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J. Pharm. Pharmacol. 1981, 33: 179–182
Communicated September 8, 1980

0220-3573/81/030179-04 $02.50/0
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A model for the pH dependence of drug-protein binding

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Change of pH alters the protein binding of drugs (Goldbaum & Smith 1954; Newbould & Kilpatrick 1960; Burney et al 1978; Vallner et al 1979). Since the free or unbound portion of a drug is generally held to be responsible for its pharmacological effects, we have sought ways of expressing pH-induced change in free concentration in practical terms. Because the more highly protein bound drugs tend to be lipophilic (Bird & Marshall 1967; Helmer et al 1968; Hansch & Dunn 1972; Chien et al 1975), we began by considering pH induced changes in relation to pH-partition theory.

In its simplest form, this theory applies to the distribution at equilibrium of a weak acid or base between two immiscible solvents, in one of which, the aqueous phase, it is partly ionized, and in the other, the lipid phase, it is non-ionized. Non-ionized solute distributes between the phases in a concentration ratio which defines the true partition coefficient (TPC) of the substance. The extent of the ionization in the aqueous phase is determined by the pK_a of the substance and pH, and these, together with the TPC, therefore govern the distribution of the solute between the phases. Fig. 1 is a computer generated plot of the concentration in the aqueous phase for substances obeying the theory, and shows the amount in the aqueous phase as a percentage of the total amount in the system, and how this varies with pH and with the TPC for a given substance. It illustrates how it is theoretically possible for large changes in concentration to occur over a narrow pH range for substances with the appropriate physiochemical characteristics. We attempted to apply this model to the partitioning of drugs between aqueous buffers and organic solvents, but a model which also allowed for the partitioning of ionized drug into the lipid phase gave an improved fit. The derivation of both of these models is given in Appendix 1.

Extension of this theory to a system such as drug-protein binding requires taking account of the fact that protein molecules are dissolved in the plasma, and therefore bound drug must be regarded as being in the lipid phase and unbound drug in the aqueous phase. In the case where non-ionized solute only binds to a single site on a single species of protein molecule, the curves in Fig. 1 are equally applicable (see Appendix 2). In this case, a considerable concentration change of free drug with pH would only occur if the drug had a high protein affinity and was in the presence of an adequate amount of protein. In vivo partitioning from tissues

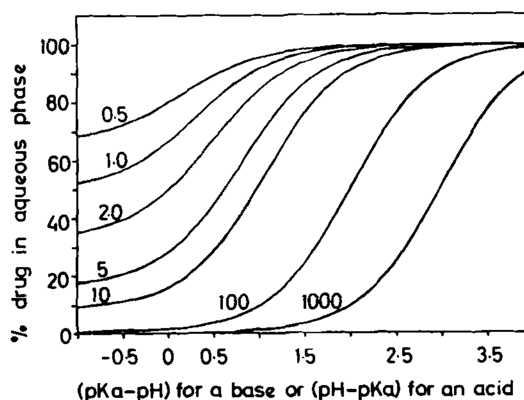


FIG. 1. Plots of the predicted concentration in the aqueous phase (expressed as a percentage of the maximum) of solutes which obey the simple pH-partition theory shown as a function of pH. Each curve is drawn using the specified value for K_u, which is the quantity ratio or volume corrected true partition coefficient for a given solute (see Appendix 1, eqn 6).

* Correspondence.

and cells apart from the plasma does not form part of this system but could change the total concentration of a given drug (Waddell & Butler 1957).

To assess whether these models could be verified experimentally for drug protein binding, we chose a lipophilic acid, fusidic acid, which has a pK_a of 5·35 and binds principally to albumin (Güttler et al 1971), and a lipophilic base, propranolol, which has a pK_a of 9·45 and binds almost exclusively to α 1-acid glycoprotein (Sager et al 1978). (±)-[^{3}H]propranolol hydrocholoride was obtained from the Radiochemical Centre and [^{3}H]diethanolamine fusidate from Leo Laboratories. Lithium-heparin plasma was obtained from six drug-free, fasting healthy volunteers and spiked with each drug to produce concentrations of 25 ng ml^{-1} propranolol and 10 μg ml^{-1} fusidic acid. Estimation of

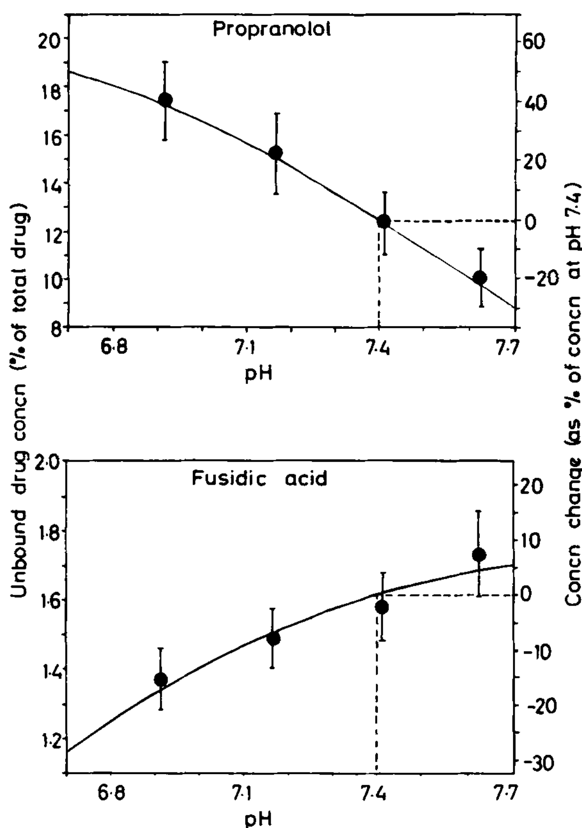


FIG. 2. Unbound concentrations of propranolol (upper figure) and fusidic acid (lower figure) plotted against pH. Curves are drawn from meaned parameters derived by fitting eqn 23 (Appendix 2) to our six sets of data. The means with s.d. of the six sets of data values are also shown. The parameter values used for the curves (s.d. of the six values in brackets) are: for propranolol: K_i = 3·72 (0·616), K_u = 371·5 (63·1) and for fusidic acid: K_i = 54·5 (3·88), K_u = 776·3 (58·0) where K_i is the ratio of bound to unbound drug in the unionized form and K_u the ratio of bound to unbound drug in the unionized form. Note pH shift from buffer values.

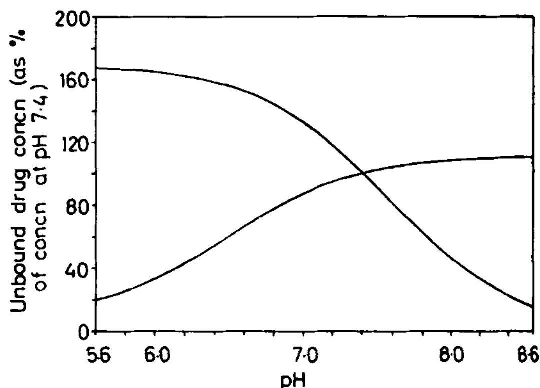


FIG. 3. The curves shown in Fig. 2 redrawn over a larger pH range to show the behaviour of the model. Over as wide a pH range as that illustrated, conformational changes would also be expected to play a part in the binding of drugs to plasma proteins.

protein binding was by equilibrium dialysis in triplicate in acrylic cells rotated 45 times min^{-1} at 37 °C for 6 h (this time having previously been established as sufficient for attainment of equilibrium), with a 11·5 μm cuprophan membrane and isotonic (280–285 mOsm kg^{-1}) Sorensen's phosphate buffer solutions at pH 6·8, 7·1, 7·4 and 7·7 (at 37 °C). Unspiked plasma was also subjected in duplicate to the same dialysis procedure to obtain the final plasma pH for each experiment (at 37 °C on an Instrumentation 413 analyser); the mean was taken as the pH of each binding estimation. After dialysis, the levels of radioactivity on each side of the membrane were measured by scintillation counting using a Packard Tri-Carb counter with an external standard and Pico-fluor scintillant. Membrane binding was measured using Packard Filter-count scintillant and was found not to vary with pH. Coefficients of variation for unbound drug percentages within assay were 2·6% for propranolol and 5·5% for fusidic acid and 0·103% for pH measurements.

Unbound drug concentrations showed little intersubject variability and were consistently related to pH over the range studied (Fig. 2). Models were fitted to the data with an optimization procedure derived from the Simplex method, published by O'Neill (1971) and the accepted pK_a values of 9·45 and 5·35. Fits of the data according to the simple model of non-ionic binding showed evident systematic errors and were not acceptable for either drug, but when the model was extended to include the binding of ionized drug, the fits illustrated in Fig. 2 were obtained (curves are drawn from mean parameters of 6 subjects). The simple lipophilic model gave values for the sum of the squared residuals which were between 80 and 2250 times that of the extended model. It is apparent that with the fits obtained, the non-ionized species of drug binds much more strongly to the protein. Fig. 3 shows the two

computer fits re-drawn over a wider pH range, in order to demonstrate the behaviour of the model.

In this experimental situation, where the protein is non-homogeneous and may itself undergo pH-induced changes in ionization and conformation (such as those described by Brand & Toribara 1975; Wilting et al 1979), and where multi-site binding may occur, we do not pretend that this model is necessarily realistic. The model required may also vary from drug to drug, and thus there is no natural parameter which quantitatively describes the effect of pH on serum or plasma protein binding. However, over the pH range of interest, the concentration change is approximately linear with pH, and hence the percentage change in free drug concentration per 0·1 pH unit (at pH 7·4) taken from the linear regression slope, appears to be a satisfactory parameter for the purposes of classification and comparison of drugs, where a more refined model cannot be applied to the data. For fusidic acid, the mean value of this parameter for the 6 sets of data was 3·10 (with a standard deviation of 0·27), and for propranolol —8·42 (1·4); the figures derived from the slope of the computer fits at pH 7·4 are 2·4 (0·21) and —9·3 (1·3) respectively. In other words, at pH 7·4 the unbound concentration of fusidic acid decreases by 2·4% per 0·1 pH unit decrease, and that of propranolol increases by 9·3% per 0·1 pH unit decrease. We suggest that this is a suitable way of expressing change in free drug concentration with pH, since it is physiologically relevant and does not require characterization or quantification of binding proteins and can also be used for drugs with multiple pK_a values. Recent work showing the negative correlation between the proportion of unbound propranolol and other drugs and the concentration of their binding proteins (Piafsky et al 1978; Sager et al 1978) serves to emphasize the importance of assessing binding in whole serum or plasma.

Many relationships between lipophilicity and biological activity have previously been investigated (Hansch & Dunn 1972; Kubinyi 1979). This study draws attention to the capacity of weak acids and bases to undergo change in unbound concentration with pH, and if they possess the appropriate TPC and pK_a, the largest changes will occur in the physiological pH range. This implies that acidosis and alkalosis could produce alterations in the duration and intensity of action of a wide variety of drugs (Levy 1976). It is also probable that drug effects could vary in magnitude in different parts of the body under the influence of pH differences, and the organ and tissue selectivity of certain drugs may be partly due to pH-dependent differences in unbound concentrations. Similarly, a consideration of the pH-dependent binding of drugs to plasma macromolecules might lead to more effective removal of drugs from the body by haemodialysis or adsorption.

APPENDIX 1

FORMULAE FOR THE pH-PARTITION THEORY

Non-ionized solute will distribute between the two phases with a concentration ratio which defines the true partition coefficient (T_u) for the solute

$$T_u = c_l/u_a \quad \ldots \quad (1)$$

where c_l is the concentration of (non-ionized) solute in the lipoid phase, and u_a is the concentration of non-ionized solute in the aqueous phase.

However,

$$u_a = f_u.c_a \quad \ldots \quad (2)$$

where c_a is the total aqueous concentration, and f_u is the fraction non-ionized at the pH in question. From the Henderson-Hasselbalch equation,

$f_u = 1/[1 + 10(pH - pK)]$ for an acid, and
$f_u = 1/[1 + 10(pK - pH)]$ for a base.

From (1) and (2),

$$c_a = c_l/f_u.T_u \quad \ldots \quad (3)$$

The total amount of solute (Q) in the system is constant, and hence:

$$Q = v_l.c_l + v_a.c_a \quad \ldots \quad (4)$$

From (3) and (4):

$$\frac{c_a}{Q/v_a} = \frac{1}{1 + (T_u.v_l/v_a).f_u} \quad \ldots \quad (5)$$

The left-hand side represents the aqueous concentration expressed as a fraction of the maximum possible, and the constant term $(T_u.v_l/v_a)$ is the volume-corrected true partition coefficient, or also represents the ratio of the quantities of non-ionized solute in the two phases. If we substitute $K_u = (T_u.v_l/v_a)$, and let c_{max} be the maximum aqueous concentration (Q/v_a), we get:

$$\frac{c_a}{c_{max}} = \frac{1}{1 + K_u.f_u} \quad \ldots \quad (6)$$

which is the equation plotted in Fig. 1 for different values of K_u.

Our experimental data on aqueous/organic solvent partitioning showed systematic deviation from equation (6) at pH values corresponding to high ionization, and hence we extended this treatment to allow the ionized species of solute to also partition into the lipid with a different 'true' partition coefficient T_i, where

$$T_i = i_l/i_a \quad \ldots \quad (7)$$

in which i represents the concentration of the ionized solute, and the definition of T_u (eqn (1) above) will need to be amended to

$$T_u = u_l/u_a \quad \ldots \quad (8)$$

We assume that ionized and non-ionized solute cannot interconvert in the lipid phase.

Using the same notation as before,

$$u_a = f_u.c_a \quad \ldots \quad (2)$$

$$i_a = (1 - f_u).c_a \quad \ldots \quad (9)$$

$$Q = v_l.(u_l + i_l) + v_a.c_a \quad \ldots \quad (10)$$

From (7), (8), (2), (9) and (10):

$$\frac{c_a}{Q/v_a} = \frac{1}{1 + (T_i.v_l/v_a) + [(T_u - T_i)(v_l/v_a).f_u]} \quad (11)$$

Substituting as in (6) gives:

$$\frac{c_a}{c_{max}} = \frac{1}{1 + K_i + (K_u - K_i).f_u} \quad .. \quad (12)$$

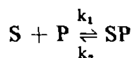
This equation gives an acceptable fit to our experimental results. It must be appreciated that the above treatment is insufficient to explain the mechanism by which ionized solute partitions into the organic phase.

APPENDIX 2

FORMULAE FOR PROTEIN BINDING MODELS BASED ON THE pH-PARTITION THEORY

Two formulae are derived here, one which only considers binding of non-ionized solute to protein, and one which encompasses binding of both non-ionized and ionized solute.

(i) *Non-ionized solute only binding*
Consider that only the non-ionized form of a solute can bind reversibly to a single site on a protein P:



At equilibrium:

$$[S][P]/[SP] = k_2/k_1 \quad .. \quad .. \quad (13)$$

Also $$[S] = f_u.c_a \quad .. \quad .. \quad .. \quad (14)$$

$$c_{tot} = [SP] + c_a \quad .. \quad .. \quad (15)$$

where c_{tot} is the total solute concentration in the system, and other notation is as before. From (13), (14) and (15):

$$\frac{c_a}{c_{tot}} = \frac{1}{1 + (k_1.[P]/k_2).f_u} \quad .. \quad .. \quad (16)$$

The term $(k_1.[P]/k_2)$ represents the ratio of bound to free non-ionized solute, and is the exact analogue of the term in equation (5).

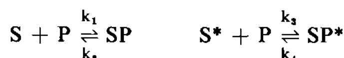
If the total protein concentration is p_{tot}

$$p_{tot} = [P] + [SP] = [P] + c_{tot} - c_a \quad (17)$$

or $$[P] = p_{tot} - c_{tot} + c_a \quad .. \quad .. \quad (18)$$

From (18) it is apparent that if $p_{tot} \gg c_{tot}$ it is valid to assume [P] is a constant independent of pH, and under these conditions the protein binding behaviour with pH will be similar to that of lipid partitioning. In our study the respective concentrations of propranolol and fusidic acid used were 0·096 and 19·34 μM and the approximate plasma concentrations of their respective binding proteins are 22·68 and 597 μM.

(ii) *Protein binding of both solute species*
Consider now that both non-ionized solute (S) and ionized solute (S*) bind with differing affinities to a single site on a protein P:



We assume that SP and SP* cannot interconvert.
At equilibrium,

$$[S][P]/[SP] = k_2/k_1 \quad .. \quad .. \quad .. \quad (19a)$$

$$[S^*][P]/[SP^*] = k_4/k_3 \quad .. \quad .. \quad .. \quad (19b)$$

$$c_{tot} = [S] + [S^*] + [SP] + [SP^*] = c_a + [SP] + [SP^*] \quad (20)$$

$$[S] = f_u.c_a; \quad [S^*] = (1 - f_u).c_a \quad .. \quad (21)$$

From (19), (20) and (21):

$$\frac{c_a}{c_{tot}} = \frac{1}{1 + ([P].k_3/k_4) + [(k_1.[P]/k_2) - (k_3.[P]/k_4)].f_u} \quad (22)$$

Again, we assume $p_{tot} \gg c_{tot}$, [P] can be assumed to be independent of pH. If we substitute $K_i = [P].k_3/k_4$ and $K_u = [P].k_1/k_2$

$$\frac{c_a}{c_{tot}} = \frac{1}{1 + K_i + (K_u - K_i).f_u} \quad .. \quad (23)$$

and this equation was used to fit our protein binding data (see Fig. 2). K_i and K_u are the equilibrium binding ratios of the two species of solute. Note that equation (23) is analogous to equation (12).

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