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ERROR INTRODUCED BY SMALL REFLECTION COEFFICIENTS IN PERMEABILITY CONSTANTS OBTAINED BY HEMOLYSIS

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SUMMARY

In 1933, M. H. Jacobs (J. Cell. Comp. Physiol. 4, 161—183) developed the theoretical basis for calculating permeability constants of nonelectrolytes passively penetrating erythrocytes from experimentally determined hemolysis times in isotonic solutions of penetrating solute. This derivation had assumed that the reflection coefficient $\sigma = 1$, whereas, usually $0 < \sigma < 1$, By comparison of Jacobs' original derivation with the equations revised to include σ, it is shown that:

$$\frac{r}{(r+1)^2} = \frac{r_0}{(r_0+\sigma^2)^2}$$

where $r = (k_1/k_2)$, the apparent ratio of solute permeability constant (k_1) to osmotic volume flow constant of water (k_2) as determined by the Jacobs approach; and $r_0 = (\omega/Lpc_s)$, the ratio of the true permeability constant (ω) to the osmotic flow calculated from the product of the pressure-filtration coefficient (Lp) and the concentration gradient (c_s). The correct ratio may be expressed as a function of the apparent ratio:

$$r_0 = B \pm \sqrt{B^2 - \sigma^4}$$

where

$$B = \left[\frac{(r+1)^2}{2r} - \sigma^2\right]$$

For large or small values of r, simpler approximations may be used:

$$r \gg 1, \qquad r_0 \approx r$$

$$r \ll 1, \qquad r_0 \approx \sigma^4 r$$

These provide $< 20\,\%$ error if $\sigma > 0.4$ and $r > 10$ or $r < 0.1$.

Corrections for $\sigma < 1$ are applied to the classical (k_1/k_2) permeability constants of ethylene glycol, glycerol and propanol for bovine erythrocytes. The σ values for monoacetin and diacetin are predicted to be 0.7 and 0.6, respectively, on the basis

of the deviation of their (k_1/k_2) constants from the expected relationship to partition coefficient and vapor pressure.

INTRODUCTION

In 1933, Jacobs [1] considerably advanced the study of passive nonelectrolyte permeability across biological membranes by relating the hemolysis time of erythrocytes suspended in an isotonic solution of penetrating solute to the permeability constant of the solute. He published a table giving the relationship among three basic parameters: (k_1/k_2), the ratio of the permeability constant of the solute to the volume-flow constant of water across the membrane; (t_h/t_o), the ratio of hemolysis time in isotonic solute to the hemolysis time in water; and (V_h/V_o), the ratio of erythrocyte volume at hemolysis to the original volume. This table was used extensively over the following 25 years to obtain permeability constants for assorted alcohols, esters and polyols across several species of erythrocyte membranes under a variety of environmental conditions.

In 1958, Kedem and Katchalsky [2], from a consideration of the irreversible thermodynamics of membrane processes restated the basic transport equations as functions of coupled gradients (see refs. 3 or 4 for a description of the relationships). Briefly, the total volume flow per unit area, J_v, and the flow of solution relative to pure solvent, J_o, may be expressed as functions of the hydrostatic pressure (Δp) and the concentration gradient of solute (Δc_s) across the membrane and several coefficients characteristic of the membrane: the pressure-filtration coefficient (L_p); a diffusion coefficient for the solute (L_D); and the reflection coefficient (σ) [5]. For an ideal semi-permeable membrane, $\sigma = 1$, but for a "leaky" membrane, which includes any membrane permeable to solute, $0 < \sigma < 1$.

The actual movement of solute across the membrane may be expressed as

$$J_s = (1-\sigma)J_v c_s + \omega RT\Delta c_s$$

where ω is a constant which contains L_D, L_p, and σ [2–4]. In this form, the expression for J_s becomes comparable to the classical expression when the first term is 0, or $\sigma = 1$:

$$\frac{ds}{dt} = \Phi_s A\Delta c_s$$

where Φ_s is the permeability constant per unit area for solutes crossing total area A of the membrane. From this, it may be deduced that ωRT is equivalent to the classical Φ_s, but that the older equation tacitly assumes that $\sigma = 1$, so that

$$J_s = \omega RT\Delta c_s = \frac{1}{A}\frac{ds}{dt}$$

The volume flow must also take into account the reflection coefficient, since

$$J_v = L_p(\Delta p - \sigma RT\Delta c_s)$$

but the classical treatment again assumes that $\sigma = 1$, and earlier expressions for flow in the absence of hydrostatic pressure $(\Delta p = 0)$ appear in the form

$$\frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{\Phi w}{c_{\mathrm{w}}}\Delta c_{\mathrm{s}} = -L_{\mathrm{P}}RT\Delta c_{\mathrm{s}}$$

where Φw is the permeability constant of water and c_{w} is the concentration of water.

Because of the assumption that $\sigma = 1$ in the derivation of Jacobs [1], it is apparent that his ratio (k_1/k_2) is not simply proportional to $(\Phi\mathrm{s}/\Phi\mathrm{w})$, or in the newer terminology, (ω/L_{P}), as originally thought, but is a more complicated function of ω, L_{P}, and σ. The inclination in recent years has been either to ignore the permeability constants obtained by the hemolysis procedure and concentrate on determining σ values, or to use them as they are, for the lack of anything better, with the proviso that they might not be exactly correct. This paper seeks to establish the relationship between the original (k_1/k_2) ratios and the true (ω/L_{p}) ratio by introducing the reflection coefficient, σ, into the derivation. The resultant equations provide a means of correcting the earlier permeability constants obtained from hemolysis times for values of σ less than one.

REVISION OF JACOB'S ORIGINAL EQUATIONS

The two basic equations originally stated by Jacobs [1] were as follows:

$$\frac{\mathrm{d}s}{\mathrm{d}t} = k_1\left(1 - \frac{s}{V}\right) \tag{1}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} = k_2\left(\frac{s+1}{V} - 1\right) \tag{2}$$

where V is the intracellular volume, s is the amount of penetrating solute inside the cell, k_1 is a proportionality constant related to the permeability constant of the solute, and k_2 is a comparable proportionality constant for the volume flow of water. For simplicity of treatment, he expressed volumes in terms of initial cell volume $(V_{\mathrm{o}} = 1)$, and concentrations in terms of isotonic equivalents ($s/V = 1$ indicating an isotonic solution). The proportionality constants k_1 and k_2 would therefore include factors for these units as well as the cell surface area being penetrated, but such factors would cancel out in (k_1/k_2) ratios. Since the area of the erythrocyte remains nearly constant as it becomes spherical in hypotonic solution, the hydrostatic pressure is assumed to be constant and only the osmotic component is included in Eqn. 2. From a further differentiation, substitution, and rearrangement, as described in some detail by Jacobs [1] a differential equation entirely in terms of volume and time may be obtained.

$$V^2\frac{\mathrm{d}^2V}{\mathrm{d}t^2} + V\frac{(\mathrm{d}V)^2}{\mathrm{d}t} + (k_1+k_2)V\frac{\mathrm{d}V}{\mathrm{d}t} - k_1k_2 = 0 \tag{3}$$

The solution of Eqn. 3 produces a complicated relationship among V, t, k_1 and k_2 which cannot be solved explicitly for (k_1/k_2); however, by a series of ingenious substitutions and approximating equations, Jacobs was able to estimate (k_1/k_2) in terms of relative hemolysis times $(t_{\mathrm{h}}/t_{\mathrm{o}})$ and hemolysis volumes $(V_{\mathrm{h}}/V_{\mathrm{o}})$.

Reexpression of Eqns. 1 and 2 to include σ and the more current terminology gives:

$$\frac{1}{A}\frac{\mathrm{d}m}{\mathrm{d}t} = \omega RT\left(c_s^{\mathrm{o}} - \frac{m}{V}\right) + (1-\sigma)c_s^{\mathrm{o}}\left(\frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t}\right) = J_s \tag{4}$$

$$\frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t} = L_{\mathrm{P}}\left[\sigma RT\left(\frac{m}{V} - c_s^{\mathrm{o}}\right) + RTc_{\mathrm{i}}\frac{(V_{\mathrm{o}})}{V} + \Delta p\right] = J_{\mathrm{v}}, \tag{5}$$

where c_s^{o} is the extracellular concentration of solute, c_{i} is intracellular concentration of impermeable solute, and m is intracellular penetrating solute, in moles (m is used in place of s to avoid confusion of units, since the latter was given in isotonic equivalents). As before, it will be assumed that the hydrostatic component, Δp, is negligible. To simplify the expressions, let

$$\omega RT = \Phi s,$$

and

$$L_{\mathrm{P}}RTc_s^{\mathrm{o}} = q.$$

Experimentally, c_s^{o} is also set equal to c_{i}, and c_s^{o} may be substituted for c_{i}. Then Eqns. 4 and 5 become:

$$\frac{1}{A}\frac{\mathrm{d}m}{\mathrm{d}t} = \Phi_s\left(c_s^{\mathrm{o}} - \frac{m}{V}\right) + (1-\sigma)c_s^{\mathrm{o}}\frac{\mathrm{d}V}{\mathrm{d}t} \tag{4'}$$

$$\frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t} = q\left[\frac{1}{V}\left(\frac{\sigma m}{c_s^{\mathrm{o}}} + V_{\mathrm{o}}\right) - \sigma\right] \tag{5'}$$

When $\sigma = 1$, Eqn. 4′ reduces to Eqn. 1, and Eqn. 5′ becomes equivalent to Eqn. 2; thus, Φ_s is comparable to k_1, and q is comparable to k_2, and ratio (k_1/k_2) should be identical to the ratio $(\Phi s/q)$. The effect of $\sigma < 1$ on this relationship can be obtained by differentiation and substitution to derive an equation completely in V and t, analogous to the maneuver used by Jacobs.

Eqns. 4′ and 5′ may be expressed in a simplified form:

$$\frac{1}{A}\frac{\mathrm{d}m}{\mathrm{d}t} = a + b\left(\frac{m}{V}\right) + c\left(\frac{1}{V}\right) \tag{4''}$$

$$\frac{1}{A}\frac{\mathrm{d}V}{\mathrm{d}t} = f + g\left(\frac{m}{V}\right) + h\left(\frac{1}{V}\right) \tag{5''}$$

where

$$a = c_s^{\mathrm{o}}[\Phi s - (1-\sigma)\sigma q]$$

$$b = [-\Phi s + (1-\sigma)\sigma q]$$

$$c = c_s^{\mathrm{o}}(1-\sigma)qV_{\mathrm{o}}$$

$$f = -\sigma q$$

$$g = (\sigma q/c_s^{\mathrm{o}})$$

$$h = qV_{\mathrm{o}}$$

The second derivative of Eqn. 5″ yields;

$$\frac{V}{A}\frac{\mathrm{d}^2V}{\mathrm{d}t^2} = g\frac{\mathrm{d}m}{\mathrm{d}t} - g\left(\frac{m}{V}\right)\frac{\mathrm{d}V}{\mathrm{d}t} - h\left(\frac{1}{V}\right)\frac{\mathrm{d}V}{\mathrm{d}t}$$

substitution of the expression for $\mathrm{d}m/\mathrm{d}t$ from Eqn. 4″ and rearrangement gives:

$$\frac{V}{A}\frac{\mathrm{d}^2V}{\mathrm{d}t^2} = gaA + \left(bA - \frac{\mathrm{d}V}{\mathrm{d}t}\right)g\left(\frac{m}{V}\right) + \left(gcA - h\frac{\mathrm{d}V}{\mathrm{d}t}\right)\left(\frac{1}{V}\right)$$

Eqn. 5″ can be solved to give $g(m/V)$ as a function of V and $\mathrm{d}V/\mathrm{d}t$, and substitution of this into the last equation will produce an expression entirely in V:

$$\frac{V}{A}\frac{\mathrm{d}^2V}{\mathrm{d}t^2} = A(ga-bf)+(b+f)\frac{\mathrm{d}V}{\mathrm{d}t} + A(gc-bh)\left(\frac{1}{V}\right) - \frac{1}{A}\left(\frac{\mathrm{d}V}{\mathrm{d}t}\right)^2$$

substitution of the values for a, b, c, f, g, and h and simplification leads to the final equation:

$$V^2\frac{\mathrm{d}^2V}{\mathrm{d}t^2} + V\left(\frac{\mathrm{d}V}{\mathrm{d}t}\right)^2 + A(\Phi_s+\sigma^2q)V\frac{\mathrm{d}V}{\mathrm{d}t} - A^2\Phi_sqV_0 = 0. \tag{6}$$

Eqn. 6 is identical in form to Eqn. 3, and by comparison of the constants,

$$(k_1+k_2) = A(\Phi_s+\sigma^2q)$$

$$k_1k_2 = A^2\Phi_sqV_0$$

Since volumes are expressed as ratios to the initial volume in Jacobs' computations, V_0 may be set equal to one, and $k_1k_2 = A^2\Phi_sq$. The ratio (k_1/k_2) as a function of Φ_s and q can be obtained by some algebraic manipulation of the last two equations.

$$k_1+k_2 = k_1 + \frac{A^2\Phi_sq}{k_1} = A(\Phi_s+\sigma^2q)$$

multiplication by k_1 yields a quadratic equation with the solution:

$$k_1 = \frac{A}{2}\{\Phi_s+\sigma^2q \pm \sqrt{(\Phi_s+\sigma^2q)^2-4\Phi_sq}\}$$

Since $(k_1/k_2) = k_1{}^2/A^2\Phi_sq$,

$$\frac{k_1}{k_2} = \frac{\{\Phi_s+\sigma^2q \pm \sqrt{(\Phi_s+\sigma^2q)^2-4\Phi_sq}\}^2}{4\Phi_sq} \tag{7}$$

This equation can be expressed more simply by dividing the numerator and denominator on the right by q^2 to convert it to a function of the (Φ_s/q) ratio:

$$\frac{k_1}{k_2} = \frac{\left\{\left(\frac{\Phi_s}{q}\right) + \sigma^2 \pm \sqrt{\left(\frac{\Phi_s}{q}+\sigma^2\right)^2 - \frac{4\Phi_s}{q}}\right\}^2}{4\left(\frac{\Phi_s}{q}\right)}$$

Let
$r = (k_1/k_2)$, the apparent ratio, and
$r_o = (\Phi_s/q)$, the true ratio; then

$$r = \frac{[(r_o+\sigma^2) \pm \sqrt{(r_o+\sigma^2)^2 - 4r_o}]^2}{4r_o} \tag{7'}$$

Expansion of the squared term and rearrangement gives

$$\frac{2r_o(r+1)}{(r_o+\sigma^2)} - (r_o+\sigma^2) = \pm\sqrt{(r_o+\sigma^2)^2 - 4r_o}$$

Squaring this equation and some further algebra finally produces a simple relationship between r and r_o:

$$\frac{r}{(r+1)^2} = \frac{r_o}{(r_o+\sigma^2)^2} \tag{8}$$

Since r is the apparent ratio (k_1/k_2) found from Jacobs' table [1], and a number of different reflection coefficients have been measured independently, the problem becomes one of calculating r_0 as a function of r and σ. First, some limiting cases may be considered.

Case I

Let $\sigma = 1$; then from Eqn. 8, r_0 could have two values, r and $1/r$. In actual fact r_0 should equal r, but it is apparent that the equation is symmetrical with respect to the reciprocal of r; thus, the expression will be identical for $r = 10$ and $r = 0.1$ and will fit either $r_0 = 10$ or $r_0 = 0.1$. Selection of the correct value for r_0 will then depend on this limiting case, since $r_0 = r$ when $\sigma = 1$.

Case II

Let $\sigma = 0$; again, two values are obtained. If $r < 1$, then $r_0 = 0$, but if $r > 1$, then

$$r_0 = \frac{(r+1)^2}{r}$$

For $r = 0.1$, for example, $r_0 = 0$, but if $r = 10$, $r_0 = 12.1$. Moreover, for $r \gg 1$, r_0 approaches r. This result is intuitively reasonable, since for the completely unselective membrane implied by $\sigma = 0$, a slowly penetrating solute ($r_0 \approx 0$) could be almost entirely carried by the volume flow, whereas a rapidly penetrating solute ($r_0 \gg 1$) would not be greatly influenced by the solution volume flow.

Case III

Let $r \ll 1$ and $r_0 \ll \sigma^2$; this condition is not uncommon for slowly penetrating solutes, since σ is often near one when $r_0 \ll 1$. In this instance $r_0 = \sigma^4 r$, and a σ of 0.9 for example, would introduce a correction factor of 0.656. Stein [3] has estimated that $r_0 = \sigma^2 r$, and this might hold over a limited range, but for $r < 0.1$, the fourth power correction would be more accurate.

Rearrangement of Eqn. 8 leads to a quadratic equation:

$$r_0^2 + \left\{2\sigma^2 \frac{(r+1)^2}{r}\right\} r_0 + \sigma^4 = 0$$

with a solution which may be expressed as

$$r_0 = B \pm \sqrt{B^2 - \sigma^4} \tag{9}$$

where

$$B = \left\{\frac{(r+1)^2}{2r} - \sigma^2\right\}$$

An approximating equation may be derived from this by use of differentials; assuming $B^2 \gg \sigma^4$, and letting $\sigma^4 = x$,

$$\frac{dr_0}{dx} = \pm\tfrac{1}{2}\sqrt{B^2 - x} \approx \pm\tfrac{1}{2}B$$

then

$$\Delta r_0 = \pm\frac{\Delta(\sigma^4)}{2B}$$

If the reference r'_0 is taken as the value for $\sigma = 0$, then $r_0 = B \pm B$, and

$$r_0 = r'_0 + \Delta r_0 = B \pm B \pm \frac{\Delta\sigma^4}{2B}$$

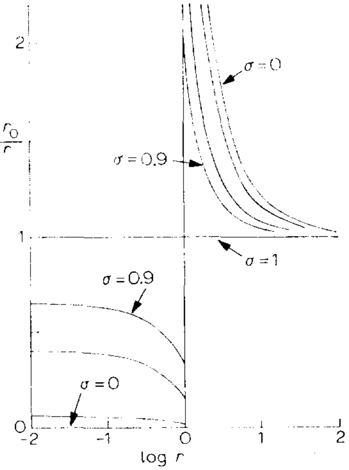

Fig. 1. Ratio (r_0/r) as a function of log(r) for reflection coefficients of 0, 0.5, 0.8, 0.9, and 1.0. Value r is the (k_1/k_2) ratio as determined by the hemolysis method of Jacobs; value r_0 is the ratio corrected for the reflection coefficient, σ (see Table I). For $\sigma = 1$, $(r_0/r) = 1$ for all values of r; for $r \ll 1$, (r_0/r) approaches σ^4; for $r \gg 1$, (r_0/r) approaches 1 for all values of σ. Curves for $\sigma = 0$ and 0.9 only are indicated; those for $\sigma = 0.5$ and 0.8 lie in between.

and the values for r_0 are:

$$r_0 = \left(2B - \frac{\sigma^4}{2B}\right), \left(\frac{\sigma^4}{2B}\right) \tag{10}$$

The first solution holds, with less than 5 % error, for values of $r > 2$; the second solution provides estimates within 5 % error for $r < 0.2$. For $0.2 < r < 2$, the exact solution in Eqn. 9 may be necessary. The ratio (r_0/r), i.e., the true $(\Phi s/q)$ divided by the apparent (k_1/k_2) ratio obtained from hemolysis times by means of Jacobs' table [1], is shown as a function of r for σ values of 1.0, 0.9, 0.8, 0.5, and 0.0 in Fig. 1. It may be noted that the effect of $\sigma < 1$ is most pronounced near $r = 1$, and that the function is actually discontinuous at $r = 1$.

CORRECTION OF PERMEABILITY CONSTANTS FOR BOVINE ERYTHROCYTES

In earlier work, Jacobs [1, 2, 6, 7] had determined (k_1/k_2) ratios for a number of alcohols, polyols and esters penetrating bovine erythrocytes and had calculated the equivalent permeability constants [7]. More recently, Farmer and Macey [8] determined σ, ωRT, and L_pRT for the penetration of ethylene glycol and glycerol into the same cells, thereby providing some means of testing the correction. The L_pRT for entry of water into cells is 0.48 cm^4/osmol/s [8]; since the values of Jacobs were derived for "isotonic" 0.3 M solutions, $c_s{}^o$ would be $0.3 \cdot 10^{-3}$ mol/cm, and $q = (0.3 \cdot 10^{-3})\,(0.48) = 1.44 \cdot 10^{-4}$ cm/s. The $RT\omega$, or Φs, for glycol is $38 \cdot 10^{-7}$ cm/s; for glycerol values of 1 and $2 \cdot 10^{-7}$ cm/s are reported [8]. From these results the $(\Phi s/q)$ or r_0 ratios may be calculated as $26 \cdot 10^{-3}$ for glycol and approximately $1 \cdot 10^{-3}$ for glycerol. These ratios are compared with the (k_1/k_2) or r ratios in Table I, along with the reflection coefficients and r values for several other penetrating solutes. It may be noted that for glycol, the r_0 value calculated by correction of r for σ comes close to the r_0 actually observed, but that the calculated glycerol r_0 is lower than the observed r_0. Propanol and erythritol are included to provide a very rapidly and a very slowly penetrating solute for comparison. In other cells, simple alcohols such as

TABLE I

OBSERVED AND CALCULATED VALUES FOR r AND r_0, FOR BOVINE ERYTHROCYTES

$r = (k_1/k_2)$, where k_1 = solute permeability constant and k_2 = osmotic flow of water as determined by the Jacobs [1] hemolysis method; $r_0 = \omega/L_pc_s{}^o$, as defined by Kedem and Katchalsky [2]; calculated r_0 obtained by correction of r for the reflection coefficient σ, as described in text.

Compound	Observed values			Calculated	
	$r \times 10^3$	$r_0 \times 10^3$	σ	(r_0/r)	$r_0 \times 10^3$
A. Propanol	3150	–	(0)	1.73	5500
B. Ethylene glycol	48	26	0.82	0.43	21
C. Glycerol	0.56	≈ 1	0.92	0.72	0.40
D. Erythritol	0.02	–	(1)	1.00	0.02
E. Monoacetin	34	–	–	–	(8.5)
F. Diacetin	310	–	–	–	(27)
Refs.	[1, 6, 7]	[8]	[8]		

propanol tend to have large permeability constants and low reflection coefficients [3, 4] and so the limiting case of $\sigma = 0$ was assumed in order to give the maximum possible correction in the propanol r_0; erythritol, on the other hand, tends to have a reflection coefficient close to 1.0, and always has a σ greater than that of glycerol, and therefore a σ of 1.0 was assumed for erythritol. Monoacetin and diacetin are included for other reasons to be discussed below.

In 1965 [9], a hypothetical model of passive nonelectrolyte transport across cell membranes was presented. The major barrier to passage was assumed to be the water/lipid interface, and it was further assumed that the solute becomes partially dissociated from both phases on passage. The consequent equations predict a linear relationship between log $(\Phi s/C_o)$ and log (P/C_o), where P is the vapor pressure of the solute, C_o is the oil/water partition coefficient, and Φs is the permeability constant. The slope of the line, γ, gave the degree of dissociation from both phases during passage, and, theoretically, lay between 0 and 1, 0 being equivalent to no dissociation and 1 to complete dissociation. Nearly linear arrays of points were found for several types of cells and various alcohols, polyols and esters. The slope for algae (*Chara*) cells was 0.4, but the slopes for bovine erythrocytes and a number of other animal cells were near 0.6, which suggested a somewhat different lipid/water interaction at the interface of the animal cell membranes. One obvious difficulty with this comparison lay in the methodology, since the Φs values for the algae were obtained by direct chemical measurement of solute entering the cells [10] and are therefore probably near the true values, whereas the permeability constants of animal cells were obtained by osmotic swelling without any correction for the reflection coefficient and are therefore suspect.

Fig. 2 illustrates the effect of the reflection coefficient correction for propanol, glycol, and glycerol on the log $(\Phi s/C_o)$ versus log (P/C_o) plot. The original Jacobs

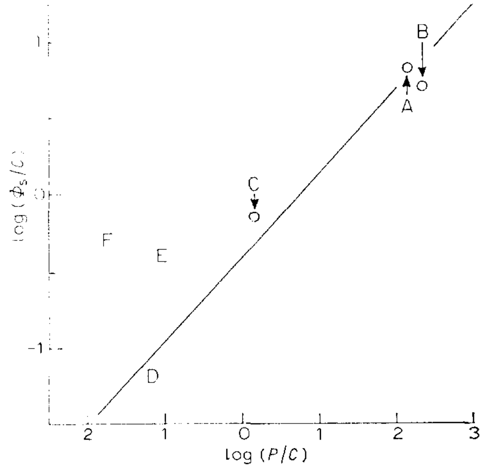

Fig. 2. Log$(\Phi s/C)$ as function of log(P/C). Φs, permeability constant; C, oil/water partition coefficient; P, vapor pressure of solute. Letter corresponds to compound in Table I and gives position of Φs as determined by the Jacobs method; arrows and points indicate correction in Φs introduced by inclusion of σ for compounds A, B, and C. Slope of line $(\gamma) = 0.53$.

permeability constants are used, as before [9], although they are somewhat lower than those of Farmer and Macey because of a higher estimate for the flow of water; however, this would have no effect on the relative position of the points. The correction reduces the scatter somewhat (although perhaps not significantly) and lowers the slope of the line, giving a γ of about 0.53. More values would be required to define γ more exactly, but these results suggest that the true permeability constants of animal cells (ωRT or r_0) would yield γ values closer to that of the algae cells. Two components, monoacetin (E) and diacetin (F) deviate widely from the line, implying unpredictably high permeability constants for these, and it was suggested previously [9] that a carrier transport system might be accelerating their penetration. An alternative explanation for the deviation is now obvious: the acetins may have relatively low reflection coefficients. If the two acetin points are lowered to the line in Fig. 2, the predicted r_0 values would be much less than the observed r values (Table I); substitution of these r and r_0 values into Eqn. 8 and solution for σ yields a σ of 0.7 for monoacetin and 0.6 for diacetin.

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