_{app})^2] / \Sigma k_{app}^2$ .

<sup>d</sup> $r_2^2 = [S k_{app}^2 - \Sigma(k_{app} - k_{app})^2] / S k_{app}^2$  where  $S k_{app}^2 = \Sigma k_{app}^2 - (\Sigma k_{app})^2 / N$  and  $N$  is the number of data points.

<sup>e</sup>The correlation coefficient for the linear regression of  $k_{app}$  vs.  $k_{app}$ .

In equation 56,  $\Sigma dev^2$  is the sum of the squared deviations, i.e.,  $\Sigma(k_{app} - k_{app})^2$ ,  $N$  is the number of data points,  $P_*$  is the number of parameters estimated, and  $C_{ii}$  is the  $i$ th diagonal element of the variance-covariance matrix of estimates. In this fitting,  $N = 27$  and  $P_* = 7$ , hence the number of degrees of freedom, namely,  $N - P_*$ , is 20. Three different measures of fit are also given in Table I; these are  $r_1^2$ ,  $r_2^2$ , and Corr.; they were calculated as shown in the footnotes to Table I. The standard deviations are small relative to the magnitude of the estimated parameters, and all three measures of fit are very close to unity, indicating excellent agreement of the observed data to the theoretical model A.

### Buccal Absorption of *n*-Alkanoic Acids in Man

Figure 3 shows the results of both the simultaneous fitting of the data for the C<sub>4</sub> through C<sub>8</sub> acids and the simultaneous fitting of the data for the

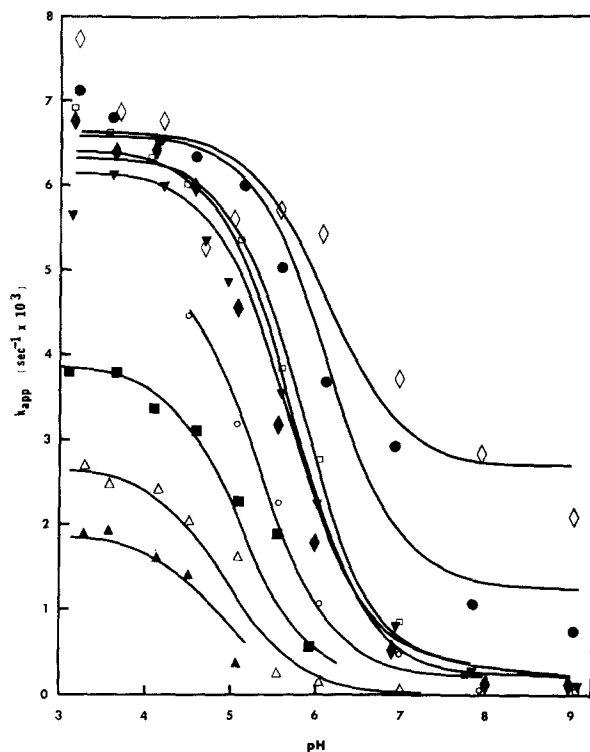


Fig. 3. Fit of the  $k_{app}$ , pH data of Beckett and Moffat (20) for buccal absorption of the  $C_4$  through  $C_{12}$  *n*-alkanoic acids to model B, based on the parameters shown in Tables II and III. Key: ▲,  $C_4$ ; △,  $C_5$ ; ■,  $C_6$ ; ○,  $C_7$ ; ◆,  $C_8$ ; ▼,  $C_9$ ; □,  $C_{10}$ ; ●,  $C_{11}$ ; and ◇,  $C_{12}$ .

$C_9$  through  $C_{12}$  acids to model B (equations 22 and 38). The lines drawn through the points are the model-predicted  $k_{app}$  values, based on the parameters listed under model B in Table II for the  $C_4$  through  $C_8$  acids and those listed in Table III for the  $C_9$  through  $C_{12}$  acids. The parameters estimated for the  $C_4$  through  $C_8$  acids using model A are also listed in Table II, but the results are not shown graphically.

### Incremental Partition Coefficients for Buccal Absorption of *n*-Alkanoic Acids in Man

As Ho and Higuchi (5) pointed out, one can calculate an incremental coefficient ( $n$ ) from the partition coefficients of *n*-alkanoic acids differing by one methylene group. The parameter  $P_u$  is the intrinsic partition coefficient of the un-ionized acid multiplied by the phase volume ratio (see equation 5). However, when one determines the ratio of two  $P_u$  values, the phase volume



**Table II.** Estimated Parameters and Measures of Fit for Simultaneous Non-linear Least-Squares Fitting of  $k_{app}$ , pH Data<sup>a</sup> for Buccal Absorption of the C<sub>4</sub> Through C<sub>8</sub> *n*-Alkanoic Acids in Man to Model A (Equations 17 and 22) and Model B (Equations 22 and 38)

Parameter	Model A <sup>b</sup>		Model B <sup>c</sup>		
	Estimate	SD <sup>d</sup>	Estimate	SD	
$k_{um}$ (sec <sup>-1</sup> × 10 <sup>3</sup> )	7.00	0.385	7.12	0.660	
$k_{im}$ (sec <sup>-1</sup> × 10 <sup>3</sup> )	—	—	4.38	74.2	
$pK_a$	4.60	0.151	4.74	0.219	
$P_u$ {	C <sub>4</sub>	0.390	0.0837	0.359	0.0718
	C <sub>5</sub>	0.6375	0.132	0.601	0.119
	C <sub>6</sub>	1.75	0.441	1.21	0.361
	C <sub>7</sub>	6.00	2.25	2.76	1.64
$P_i$ {	C <sub>8</sub>	13.5	5.92	9.02	7.15
	C <sub>4</sub>	—	—	0.0000735	0.00266
	C <sub>5</sub>	—	—	0.00633	0.108
	C <sub>6</sub>	—	—	0.0409	0.785
	C <sub>7</sub>	—	—	0.0523	0.960
	C <sub>8</sub>	—	—	0.0523	0.943
Measures of fit <sup>d</sup>					
$r_1^2$	0.980		0.991		
$r_2^2$	0.950		0.976		
Corr.	0.982		0.988		

<sup>a</sup> $k_{app}$  values were calculated by means of equation 55 from values of percent absorbed in 5 min, kindly supplied by Beckett as data plotted in Fig. 4 of the paper of Beckett and Moffat (20).

<sup>b</sup>Since there were 36 data points and seven parameters, there were 29 degrees of freedom.

<sup>c</sup>Since there were 36 data points and 13 parameters, there were 23 degrees of freedom.

<sup>d</sup>See footnotes to Table I.

ratio cancels. Hence the ratio of  $P_u$  values is equivalent to the ratio of intrinsic partition coefficients for the two acids between the membrane and the aqueous contents of the buccal cavity. This is indicated by equation 57:

$$n = \frac{(P_u)_{j+1}}{(P_u)_j} = \frac{(V_m/V_w)K_{j+1}}{(V_m/V_w)K_j} = \frac{K_{j+1}}{K_j} \quad (57)$$

where  $j$  and  $j + 1$  are the carbon numbers of two *n*-alkanoic acids differing by one methylene group. The values of  $n$  which were calculated by application of equation 57 are shown in Table IV. The average value of  $n$  calculated for the C<sub>4</sub> to C<sub>8</sub> acids by this method and for model B is 2.31. Applying their aqueous diffusion layer model, Ho and Higuchi (5) reported an average value of 2.33 using the same method and for the same acids. Hence the two entirely different models yield the same average value of  $n$  for these five

**Table III.** Estimated Parameters and Measures of Fit for Simultaneous Nonlinear Least-Squares Fitting of  $k_{app}$ , pH Data<sup>a</sup> for Buccal Absorption of the C<sub>9</sub> Through C<sub>12</sub> *n*-Alkanoic Acids in Man to Model B (Equations 22 and 38)

Parameter	Estimate <sup>b</sup>	SD <sup>c</sup>	
$k_{um}$ (sec <sup>-1</sup> × 10 <sup>3</sup> )	6.78	0.345	
$k_{im}$ (sec <sup>-1</sup> × 10 <sup>3</sup> )	6.95	6.22	
$pK_a$	4.67	0.367	
$P_u$ {	C <sub>9</sub>	10.0	7.61
	C <sub>10</sub>	15.0	12.5
	C <sub>11</sub>	34.7	32.5
	C <sub>12</sub>	45.0	44.2
$P_i$ {	C <sub>9</sub>	0.0524	0.0640
	C <sub>10</sub>	0.0344	0.0539
	C <sub>11</sub>	0.214	0.247
	C <sub>12</sub>	0.634	0.947
Measures of fit <sup>c</sup>			
$r_1^2$	0.992		
$r_2^2$	0.965		
Corr.	0.983		

<sup>a</sup>See footnote *a* to Table II.

<sup>b</sup>Since there were 40 data points and 11 parameters, there were 29 degrees of freedom.

<sup>c</sup>See footnotes to Table I.

*n*-alkanoic acids. The average value of *n* for the same five acids, when evaluated by model A, gave the slightly higher value of 2.48.

The calculation of individual values of *n* from the  $P_u$  values of pairs of *n*-alkanoic acids differing by one carbon atom is subject to variation due to errors in both of the  $P_u$  values. The value of *n* may be estimated from all the  $P_u$  values simultaneously by application of equations 58 and 59:

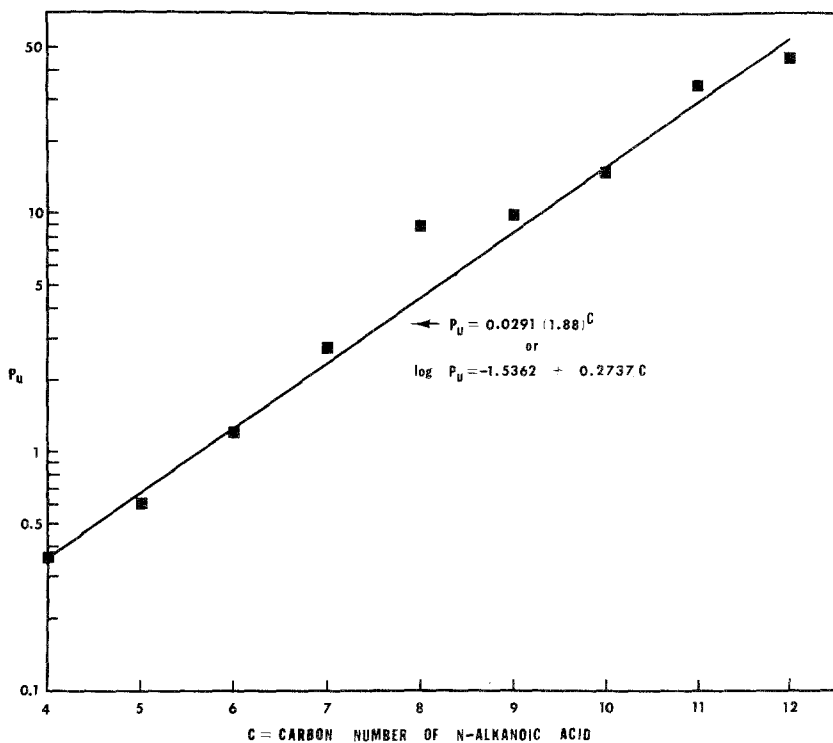
$$P_u = a \cdot n^C \quad (58)$$

$$\log P_u = \log a + (\log n) \cdot C \quad (59)$$

In equations 58 and 59, *a* is a constant and *C* is the carbon number of the acid. In conformity with equation 59, the  $P_u$  values of the C<sub>4</sub> to C<sub>12</sub> *n*-alkanoic acids, evaluated by model B, are plotted semilogarithmically against the carbon number of the acid in Fig. 4. Using all nine points, the least-squares line had an intercept of  $\log a = -1.5027$ , whence  $a = 0.0314$ , and a slope of  $\log n = 0.2737$ , whence  $n = 1.88$ ; the correlation coefficient was 0.988. Since the  $P_u$  value for the C<sub>8</sub> acid departed considerably from the trend of the other points, the least-squares line was also estimated for eight points (excluding the  $P_u$  value for the C<sub>8</sub> acid). The latter line had an intercept of

**Table IV.** Incremental Partition Coefficients for Un-ionized Molecules of *n*-Alkanoic Acids in the Buccal Absorption Test

Acids	<i>n</i>	
	Model A	Model B
C <sub>5</sub> /C <sub>4</sub>	1.63	1.67
C <sub>6</sub> /C <sub>5</sub>	2.75	2.01
C <sub>7</sub> /C <sub>6</sub>	3.43	2.28
C <sub>8</sub> /C <sub>7</sub>	2.10	3.27
C <sub>9</sub> /C <sub>8</sub>	—	1.11
C <sub>10</sub> /C <sub>9</sub>	—	1.50
C <sub>11</sub> /C <sub>10</sub>	—	2.31
C <sub>12</sub> /C <sub>11</sub>	—	1.30
Average of C <sub>4</sub> to C <sub>8</sub>	2.48	2.31
Average of C <sub>4</sub> to C <sub>12</sub>	—	1.93


**Fig. 4.** Semilogarithmic plot of  $P_u$  against carbon number of *n*-alkanoic acid when data are evaluated by model B.

$\log a = -1.5362$ , whence  $a = 0.0291$ , and a slope of  $\log n = 0.2737$ , whence  $n = 1.88$ ; the correlation coefficient was 0.997. The second line is the one drawn through the points in Fig. 4.

The incremental partition coefficient of 1.88 implies that the membrane of the buccal cavity is not strongly nonpolar. Ho and Higuchi (5) point out (a) that the butanol/water system would probably yield a value near 2.3 at 37°C and (b) that incremental constants from 1.7 to 2.5 per unshielded CH<sub>2</sub> group among chosen homologous pairs of ether, alcohol, amide, and ester molecules have been reported from permeation determinations using the plant cell *Chara ceratophylla*.

### Gastrointestinal Absorption of Barbitol and Sulfaethidole in Rat Intestine

Figure 5 shows the results of the individual fittings of the  $k_{app}$ , pH data for barbitol and sulfaethidole in rat small intestine. The lines drawn through the points are the model-predicted  $k_{app}$  values based on the parameters shown in Table V. In these two cases, the measures of fit  $r_1^2$ ,  $r_2^2$ , and Corr. are close to unity, but the standard deviations are excessive relative to the magnitude of the estimates. This is not really a reflection of poor fits to the model, but rather mainly a reflection of the fact that there were only 4 and 5

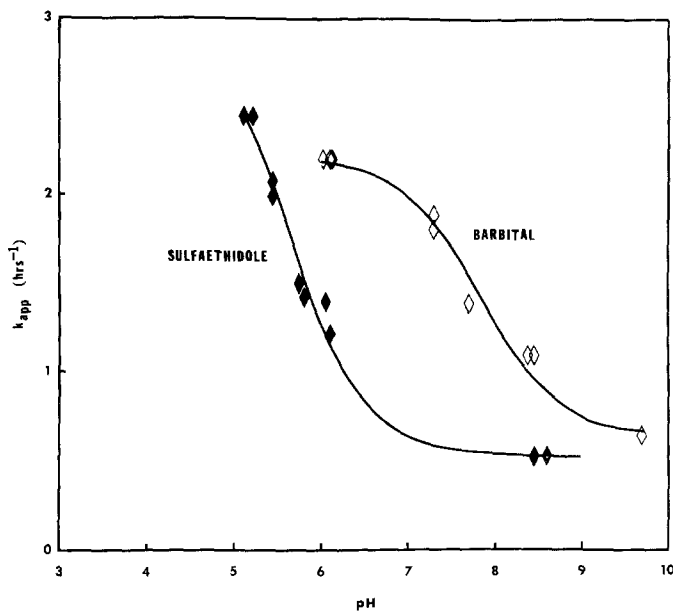


Fig. 5. Fit of the  $k_{app}$ , pH data of Crouthamel *et al.* (7) for barbitol and sulfaethidole in rat small intestine to model B, based on the parameters shown in Table V.

degrees of freedom for the fitting of the barbital and sulfaethidole data, respectively. This may be inferred by comparing the magnitudes of the standard deviations in Table I to III with those in Table V. The problem of the relationship of the magnitude of experimental error, the number of degrees of freedom, and the magnitude of standard deviations of estimated parameters has been discussed by Atkins (26) and agrees with the above interpretation.

Suzuki *et al.* (4) reported that, when using their model, the diffusion coefficients for the barbiturates were smaller than those for the sulfonamides by a factor of 10 and that this could not easily be explained by the usual Stokes-Einstein diffusion equation. Based on diffusion theory, the  $k_{um}$  of models A and B in this report would be given by equation 60:

$$k_{um} = (D_{um} \cdot A)/(h \cdot V) \quad (60)$$

where  $D_{um}$  is the diffusion coefficient for the un-ionized acid out of the membrane,  $A$  is the effective surface area of the membrane,  $h$  is the effective thickness of the membrane-blood interface, and  $V$  is the effective volume of

**Table V.** Estimated Parameters and Measures of Fit for Nonlinear Least-Squares Fitting of  $k_{app}$ , pH Data for Crouthamel *et al.* (7) for Gastrointestinal Absorption of Barbital and Sulfaethidole in Rat Intestine to Model B (Equations 22 and 38)

Parameter	Drug			
	Barbital		Sulfaethidole	
	Estimate	SD <sup>a</sup>	Estimate	SD
$k_{um}$ (hr <sup>-1</sup> )	3.63 <sup>b</sup>	11.0	5.82 <sup>b</sup>	30.4
$k_{im}$ (hr <sup>-1</sup> )	14.5 <sup>c</sup>	1626.0	14.3 <sup>c</sup>	1608.0
$P_u$	1.55	12.0	1.08	11.8
$P_i$	0.0475	1.56	0.0388	4.54
$pK_a$	7.44	1.45	5.33	4.22
Measures of fit <sup>d</sup>				
$r_1^2$	0.998		0.997	
$r_2^2$	0.979		0.983	
Corr.	0.990		0.992	

<sup>a</sup>Standard deviation of estimated parameter. These are large in these fittings, since there were only nine data points for barbital and ten for sulfaethidole, providing only 4 and 5 degrees of freedom, respectively.

<sup>b</sup>The  $k_{um}$  values of 3.63 and 5.82 hr<sup>-1</sup> correspond to values of 1.08 and 1.62 sec<sup>-1</sup> × 10<sup>3</sup>, respectively.

<sup>c</sup>The  $k_{im}$  values of 14.5 and 14.3 hr<sup>-1</sup> correspond to values of 4.02 and 3.97 sec<sup>-1</sup> × 10<sup>3</sup>, respectively.

<sup>d</sup>See footnotes to Table I.

the membrane. Since barbital and sulfaethidole were studied in the rat under the same experimental conditions, the ratio of the  $k_{um}$  values for these two compounds should equal the ratio of the diffusion coefficients. the ratio of  $k_{um}$  for barbital/ $k_{um}$  for sulfaethidole is  $3.63/5.82 = 0.62$ , which appears to be a more reasonable ratio than that reported by Suzuki *et al.* (4).

In the footnotes to Table V, the rate constants  $k_{um}$  and  $k_{im}$  for barbital and sulfaethidole are given with dimensions of  $\text{sec}^{-1} \times 10^3$  for comparison with data given in Tables I to III. The values of  $k_{um}$  of  $7.48 \text{ sec}^{-1} \times 10^3$  for the *o*-, *m*-, and *p*-toluic acids,  $7.12 \text{ sec}^{-1} \times 10^3$  for the  $C_4$  to  $C_8$  *n*-alkanoic acids, and  $6.78 \text{ sec}^{-1} \times 10^3$  for the  $C_9$  to  $C_{12}$  *n*-alkanoic acids for buccal absorption in man are about seven times the  $k_{um}$  value of  $1.08 \text{ sec}^{-1} \times 10^3$  for barbital and about 4.3 times the  $k_{um}$  value of  $1.62 \text{ sec}^{-1} \times 10^3$  for sulfaethidole in rat intestine. The  $k_{im}$  value of  $4.38 \text{ sec}^{-1} \times 10^3$  for buccal absorption of the  $C_4$  to  $C_8$  *n*-alkanoic acids in man is very similar to the  $k_{im}$  values of 4.02 and  $3.97 \text{ sec}^{-1} \times 10^3$  for absorption of barbital and sulfaethidole, respectively, in rat small intestine. It is also of interest that the  $P_u$  values of 1.55 and 1.08 for barbital and sulfaethidole, respectively, are closest to the value of  $P_u$  of 1.21 for hexanoic acid (see  $C_6$  under model B in Table II).

### Relative Values of Intrinsic Partition Coefficients for Ions

The  $P_i$  values of 0.0475 and 0.0388 for barbital and sulfaethidole, respectively, in rat intestine are very similar to the  $P_i$  values of 0.0409, 0.0523, 0.0523, 0.0524, and 0.0344 estimated for the  $C_6$ ,  $C_7$ ,  $C_8$ ,  $C_9$ , and  $C_{10}$  *n*-alkanoic acids, respectively, in the buccal absorption test. There is no uniform change in  $P_i$  values with increase in the number of methylene groups of the *n*-alkanoic acids as for the  $P_u$  values (see Fig. 4). The  $P_i$  value for the  $C_4$  acid is extremely small, there is some increase for the  $C_5$  acid, then the  $P_i$  values are essentially the same for the  $C_7$  through  $C_{10}$  acids, then there is an abrupt increase for the  $C_{11}$  and  $C_{12}$  acids (see Tables II and III). Fitting of the  $C_4$  through  $C_8$  *n*-alkanoic acid data to model B resulted in improvement of fit by all three measures of fit (see Table II). Also, the  $P_u$  values, estimated using model A, are all higher for the  $C_4$  through  $C_8$  acids and do not fall on the line, shown in Fig. 4, based on model B. These points suggest that absorption of ions should be taken into consideration for all the *n*-alkanoic acids studied so far.

The fact that the standard deviations of the estimated  $P_i$  values are relatively much larger in Table II than in Table III is probably a reflection that in fitting the  $C_4$  through  $C_8$  acids to model B the data supplied little information about the asymptotic nature of  $k_{app}$  at high pH.

## DISCUSSION

### Treatment of Ion Absorption in Model B

The buccal absorption data of the *o*-, *m*-, and *p*-toluic acids, evaluated in this report, can be explained solely on the basis of absorption of the unionized molecules. However, it seems unlikely that the buccal absorption data of the *n*-alkanoic acids (particularly the higher members, C<sub>9</sub> to C<sub>12</sub>) and the gastrointestinal absorption data of barbital and sulfaethidole can be explained without invoking the concept that ions are absorbed. Attempts to fit the latter data to equations 17 and 22 were unsuccessful.

The investigations of Turner *et al.* (27), and the literature they summarized, indicate that certain ionized drugs do pass through the *in vitro* intestine of the rat. Recently, Lanman *et al.* (28) demonstrated first-order disappearance of the ions of hippuric acid, sulfanilic acid, phenol red, and *p*-aminohippuric acid from rat intestine *in vivo*, and they reported that the anions were absorbed at rates which ranked in the same order as the apparent chloroform/water partition coefficients measured at pH 7.4.

A conventional model is the aqueous pore-lipoid film model of biological membranes. According to this model, most of the diffusion occurs through the lipid film with hydraulic flow passing through the channels, either intracellular or intercellular (29). Past investigations (30–32) have indicated that there is apparently a species difference in the size of the pores or channels. Höber and Höber (30) reported that in the rat only small molecules, with molecular weight about 180 or less (corresponding to a molecular radius of about 4 Å) diffuse through water-filled pores. Lifson and Hakim (31) estimated a functional pore radius of 10 to 15 Å in the dog. Fordtran *et al.* (32) estimated the effective pore radius to be 7 to 8.5 Å in the jejunum and 3.0 to 3.8 Å in the ileum of the human small intestine. One could assume that small organic ions are absorbed through water-filled pores or channels in an analogous manner to the non-lipid-soluble small molecules studied in the previous investigations (30–32). However, if the effective pore diameter in the buccal membrane of man is assumed to be of the same order of magnitude as those estimated by Fordtran *et al.* (32) in the human small intestine, one would not expect the large anions of the higher *n*-alkanoic acids to be absorbed in this manner. Also, the data of Beckett and Moffat (20) strongly suggest a disproportionate but gradual increase in absorption of ions as the *n*-alkanoic acid series is ascended. We thus chose an alternative to the pore theory to account for ion absorption.

Vacek *et al.* (33) studied the paper chromatographic behavior of a series of chlorophenols using Whatman No. 3 paper impregnated with 10% olive oil in benzene and buffers of different pH as the mobile phase. They found that at low pH values, the  $R_F$  value (in our symbolism) was given by

equation 61:

$$R_F = 1 - f_E = 1/(1 + f_u P'_u) \quad (61)$$

where  $P'_u$  is given by equation 5 except that the volume ratio is replaced by the areas of cross-section of both phases on the paper. It should be noted that equation 61 is readily obtained from equation 20. However, to explain the chromatographic behavior of the chlorophenols when  $pH > pK_a$ , the authors had to define a distribution coefficient for the anions and derive an equation, which in our symbolism is equation 62 and which is readily obtained from equation 40:

$$R_F = 1 - f_E = 1/[1 + f_u P'_u + (1 - f_u) P'_i] \quad (62)$$

where  $P'_i$  is given by equation 28, except that the volume ratio is replaced by the areas of cross-section of both phases on the paper. In relating the equations above to the equations for model B, it is implicit that olive oil in the *in vitro* chromatographic system is analogous to the membrane.

In deriving the equations for model B, we chose to treat ion absorption *in vivo* as a partitioning process analogous to the *in vitro* chromatographic system of Vacek *et al.* (33). This implies that the organic ions partition into the membrane and transfer out of the membrane in an analogous manner to that of the un-ionized molecules, but the exact mechanism is not specified by the theory. This assumption is supported by the results and correlation of Lanman *et al.* (28) and the opinion expressed by Beckett and Moffat (20) with respect to the higher *n*-alkanoic acids. Ling (34) conceives that the gastrointestinal membrane consists largely of water and that the water in the cell is adsorbed as polarized multilayers on the proteins, which lowers the activity of water within the cell. This treatment suggests that the membrane does not really have the character of a nonpolar "oil" or organic solvent, as has frequently been assumed in the past, but that it may be much more polar. Partitioning of organic ions into such a membrane appears reasonable.

The possibility of ion-pair absorption also exists for some drugs. Investigations of Perrin and Vallner (35) and Suzuki *et al.* (36) strongly suggest that ion-pair absorption occurs with some amphoteric and basic drugs. The paper of Doyle and Levine (37) suggests how equation 38 would have to be modified to incorporate absorption of ion pairs *in vivo*.

### Omission of Consideration of One of the Aqueous Diffusion Layers

The existence of the aqueous diffusion layer or unstirred water layer on the lumen side of gastrointestinal and buccal membranes is not denied by our treatment, but rather just not taken into consideration. Several recent articles have discussed the possible role of the unstirred water layer in



membrane transport (38–42). The models of Suzuki *et al.* (4), Ho and Higuchi (5), Ho *et al.* (46), and Flynn and Yalkowsky (43) incorporated a consideration of the aqueous diffusion layer in absorption and transport through membranes *in vitro*. Wilson *et al.* (40) studied the uptake of bile acid and fatty acid from monomer solutions and of fatty acid from micellar solutions across the rat jejunal brush border. They concluded that during the absorption of these substances from monomer solutions the cell membrane primarily is rate-limiting, while when the fatty acid is dissolved in a bulky micelle the diffusion of the large micelle across the unstirred layer is rate-limiting. The *in vivo* data evaluated in this report all arose from administration of drugs in monomer solutions. Although our derivations disregard the aqueous diffusion layer on the lumen side of the membrane, they do not necessarily disregard the aqueous diffusion layer on the blood side of the membrane. The assumption is that the rate-limiting step is transport out of the membrane into the systemic circulation and that this is independent of the partition coefficient. This is different than the treatment of Davson and Danielli (45) and appears to make the treatment unique. Although the mechanism is not specified, transport out of the membrane could involve the aqueous diffusion layer on the blood side of the membrane. It is of interest that the models of Suzuki *et al.* (4), Ho and Higuchi (5), and Ho *et al.* (46) disregard the aqueous diffusion layer on the blood side of the membrane but take into consideration the aqueous diffusion layer on the lumen side of the membrane.

### Comparison of Estimated $pK_a$ 's from *in vivo* Data with Those Determined *in vitro*

Table VI compares the  $pK_a$ 's estimated by fitting the  $k_{app}$ , pH data obtained *in vivo* at 37°C with the  $pK_a$ 's determined *in vitro* at 25°C. With two exceptions, namely, *o*- and *m*-toluic acids, the *in vitro*  $pK_a$  is higher than the estimated *in vivo*  $pK_a$ . Temperature, alone, has variable effects on the  $pK_a$  measured *in vitro* (47). The differences in  $pK_a$ , shown in Table VI, may be expected on the basis of ionic strength, salt effects, colloidal effects, etc. (48), and experimental error in fitting the  $k_{app}$ , pH data.

### CONCLUSIONS

The new physical models, embodied in equations 17, 22, and 38 of this report, appear to be equally as successful, if not more successful than the aqueous diffusion layer models of Suzuki *et al.* (4), Ho and Higuchi (5), and Ho *et al.* (46) in analyzing gastrointestinal and buccal absorption data so far collected in animals and man. This does *not* imply that one theory is correct and

**Table VI.** Comparison of Estimated  $pK_a$ 's from *in Vivo* Data (37°C) with Those Determined *in Vitro* (25°C)

Compound	Model	Estimated from <i>in vivo</i> data (37°C)	<i>In vitro</i> (25°C)	$\Delta pK_a^d$	
<i>o</i> -Toluic acid	A	4.04	3.92 <sup>a</sup>	-0.12	
<i>m</i> -Toluic acid	A	4.26	4.24 <sup>a</sup>	-0.02	
<i>p</i> -Toluic acid	A	4.27	4.33 <sup>a</sup>	0.06	
C <sub>4</sub> -C <sub>8</sub> <i>n</i> -alkanoic acids	{	A	4.60	4.84 <sup>b</sup>	0.24
		B	4.74	4.84 <sup>b</sup>	0.10
C <sub>9</sub> -C <sub>12</sub> <i>n</i> -alkanoic acids	B	4.67	4.84 <sup>b</sup>	0.17	
Barbital	B	7.44	7.9 <sup>c</sup>	0.46	
Sulfaethidole	B	5.33	5.5 <sup>c</sup>	0.17	

<sup>a</sup>Reported by Beckett and Moffat (20).

<sup>b</sup>The average of  $pK_a$ 's of 4.82 for *n*-butyric acid and 4.85 for *n*-octanoic acid cited by Beckett and Moffat (20).

<sup>c</sup>Reported by Crouthamel *et al.* (7).

<sup>d</sup>Difference between *in vitro*  $pK_a$  at 25°C and estimated *in vivo*  $pK_a$  at 37°C.

the other incorrect. To the authors, it implies that the appropriate model cannot be chosen on the basis of the type of data which have been collected to date and that we need more definitive data to make a decision. In essence, the new equations quantitate the *pH*-partition hypothesis (1,2). They could also allow quantitation of renal reabsorption of acids and bases.

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