J. Membrane Biol. 77, 255–264 (1984)

The Journal of
Membrane Biology

Monocarboxylic Acid Permeation Through Lipid Bilayer Membranes

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Summary. The membrane permeability coefficients for the homologous monocarboxylic acids, formic through hexanoic, as well as benzoic and salicylic, were determined for egg phosphatidylcholine-decane planar bilayer membranes. The permeabilities of formic, acetic and propionic acid were also determined for "solvent-free" phosphatidylethanolamine bilayers. Permeability coefficients were calculated from tracer fluxes measured under otherwise symmetrical conditions, and precautions were taken to ensure that the values were not underestimated due to unstirred layer effects. The relation between the nonionic (HA) permeability (P^m) and the hexadecane/water partition coefficient (K_p) was: $\log P^m = 0.90 \log K_p + 0.87$ (correlation coefficient = 0.996). Formic acid was excluded from the analysis because its permeability was sixfold higher than predicted by the other acids. The permeabilities for "solvent-free" membranes were similar to those for decane-containing membranes. The exceptionally high permeability of formic acid and the high correlation of the other permeabilities to the hexadecane/water partition coefficient is a pattern that conforms with other nonelectrolyte permeabilities through bilayers. Similarly, the mean incremental free energy change per methylene group ($\delta\Delta G/-CH_2-$) was -764 cal mol^{-1}, similar to other homologous solutes in other membrane systems. However, much less negative $\delta\Delta G$ values (-120 to -400 cal mol^{-1}) were previously reported for fatty acids permeating bilayers and biological membranes. These values are due primarily to unstirred layer effects, metabolism and binding to membranes and other cell components.

Key Words fatty acid · weak acid · membrane permeability · lipid bilayer · partition coefficient

Introduction

A review of the literature on nonelectrolyte permeability suggests that the nonionic forms of monocarboxylic acids are unusual permeants because their apparent permeability coefficients are relatively insensitive to increasing chain length. This implies that the permeability barrier is more hydrophilic toward monocarboxylic acids than to other nonelectrolytes, thus suggesting a different rate-limiting step for permeation. For example, the fatty acid permeabilities through planar lipid bilayers reported by Wolosin and Ginsburg (1975) are proportional to the decane/water partition coefficient raised to the 0.38 power, meaning that the rate-limiting barrier behaves as though it is much more hydrophilic than decane. In contrast, the permeability to other nonelectrolytes including butyric acid is proportional to the first power of the hexadecane/water partition coefficient (Finkelstein, 1976; Orbach & Finkelstein, 1980).

Reported patterns of short-chain fatty acid permeabilities of biological membranes are extremely variable. In general, fatty acids appear to permeate a relatively hydrophilic barrier, based on the small incremental free-energy change ($\delta\Delta G$) with the addition of a methylene group. For example, reports from Dietschy's lab (reviewed by Dietschy, 1978; Thompson & Dietschy, 1981) on a variety of membranes give $\delta\Delta G$'s near zero for acetic-hexanoic acids and about -300 cal/mol for hexanoic-tetradecanoic acids. However, more polar solutes, e.g., amides and diols, give $\delta\Delta G$'s of -600 to -800 cal/mol (Finkelstein, 1976; Wright & Bindslev, 1976; Orbach & Finkelstein, 1980).

In light of the large variability and unusual patterns of fatty acid permeation in biological membranes, it is important to establish permeability coefficients and permeability patterns in lipid bilayer membranes. The bilayer system is useful because true membrane permeabilities can be measured despite two factors which complicate biological membrane studies. Firstly, the nonionic weak acid (HA) is often highly permeant, so the unstirred layer permeability

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(P^{ul}) is a major component of the total permeability. Secondly, the relatively impermeant ionic form (A^-) "facilitates" the diffusion of tracer through the unstirred layers. Fortunately, this provides a way of measuring the membrane permeability (P^m) even when $P^m > P^{ul}$ (Gutknecht & Tosteson, 1973).

In a recent paper (Walter, Hastings & Gutknecht, 1982), we established criteria to determine P^m for HA using butyric acid as an example. Our value for butyric acid permeability was 60-fold greater than that reported by Wolosin and Ginsburg (1975) on a similar bilayer system. The discrepancy was shown to be due to inadequate unstirred layer corrections. Thus we hypothesized that weak acid permeabilities are governed by the same rules as other nonelectrolytes and that the apparently less negative values of $\delta\Delta G/-CH_2-$ are due to unstirred layer effects and possibly other factors related to hydrophobicity or metabolism of weak acids.

In this study we measured the permeabilities of short-chain fatty acids and benzoic and salicylic acids through lipid bilayer membranes. The primary objective was to establish the permeability pattern and find out whether these permeant weak acids show normal $\delta\Delta G$ values with the addition of $-CH_2-$ groups. We also wished to provide baseline permeability data for comparison with biological membranes where nonionic diffusion, carrier-mediated transport of HA or A^-, and paracellular transport are all possible and difficult to distinguish.

Materials and Methods

Membrane Formation

Lipid bilayer membranes were made by the brush technique of Mueller and Rudin (1969) from a solution of egg phosphatidylcholine (PC) in decane (25 to 30 mg/ml) (Lipid Products, Surrey, England, or Avanti Biochemicals, Birmingham, Ala.). The decane (Aldrich Chemical Co., Milwaukee, Wis.) was passed over an alumina column before use. Membranes were formed on a 1.6 mm^2 hole in a polyethylene partition separating two magnetically stirred, 1.25 ml chambers. Ag/AgCl electrodes were placed on either side of the membrane and membrane resistances were calculated from current/voltage relationships. The membranes were monitored visually using a low-power microscope for estimates of the bilayer membrane area. The membranes were held planar by adjusting the volume in the rear chamber with a micrometer syringe.

The aqueous solution was usually 100 mM NaCl plus 1 to 10 mM unlabeled acid and 5 to 10 mM of appropriate buffer (MES, PIPES, HEPES, Tricine or CAPS, Sigma Chemical Co., St. Louis, Mo.). The weak acid dissociation constants were corrected for ionic strength as described by Perrin and Dempsey (1974). The temperature was 22 ± 2 °C.

For some experiments, large "solvent-free" membranes were used (Montal & Mueller, 1972). A 0.8 mm^2 hole was punched in 1 mil (25 μm) Teflon, which was then mounted in a Teflon chamber divided into two 3-ml compartments. The hole was wiped with squalane (Eastman, Rochester, N.Y.) and then bacterial phosphatidylethanolamine (PE) in pentane was layered on the aqueous surface in each compartment. The membrane was formed by slowly raising the two aqueous solutions above the hole. Membrane capacitance and current-voltage relationships were monitored routinely.

Flux Measurements and Permeability Calculations

One-way fluxes were measured by means of tracers under symmetrical conditions. As soon as the membranes were optically black, 0.5 to 10 μCi of ^{14}C-labeled acid were injected into the rear chamber. The trans compartment was perfused continuously at 0.5 to 1.5 ml min^{-1}. The perfusate was collected in a vacuum trap at 3-min intervals for about 30 min. The flux measurement generally reached steady state between 3 and 6 min after injection of tracer. The rear chamber was sampled periodically using a 10-μl syringe. Samples were counted in a liquid scintillation counter. Fluxes were calculated from the equation:

$$J = \frac{^{14}C^{trans}}{tAS^{cis}} \tag{1}$$

where J is the one-way flux in mol cm^{-2} sec^{-1}, $^{14}C^{trans}$ is the total amount of tracer (μCi) entering the front compartment during the time interval t (sec), A is the membrane surface area (cm^2) and S^{cis} is the specific activity of labeled acid in the rear compartment (μCi mol^{-1}). Tracers were obtained from ICN (Irvine, Calif.) or New England Nuclear (Boston, Mass.).

Solute fluxes across Montal-Mueller membranes were determined by measuring the rate of accumulation of tracer in the trans compartment. Tracer was injected into the cis compartment and 50 to 100 μl samples were taken from the trans compartment at regular intervals. The volume removed from the trans compartment was replaced by an equal volume of cold buffer. The total amount of tracer in the trans compartment was calculated from the concentration in the sample aliquot and the volume of the compartment was corrected for dilution with cold buffer. The amount of tracer increased linearly with time and the flux was determined from:

$$J = \frac{d^{14}C^{trans}}{dt}\frac{1}{S^{cis}A} \tag{2}$$

where $d^{14}C^{trans}/dt$ is the rate of increase in total tracer in the trans compartment.

Permeability coefficients were determined from the fluxes using the relationship developed for solutes with one permeant (HA) and one relatively impermeant (A^-) molecular form (Gutknecht, Bruner & Tosteson, 1972; Walter et al., 1982):

$$\frac{1}{J} = \frac{1}{P^{ul}_{HA}[HA] + P^{ul}_{A^-}[A^-]} + \frac{1}{P^m[HA]} \tag{3}$$

where P^{ul} is the unstirred layer permeability coefficient for HA or A^- and P^m is the membrane permeability coefficient for HA. Equation (3) is applicable only when pH throughout the unstirred layer is constant.

The difference between P^{ul}_{HA} and $P^{ul}_{A^-}$ is very small, so the denominator of the first term in Eq. (3) can be written: $P^{ul}[A_T]$, where $[A_T]=[HA]+[A^-]$. Thus, Eq. (3) can be rewritten in a linearized form:

$$\frac{[A_T]}{J}=\frac{[A_T]}{P^m[HA]}+\frac{1}{P^{ul}}. \quad (4)$$

The value for P^m is obtained from the slope of the line resulting from fluxes determined at several pH's.

The assumption that only HA contributes significantly to the transmembrane flux was confirmed by comparing the measured flux to the A^- flux (J_{A^-}) predicted by assuming that all the membrane conductance (G) was due to A^- (Hodgkin, 1951):

$$J_{A^-}=\frac{RTG}{z^2F^2} \quad (5)$$

where R, T, z and F have their usual meanings. For all the test solutes, the calculated J_{A^-} was less than 2% of the measured flux.

Partition Coefficients

The hexadecane/water partition coefficients (K_p) (mol/vol ratio) were determined by equilibrating an aqueous solution (100 mM HCl) containing ^{14}C-labeled solute with hexadecane (Eastman, Rochester, N.Y.) that had been passed through an alumina column. The aqueous solution and hexadecane were added to a capped centrifuge tube, mixed on a vortex and held on a gyrotary shaker at room temperature (22 to 25 °C) for at least 30 min. The samples were then centrifuged at 6,600 × g for 30 min (a procedure shown to remove any emulsified water from the hexadecane phase). An aliquot of the hexadecane phase was removed for liquid scintillation counting. Then all the remaining hexadecane was removed and 10 µl of the aqueous phase was taken for counting. Fresh hexadecane was layered over the remaining aqueous phase, and the equilibration and sampling process were repeated until the K_p became constant. This process removed all impurities more hydrophobic than the test solute. Generally no hydrophobic impurities were detected.

Results

Table 1 shows partition coefficients and permeability coefficients of weak acids through egg PC-decane (Mueller-Rudin) bilayers and bacterial PE-squalane (Montal-Mueller) bilayers. Permeabilities of Mueller-Rudin membranes were determined from fluxes measured at several pH's by means of Eqs. (3) and (4). Permeabilities of Montal-Mueller membranes were determined at only one pH, at least 0.5 pH units higher than that required to eliminate unstirred layer effects in the Mueller-Rudin system. The unstirred layer thicknesses were similar (*ca.* 200 µm) in both chambers. Although there are fewer data for the Montal-Mueller membranes, the permeabilities are generally similar to the decane-containing membranes. We also measured acetic acid permeability of bacterial PE-decane bilayers and obtained (3.4 ± 0.3)

Table 1. Hexadecane/water partition coefficients and membrane permeability coefficients of weak acids in two types of lipid bilayer membranes

Acid	K_p	P^m (cm sec^{-1})	
		Mueller-Rudin (egg PC-decane)	Montal-Mueller (bacterial PE-squalane)
Formic	$(1.2\pm0.3)\times10^{-4}$ $n=6$	$(1.1\pm0.2)\times10^{-2}$ $n=9$	4.3×10^{-3} $n=1$
Acetic	$(3.7\pm0.4)\times10^{-4}$ $n=12$	$(6.6\pm1.3)\times10^{-3}$ $n=4$	$(6.9\pm1.8)\times10^{-3}$ [a] $n=3$
Propionic	$(2.3\pm0.4)\times10^{-3}$ $n=6$	$(2.6\pm1.0)\times10^{-2}$ $n=7$	$(3.5\pm1.2)\times10^{-2}$ $n=2$
Butyric	$(8.7\pm0.6)\times10^{-3}$ $n=7$	$(9.5\pm0.5)\times10^{-2}$ $n=42$	
Hexanoic	$(1.4\pm0.1)\times10^{-1}$ $n=6$	1.1 ± 0.2 $n=7$	
Benzoic	$(5.3\pm0.4)\times10^{-2}$ $n=6$	$(5.5\pm1.7)\times10^{-1}$ $n=7$	
Salicylic	$(6.1\pm0.9)\times10^{-2}$ $n=6$	$(7.7\pm1.0)\times10^{-1}$ $n=10$	

[a] The permeability of bacterial PE-decane bilayers to acetic acid was $(3.4\pm0.3)\times10^{-3}$ cm sec^{-1} ($n=3$).

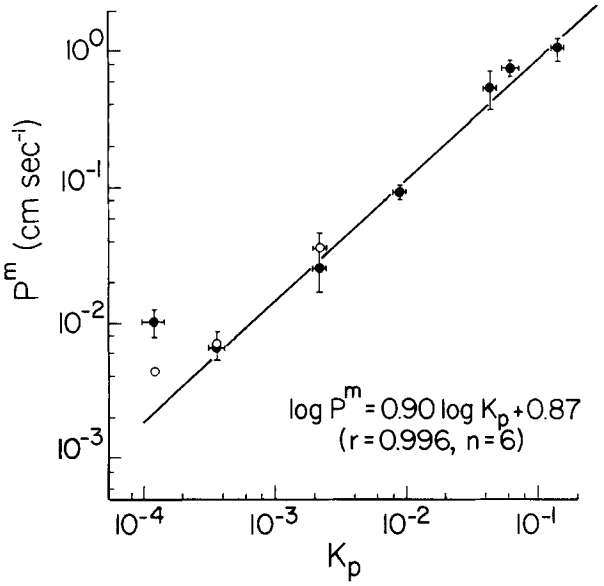

Fig. 1. Relation between monocarboxylic acid permeability and hexadecane/water partition coefficient. Closed circles represent egg phosphatidylcholine-decane membranes. The linear regression equation (excluding formic acid) is $\log P^m = 0.90 \log K_p + 0.87$ ($r = 0.996$). The open circles represent permeabilities of "solvent-free" phosphatidylethanolamine bilayers and are not included in the regression analysis. The error bars indicate standard deviations

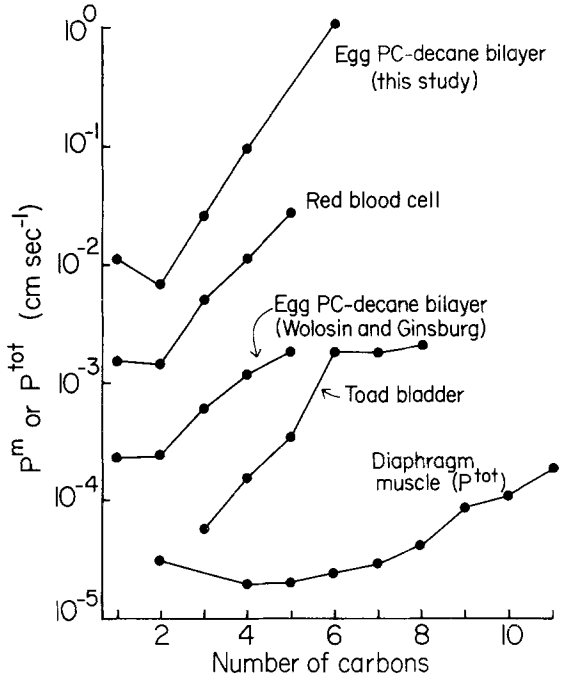

Fig. 2. Fatty acid permeabilities of several types of membranes. Except for diaphragm muscle, the values reported are nonionic (HA) permeabilities (P^m or P^{app}). Values for diaphragm muscle are reported as P^{tot}, defined as $J/[A_T]$, because the authors made no assumptions about the permeant species (Schwartz & Dietschy, 1980). Some of the permeabilities are "corrected" for unstirred layers, and the methods of correction are evaluated in the text. Red cell data are from Klocke et al. (1972) and toad bladder data are from Rosen et al. (1964)

$\times 10^{-3}$ cm sec^{-1} (Table 1), slightly lower than P^m for "solvent-free" PE bilayers, i.e., $(6.9 \pm 1.8) \times 10^{-3}$ cm sec^{-1}.

All of our values for acetic acid permeability are slightly higher than the value for egg PC liposomes reported by Alger and Prestegard (1979), who used nuclear magnetic resonance techniques. We calculated a liposome permeability of 1.4×10^{-3} cm sec^{-1} at 25 °C from their value of $(5 \pm 2) \times 10^{-4}$ cm sec^{-1} at 7 °C and their activation energy for permeation of 10.4 kcal mol^{-1}. Their lower value may be due partly to the choice of pK and/or the presence of some multilamellar liposomes.

Figure 1 shows the relation between monocarboxylic acid permeability and hexadecane/water partition coefficient, plotted according to the equation:

$$\log P^m = a \log K_p + b. \quad (6)$$

The line is the least-squares fit of the data from the Mueller-Rudin membranes, excluding formic acid which has a sixfold higher permeability than predicted by the other solutes. The correlation coefficient is 0.996 and the slope of the line is nearly 1.0, indicating that P^m and K_p are linearly related. Permeabilities for the solvent-free membranes fit the same pattern, and the formic acid P^m is also higher than predicted. Benzoic and salicylic acids fall on the regression line, signifying that the correlation is not restricted to the homologous straight-chained fatty acids. Benzoic and salicylic acids have similar P^m's and K_p's despite the presence of an additional hydroxyl group in salicylic acid. The apparent noneffect of the additional $-OH$ is due to its position adjacent to the carboxyl group allowing internal hydrogen bonding (Pauling, 1960). Additional hydroxyl groups in the *meta* or *ortho* positions would be expected to greatly decrease the K_p and P^m.

Fatty acid permeabilities of egg PC-decane bilayers are plotted as a function of the alkyl chain length in Fig. 2. For solutes larger than formic acid, P^m increases by a factor of 3.5 to 3.9 per methylene group. The slope of the line

Table 2. Incremental free energy changes with the addition of a methylene group to homologous fatty acids and alcohols[a]

System	$\delta\Delta G/-CH_2-$ (cal mol^{-1})	Reference
K_p, hexadecane/water		
C_2-C_6	-898 ± 159	This study
K_p, octanol/water		
C_2-C_5	-715 ± 107	Wolosin and Ginsburg (1975)
K_p, red cell ghost/water[b]		
C_4-C_7	-663 ± 152	Roth and Seeman (1972)
K_p, liposome/gas[c]		
C_1-C_4 (alkanes)	-878 ± 87	Miller et al. (1977)
P^m, egg PC-decane bilayer C_2-C_6	-764 ± 54	This study
P^m, bacterial PE-squalane		
C_2-C_3	-968	This study
P^m, egg PC-decane bilayer C_2-C_5	-397 ± 147	Wolosin and Ginsburg (1975)
P^m, red cell		
C_2-C_5	-591 ± 133	Klocke et al. (1972)
P^m, toad bladder		
C_3-C_6	-679 ± 274	Rosen et al. (1964)
C_6-C_8	-37 ± 57	
P^m, jejunum		
C_2-C_8	-118 ± 156	Sallee and Dietschy (1973)
C_8-C_{12}	-300 ± 96	
$C_{12}-C_{18}$	-695	Sallee (1979)
C_3-C_{10}	-213 ± 157	Jackson et al. (1978)

[a] Representative data were selected from a large literature on fatty acids and are not intended to be comprehensive. Values of $\delta\Delta G$ were calculated from Eq. (7) and are expressed as the mean $\pm$SD. $T=25$ °C for all values except red cell and jejunum, which were 37 °C.

[b] These partition coefficients include all membrane-associated solute and thus are not perfectly representative of partitioning into the hydrocarbon core of the membrane (*see* Katz & Diamond, 1974).

[c] This K_p is for alkanes which partition into the hydrocarbon core of the bilayer.

in Fig. 2 reflects the mean incremental free energy change per methylene group ($\delta\Delta G/-CH_2-$), which was calculated according to Diamond and Wright (1969):

$$\delta\Delta G = RT \ln (P_1^m/P_2^m) \tag{7}$$

where P_1^m and P_2^m are the membrane permeability coefficients for two solutes that differ by only a methylene group. The results are given in Table 2. For acetic through caproic acids, $\delta\Delta G/-CH_2-=-764$ cal mol^{-1} for decane-containing bilayers and -898 cal mol^{-1} for the hexadecane/water K_p's. For "solvent-free" bilayers $\delta\Delta G=-968$ cal mol^{-1} based on a single ratio of acetic to propionic acid permeability coefficients.

Discussion

Monocarboxylic Acids are "Normal" Solutes

The permeability coefficients of monocarboxylic acids correlate well with and are linearly related to the hexadecane/water partition coefficients (Fig. 1). The one exception, formic acid, has a higher P^m than predicted by its K_p, similar to another one-carbon nonelectrolyte, formamide (Gallucci, Micelli & Lippe, 1971; Finkelstein, 1976). The "high" permeabilities for the first members of homologous series seem to be analogous to the "high" permeability reported for water (e.g., Finkelstein, 1976) and are probably due to a size-selective step in the permeation process (Walter & Gutknecht, *manuscript in preparation*). The monocarboxylic acid permeabilities conform to the pattern established for other nonelectrolytes (Orbach & Finkelstein, 1980) and, therefore, are "normal" solutes.

One theoretical basis for the correlation between P^m and K_p is a simplified description of membrane permeation (Diamond & Wright, 1969):

$$P^m = \frac{K_p^m D^m}{d} \tag{8}$$

where K_p^m and D^m are the average partition and diffusion coefficients for the solute in the membrane interior and d is the membrane thickness. A high correlation between P^m and K_p into a model solvent indicates that the membrane selectivity is determined by the same forces which determine K_p. Thus, our data indicate that the bilayer membrane barrier is almost as hydrophobic as hexadecane. The three values from the "solvent-free" membranes are almost identical to the values for decane-containing membranes, indicating that the membrane hydrophobicity inferred from Fig. 1 is not caused by the presence of decane.

The short-chain fatty acid permeabilities increase by a factor of 3.5 to 3.9 per methylene group (Table 1). The incremental free energy per $-CH_2-$ for partitioning into hexadecane and for permeation across both types of lipid

bilayers ranges from -764 to -968 cal mol^{-1}, independent of alkyl chain length over the range of C_2 to C_6 (Table 2). Our values compare favorably with $\delta\Delta G/-CH_2-$ of -878 cal mol^{-1} for partitioning of alkanes into phosphatidylcholine/phosphatidic acid (96:4) liposomes and -663 cal mol^{-1} for fatty acid partitioning into red cell ghosts (Table 2). Other classes of nonelectrolytes and other types of membranes also give $\delta\Delta G$'s within the range we observed (e.g., Wright & Bindslev, 1976). From the data of Finkelstein (1976) and Orbach and Finkelstein (1980), we calculated $\delta\Delta G/-CH_2-$ ranging from -686 to -809 cal mol^{-1} for diols and amides permeating membranes made of egg PC-decane, egg PC-cholesterol-decane or sphingomyelin-cholesterol-decane.

In contrast, the fatty acid permeabilities reported by Wolosin and Ginsburg (1975) increase slowly with chain length (Fig. 2), and $\delta\Delta G/-CH_2-$ decreases with chain length, giving an average value of -397 cal mol^{-1} (Table 2). However, all of Wolosin and Ginsburg's fatty acid permeabilities are underestimated due to inadequate unstirred layer corrections (Walter et al., 1982). The degree of underestimation increases with chain length (Fig. 2), resulting in lower absolute values for $\delta\Delta G$ and shallow slopes when the data are plotted as in Fig. 1. For example, Wolosin and Ginsburg's fatty acid data (C_2 to C_5) yield slopes of 0.38 for decane and 0.56 for octanol. Thus, inadequate unstirred layer corrections cause the membrane barrier to appear more hydrophilic than it actually is.

Criteria for Membrane-Limited Fluxes of Fatty Acids

Permeability Patterns

If fatty acid fluxes across cell membranes are limited by diffusion of HA across the lipid core of the membrane, then permeabilities of fatty acids should increase log-linearly with acyl chain length, and values of $\delta\Delta G/-CH_2-$ should be fairly constant and in the range of -600 to -800 cal mol^{-1}. Different permeability patterns within a homologous series suggest either that permeation occurs by more than one pathway or that true membrane permeabilities are masked by other rate-limiting processes, e.g., unstirred layer permeation, metabolism or nonmetabolic binding.

Klocke, Andersson, Rotman and Forster (1972) determined weak acid permeabilities of red blood cell membranes by observing external pH changes as fatty acids were taken up by cells in a continuous flow system. Their method allowed measurement of initial rates of uptake, and the external unstirred layer thickness was only 1 to 2 μm. $\delta\Delta G/-CH_2-$ calculated from their data is -591 cal mol^{-1}, slightly lower than our value for lipid bilayers (Table 2). The difference may be real or due to a small unstirred layer effect on the red cell butyric and valeric acid permeabilities. However, the red cell permeability pattern is generally similar to the pattern for PC-decane bilayers (Fig. 2). Earlier fatty acid permeabilities reported by Green (1949) were underestimated due to unstirred layer effects and other factors discussed by Klocke et al. (1972).

Studies on weak acid/base transport frequently report the apparent nonionic permeability (P^{app}), which for weak acids is defined as $J/[HA]$. P^{app} is the value one would calculate directly from tracer fluxes without including the unstirred layer term in Eq. (3); thus, $P^{app} < P^m$ if unstirred layer effects are significant. An expression for P^{app} is obtained by multiplying both sides of Eq. (4) by $[HA]/[A_T]$ and substituting D/δ for P^{ul} to give

$$\frac{[HA]}{J} = \frac{[HA]\,\delta}{[A_T]\,D_{aq}} + \frac{1}{P^m} \tag{9}$$

where $[HA]/J = 1/P^{app}$ and D_{aq} is the aqueous diffusion coefficient. Figure 3 shows how P^{app} deviates increasingly from P^m as acyl chain length increases, δ increases and pH decreases. If chain length increases at constant pH, the tracer flux will be limited eventually by the unstirred layer, because P^m increases much faster than D_{aq} decreases. The "critical" chain length at which P^{app} becomes nearly constant is a function of P^{ul}/P^m and $(pH - pK_a)$. Failure to recognize the cause of the nonlinear relationship described by Eq. (9) has led to the erroneous conclusions that either A^- is crossing the membrane or that P^m is pH dependent (Bean, Shepherd & Chan, 1968; Inui et al., 1977).

An example of the pattern shown in Fig. 3 is the series of fatty acid permeabilities reported for toad bladder at pH 6.0 (Rosen, Leaf & Schwartz, 1964). In toad bladder P^{app} (i.e., $J/[HA]$) increases steeply for fatty acid chain lengths C_3 to C_6, then levels off around C_6 to C_8 (Fig. 2). The values of $\delta\Delta G/-CH_2-$ for C_3 to C_6 are about -679 cal mol^{-1}, similar to lipid bilayers

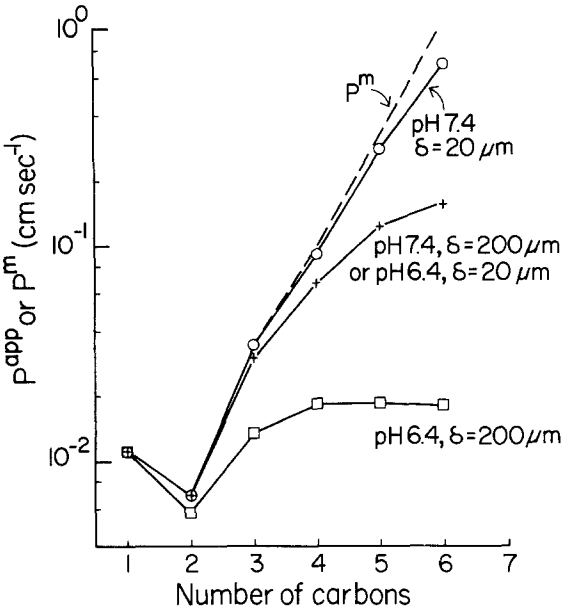

Fig. 3. Membrane permeabilities of PC-decane bilayers (Table 1) are used to calculate apparent permeability coefficients (P^{app}) of fatty acids as a function of chain length, unstirred layer thickness (δ) and pH. $P^{app}=J/[HA]$, which was calculated by Eq. (9), using measured values of P^m, and two different values of δ and pH. $[HA]/[A_T]$ was determined from the pK_a of each acid. Aqueous diffusion coefficients (D_{aq}) were taken from Bidstrup and Geankoplis (1963). For comparison, the true membrane permeabilities (P^m) are shown also (dashed line)

and red cell membranes (Table 2). Thus, P^{app} probably represents P^m over the range of C_3 to C_6. However, the nearly constant values of P^{app} over the C_6 to C_8 range probably reflect an unstirred layer limited permeability as described by Eq. (9). The two orders of magnitude difference between toad bladder, red cell and bilayer permeabilities (Fig. 2) reflects the unusually low permeability of the toad bladder to most nonelectrolytes (Bindslev & Wright, 1976). The intrinsically low permeability also causes the "critical" chain length at which P^{ul} becomes rate limiting to be C_6 to C_8 rather than C_4 to C_7 as suggested by the model curves in Fig. 3, which are based on lipid bilayer permeabilities.

Equilibration Rates in Cells and Tissues

Permeabilities are often calculated from the rates of net uptake of solutes into cell suspensions or isolated tissue. For example, Klocke et al. (1972) measured fatty acid permeabilities of red cells by using a continuous flow system and taking advantage of the rapid dissociation of HA inside the cell. The initial half-time ($t_{1/2}$) for equilibration of pentanoic acid, their most permeant solute, was about 0.02 sec. In isolated intestinal cells the $t_{1/2}$ for octanoic acid efflux was too fast to measure at 0 °C ($t_{1/2}<15$ sec), and propionic acid efflux was too fast to measure at 37 °C ($t_{1/2}=1$ to 2 min at 0 °C; $E_a \simeq 13$ kcal mol^{-1}) (Lamers, 1975). From the published values for intestinal permeabilities, we estimate that all fatty acids larger than pentanoic should equilibrate with $t_{1/2}$'s <20 sec, assuming that about 12% of the cell surface is accessible to fatty acid, pH = 7.4, $T=37$ °C and the unstirred layer thickness is 150 μm (Jackson, Williamson, Dombrowski & Garner, 1978). In contrast, most investigators studying fatty acid uptake by intestinal tissue observe constant and rather slow rates of uptake over sampling periods of 1 to 8 min (e.g., Sallee & Dietschy, 1973; Westegard & Dietschy, 1974; Jackson et al., 1978; Sallee, 1979; Thompson, 1980, 1982, 1983). Furthermore, apparent activation energies for uptake of fatty acids by intestine (C_6 to C_{10}) are very low, i.e. <4.4 kcal mol^{-1} (Sallee & Dietschy, 1973). Nevertheless, all of these investigators argue that the rate-limiting step in fatty acid uptake is diffusion through the plasma membrane. However, this argument assumes either that influx measurements are complete before the tracer concentration in the cytoplasm becomes significant or that fatty acids are removed from the cytoplasmic pool by metabolism and/or sequestration at rates exceeding the transmembrane influx. These assumptions were not verified in any of the above studies on intestine nor in similar studies of fatty acid uptake by diaphragm muscle Schwartz & Dietschy, 1980) and adipocytes (Sherrill & Dietschy, 1975).

Figure 2 shows the permeability patterns for fatty acid uptake by diaphragm muscle; roughly similar patterns were observed in intestine and adipocytes. In every case, the pattern is characterized by a shallow slope which increases with chain length (*see also* Table 2). A major conclusion from these studies is that the cell membrane barrier to fatty acids is relatively polar, similar to isobutanol in its solvent properties (Dietschy, 1978; Schwartz & Dietschy, 1980). However, this permeability pattern, together with the low temperature coefficient, first-order kinetics, constant rates of uptake over several minute incubation periods, and high levels of accumulation of medium- to long-chain fatty acids, suggest that some process(es) other than transmembrane diffusion is (are) limiting the rate of uptake of most fatty acids.

That the cell membrane is not rate-limiting for uptake of C_{10} to C_{16} fatty acids was clearly shown by DeGrella and Light (1980*a*, *b*), who studied suspended rat heart myocytes. These authors were able to identify saturable (metabolic) and nonsaturable (nonmetabolic) components of total uptake. The saturable components (oxidation and esterification) were independent of chain length. The nonsaturable component was accumulation of free fatty acids into a hydrophobic region of the cell, deduced from the $\delta\Delta G/-CH_2-$ of -650 cal mol^{-1}. Only at very low concentrations (0.1 μM) or low temperature was the total uptake rate apparently limited by transmembrane diffusion. From their uptake rates at low concentrations we estimate a minimum "permeability" for decanoic acid of approximately 200 nmol min^{-1} (mg protein)$^{-1}$ which is 2 to 4 orders of magnitude higher than the values reported for isolated adipocytes, intestine and muscle (Sallee & Dietschy, 1973; Sherrill & Dietschy, 1975; Jackson et al., 1978; Schwartz & Dietschy, 1980; Thompson, 1982).

The rather low and relatively constant permeabilities of short- and medium-chain fatty acids in intestine, adipocytes and diaphragm muscle (Fig. 2) might suggest unstirred layer-limited uptake. However, Sallee and Dietschy (1973) showed that short-chain fatty acid permeabilities are not altered by the stirring rate. Furthermore, the unstirred layer model predicts a hyperbolic relationship between apparent permeability and chain length (Fig. 3), whereas the observed relationship in cell and tissue uptake experiments is always concave upward (Fig. 2) (Sallee & Dietschy, 1973; Sherrill & Dietschy, 1975; Sallee, 1979; Schwartz & Dietschy, 1980; Thompson, 1980, 1982, 1983). A third argument against the unstirred layer model is that isolated adipocytes show short-chain fatty acid permeabilities about 100 times lower than intestine or diaphragm muscle, although the unstirred layer is much smaller in suspended cells than in intestine or muscle (Sallee & Dietschy, 1973; Sherrill & Dietschy, 1975; Schwartz & Dietschy, 1980). Thus, we conclude that unstirred layers alone cannot explain the permeability pattern shown in Fig. 2. However, unstirred layer effects do become significant as chain length increases (Jackson et al., 1978; Schwartz & Dietschy, 1980; Thompson, 1980). Unfortunately, the unstirred layer corrections used by Dietschy, Thompson and coworkers are inadequate because (1) they neglect the effects of chemical reactions in the unstirred layers as described by Eq. (3) and (2) their solutions are buffered primarily by CO_2/HCO_3^-, which reacts too slowly to ensure a constant unstirred layer pH in the presence of net acid/base transport (Gutknecht, Bisson & Tosteson, 1977).

The uptake rates of fatty acids by cells and tissues during short-term incubations are probably controlled by a combination of metabolic and nonmetabolic processes which result in shallow slopes and increasingly negative values of $\delta\Delta G/-CH_2-$ as chain length increases (Fig. 2). Very small fatty acids (C_2 and C_3) may be absorbed as A^- as well as HA (Lamers, 1975), which tends to elevate the short-chain part of the curve. Unstirred layer effects become increasingly important for medium-chain fatty acids, which tend to depress the midportion of the curve. Long-chain (C_{12} to C_{18}) fatty acids show $\delta\Delta G/-CH_2-$ values approaching -700 cal mol^{-1} (Table 2). However, these values were obtained with micellar solutions which "facilitate" fatty acid diffusion through the external unstirred layer (Sallee, 1979). The increasingly negative $\delta\Delta G$ values for fatty acids longer than C_5 are probably due primarily to increasing amounts of nonmetabolic accumulation in membranes and other hydrophobic sites in the cell. Using the uptake rates of Sallee and Dietschy (1973) or Jackson et al. (1978) and the cell water estimates of Lamers and Hulsmann (1975), we calculate that for fatty acids longer than C_5 the intracellular "concentration" will exceed the extracellular concentration within 5 min. Lamers and Hulsmann (1975) made similar calculations for octanoic acid in isolated intestinal cells. Nonmetabolic domination of long-chain fatty acid uptake was clearly demonstrated in isolated intestinal cells in which the rate of palmitate accumulation during 5-min incubations was not affected by metabolic inhibitors or even by killing the cells (Haag, Bierbach & Holldorf, 1976). Although the exact sites of cellular accumulation are not known, it seems clear that nonmetabolic binding, not transmembrane diffusion, dominates the uptake of most medium- and long-chain fatty acids, especially at millimolar concentrations which are much higher than the K_m's for metabolic product formation (DeGrella & Light, 1980*a*, *b*).

Conclusions

Fatty acid permeation through lipid bilayer membranes is due to nonionic (HA) diffusion

and is proportional to the hexadecane/water partition coefficient for HA, similar to the permeability pattern of other nonelectrolytes in lipid bilayers. Biological membrane data are consistent with the hypotheses that (1) diffusion of HA is the primary mechanism for fatty acid uptake and (2) the lipid barrier is similar in polarity to synthetic bilayer membranes. The great discrepancies among published values for permeabilities, permeability patterns and values of $\delta\Delta G/-CH_2-$ for fatty acids are due primarily to the hydrophobic nature of these solutes. This causes unstirred layer limitations of uptake rates and nonmetabolic accumulation in membranes and other hydrophobic regions of the cell. Fatty acids are anabolic as well as catabolic substrates. Thus, they accumulate due to biochemical processes that may be mistaken for membrane-limited uptake. In complex tissues such as intestine and muscle, the true membrane permeabilities to most fatty acids cannot be measured. However, fatty acid permeabilities and permeability patterns in lipid bilayers can serve as useful starting points for studies of fatty acid transport in complex biological membranes and tissues.

We thank Dr. Ramon Latorre for the use of his laboratory at the Harvard Medical School, Dr. Sidney Simon for helpful advice and discussions, and Dr. David Keifer for reviewing an early version of the manuscript. This work was supported by National Institutes of Health grants GM 28844 and ES 02289.

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Received 26 April 1983; revised 11 July 1983