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Facilitated Diffusion and Membrane Permeation of Fatty Acid in Albumin Solutions

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Abstract

Facilitated transport is characteristic of most living systems, and usually involves a series of consecutive adjacent transfer regions, each having different transport properties. As a first step in the analysis of the multiregional problem, we consider in a single unstirred layer the facilitated diffusion of fatty acid (F) in albumin (A) solution under conditions of slow versus rapid association–dissociation, accounting for differing diffusivities of the albumin–fatty acid complex (AF). Diffusion gradients become established in an unstirred layer between a source of constant concentration of A , AF , and F in equilibrium, and a membrane permeable to F . The posited system does not reduce to a thin- or thick-layer approximation. The transient state is prolonged by slower on/off binding rates and by increasing the thickness of the unstirred layer. Solutions to transient and steady state depend the choice of boundary conditions, especially upon for thin regions. When there are two regions (each with its specific binding protein) separated by a permeable membrane, the steady-state fluxes and concentration profiles depend on the rates of association and dissociation reactions, on the diffusion coefficients, local consumption rates, and on the membrane permeability. Sensitivity analysis reveals the relative importance of these mechanisms.

Keywords

Palmitate–albumin binding; Membrane transport; Blood–tissue exchange processes; Plasma protein; Capillary permeability; Cytosolic carrier protein; Boundary layer fluxes

INTRODUCTION

Facilitated, or carrier mediated, diffusion mechanisms play a crucial role in accelerating transport across membranes and through intracellular regions in many biological transport systems. All these transport processes involve a series of passive semipermeable or active barriers that separate the different regions, and each region has typical components such as protein carriers, catalytic or synthesizing enzymes, and reaction products or metabolites. Commonly, one or more of the regions is unstirred, and if the solute binds or adsorbs to a protein, solute flux depends on the mobility of the binding protein.^{3,14,28,40,43} Classes of solutes that are transported in a bound, nontoxic form are those that might cause damage if in the free form, e.g., calcium inside cells or solutes are carried on specialized proteins in the plasma and released only via localized receptors, e.g., retinone and testosterone. Common examples, considering primarily the diffusional facilitation, are free fatty acid transport from

the plasma through the endothelial cell and the interstitial tissue to the myocardial cell, oxygen diffusion in intracellular hemoglobin (Hb) and myoglobin (Mb) solutions, and calcium diffusion in parvalbumin solutions.

Fatty acid transport in the heart will serve as a focal example³⁶ for our model analysis. Over 99% of the fatty acid in the plasma is bound to albumin; this binding reduces the concentration of free fatty acid to levels that do not damage cell membranes. Both free and albumin-bound fatty acid diffuse through the stagnant plasma layer near the capillary wall. The fatty acid is separated from albumin at the endothelial membrane, perhaps via special receptor/transporter sites,³⁶ transferred across the membrane and released into the cytosol of endothelial cells where it is complexed with another binding protein, fatty-acid binding protein (FABP). Although we once thought that there was no significant concentration of fatty-acid binding protein in cardiac endothelial cells,¹⁸ more recent evidence for it is clear.¹⁹ Transport via clefts between endothelial cells is thought to be negligible because albumin cannot pass through the clefts and the fraction of fatty acid which is free is so small.⁴ Next, the fatty acid is transferred across the abluminal endothelial membrane to the interstitial space, where it is again bound to albumin. Finally, it permeates the myocyte sarcolemma and enters the myocyte cytosol where it attaches to cardiac cell fatty-acid binding protein or is chemically transformed. Because such a high fraction of fatty acid is continuously bound during each of these stages, the contribution of bound fatty acids to the amount of fatty acid being transferred far exceeds that of the free form despite the slower diffusion of the bound forms.

Indicator dilution experiments by Rose and Goresky²⁴ demonstrated for palmitic acid high rates of unidirectional flux from blood to tissue in the hearts of anesthetized dogs. Van der Vusse *et al.*³⁵ found that transport from the blood was a saturable process, such that the fractional flux (moles transported per mole available in the blood) diminished as the concentration of the albumin-fatty acid complex in the blood increased. Although Weisiger, Pond, and Bass⁴⁰ argued that this might be due to interactions between fatty acids and free sites on albumin in an unstirred buffer layer adjacent to the endothelial wall, this seems less likely than saturation of receptor sites for the albumin-fatty acid complex, sites that are found in abundance on endothelial surfaces.^{10,12,25,26} The conductance or permeability-surface area product for the transport of unbound fatty acid across the endothelial luminal membrane was found to be about $6 \text{ ml g}^{-1} \text{ min}^{-1}$,³⁵ a value too small to account for more than a small fraction of the total fatty acid flux in the heart, where the extraction is usually 50% or so, up to 70%.^{2,8,17} Bassingthwaite *et al.*⁴ and Van der Vusse *et al.*³⁵ reached the working conclusion that intraregion facilitated diffusion (of free and bound fatty acid) and an additional mechanism at the membrane favoring the unbundling of fatty acid from the protein were both critical to achieving the high fluxes observed *in vivo*.

The working hypothesis that a membrane acceptor site is involved^{4,38} accords counter to that of Sorrentino *et al.*,³² Fleischer *et al.*,⁹ Weisiger and Ma,³⁹ Weisiger *et al.*,^{40,41} and Zakim,⁴⁴ whose experiments and analyses led them to conclude that no membrane protein was needed to facilitate the dissociation of the fatty acid from albumin at the membrane, but emphasized that the rate of dissociation was limiting. The equilibrium dissociation constant observed by Spector *et al.*³³ for palmitate on albumin's highest affinity site was $3 \times 10^{-8} \text{ M}$. Lower affinity sites carry a small fraction of the fatty acid unless the molar ratio of fatty acid to albumin exceeds 0.1 (see Refs. 33 and 41), while a typical physiological ratio is about 1, so more than one site is usually occupied. The rates of dissociation were found to be 0.06 s^{-1} at 25 °C by Svenson *et al.*³⁴ and 0.14 s^{-1} at 37 °C by Weisiger and Ma.³⁹ These values imply that the binding reaction or association rates are about $0.2\text{--}0.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. The observations of Daniel *et al.*⁷ give even slower rates of dissociation from albumin, e.g., 0.035 s^{-1} for palmitate.

The processes that govern fatty acid transport within different regions and across membrane surfaces are sufficiently complex that quantifying descriptions, mathematically defined hypotheses, are needed to elucidate the possible and probable ranges of parameters of the physicochemical system. The need to account for unstirred layers of solution adjacent to membranes is clear.^{6,13,14,28} Clearly, models assuming instantaneous equilibrium dissociation between plasma fatty acid and albumin in the unstirred layer or at the membrane surface cannot be used to assess the effects of slow association and dissociation. The conclusion of Van der Vusse *et al.*³⁵ that a membrane receptor should be considered was based on the experimental observation that the transport of free unbound fatty acid was too low to account for fluxes observed in the presence of albumin.

Such arguments are dependent on modeling analysis. A number of relevant models are of interest. Weisiger *et al.*⁴⁰ presented an analytical solution to the one-dimensional steady-state problem. Assuming that the protein is in great excess so that its concentration is practically constant, they had only two independent variables to deal with: the concentrations of the free and the bound ligands. In another work, Weisiger *et al.*⁴¹ have formulated an extended perfusion model of the hepatic sinusoid for those two independent concentrations (in their experiments the oleate to protein molar ratio equals 0.1). Schwab and Goresky^{27,28} have used Weisiger *et al.*'s equations and boundary conditions in modeling diffusion into a polyethylene sheet,⁴¹ and in formulating the effect of unstirred layer on the uptake of the ligand in the liver.²⁸ They formulated the transient case for the free and bound forms of the ligand. Sen²⁹ dealt with the problem of carrier-facilitated transport through thin films (in the oxyhemoglobin or myoglobin system) by assuming, as did Weisiger *et al.*,⁴⁰ that the carrier is not an independent variable, thus reducing the model to two equations. Sen's asymptotic expansions are not applicable for a thick layer (the plasma) and, as suggested by Wittenberg and Wittenberg⁴³ one cannot settle for thin-layer approximations when dealing with physiological systems. Gonzalez-Fernandez and Attal¹¹ used a numerical approach to a similar problem, employing a piece-wise linearization of the equations. Jacques¹⁵ and Smith *et al.*³⁰ developed numerical and asymptotic methods for the steady-state diffusion problem. Their solution is not restricted to a thin layer, but accounts for only two diffusion coefficients: for the free ligand and for the complex. These models are inappropriate when the protein/carrier concentration is an independent variable that changes with position and time, whether or not its diffusion coefficient differs from that of the bound complex. Considering the fatty acid transport problem, we note that the typical parameter values of this system fall between those for the very thin and the very thick layer (asymptotic cases). Consequently, the expansions that are cited for these two extreme cases are not exactly appropriate. Asymptotic expansions for the steady-state facilitated diffusion through thick slab have been given by Ward,³⁷ arbitrarily given by Murray,²² and by Mitchell and Murray.²⁰ All these solutions are based on the assumption that there are only two independent variables. Kolkka Salathé¹⁶ presented a general solution for the steady-state diffusion in a single region, but did not tackle the two-region problem considered here. Schwab and Goresky²⁸ extended their approach and that of Weisiger *et al.*⁴¹ by considering the problem in the context of a gradient in ligand concentration along the length of a capillary.

The present study focuses on time-dependent numerical solutions. The reasons are to allow the computation of transient states and to show a simple approach that can be readily extended to more complex situations such as combinations of boundary-layer facilitated diffusion and specialized membrane transport systems.

Most of the published theoretical analyses deal only with one-region diffusion, but in the biological setting there is transfer through regions in series. Bassingthwaite, Wang, and Chan⁵ considered a multiregion diffusion problem, the mass transport in a capillary-tissue region composed of four radially ordered domains. They assumed that the convection in the

longitudinal direction is the dominant mechanism, with axial diffusion in all regions, and neglected variation in concentrations arising from diffusion in the radial direction. These radial variations within the different regions and the phenomena that occur at the membranes are considered in this paper.

The mechanisms that govern mass transport through the various regions may be different: different binding proteins, different reaction rates with binding sites, or of consuming reactions, etc. Consequently, their relative importance changes. Most of all, the controlling mechanisms of adsorption at the membranes' surfaces and transport the membrane across vary.^{9,31,32,38} Gutknecht *et al.*¹³ showed that transmembrane transport of bromine was via the unionized form but that, like albumin-bound *F*, the diffusional transport of the ionized form in the stagnant layer contributed greatly.

This composite situation calls for the formulation of a general type of model made of building blocks that can be used to simulate different kinds of domains by substituting the appropriate parameters, yet simple enough so as to be connected to other building blocks and create a continuous chain of domains, each with its particular mass transport mechanisms. To this end, we extend from a one-region model to consider two adjacent regions, each with its different proteins and diffusion constants. The mass transfer at the membrane separating the two domains affects the concentration profiles within the regions, and alternative boundary conditions are therefore studied. Here we simulate a simple linear transport through the membrane, whereby the flux is proportional to the concentration gradient. This provides a basis for subsequent developments involving carrier-mediated membrane transport of membrane-receptor mediated events.

MODEL FORMULATION

The present modeling is intended to demonstrate the relative importance of the different mechanisms involved, and serves as a building block for more advanced models. We start with what seems to be a very simple problem of transport in a "slab" of solution containing three species, i.e., albumin (*A*), free fatty acid (*F*), and their complex (*AF*), with concentrations C_A , C_F , and C_{AF} , respectively. This layer is open on one side ($x=0$) to a bulk fluid where the supply of constituents comes from and is bound at the other end ($x=x_1$) by a membrane with permeability *P*. The concentration profiles of the constituents in this layer are determined by association and dissociation reactions, by the diffusion of all three species through the slab, by local consumption, and by the membrane permeability. Next, we deal with the two-region model, where a second region that contains binding protein (*B*), free fatty acid and their complex (*BF*) receives *F* by permeation from the first regions, and loses *F* across a second membrane at the far end at x_2 . The first region may be considered the plasma unstirred layer and the second the endothelial cell. Numerical results and sensitivity analysis follow the formulation of the models.

The System of Equations for the One-Region Model

The unsteady-state set of diffusion equations for the concentrations of the three constituents is given by the following set of partial differential equations in the range of $0 < x \leq x_1$:

$$\frac{\partial C_A}{\partial t} = D_A \frac{\partial^2 C_A}{\partial x^2} - k_{+1} C_A C_F + k_{-1} C_{AF}, \quad (1)$$

$$\frac{\partial C_F}{\partial t} = D_F \frac{\partial^2 C_F}{\partial x^2} - k_{+1} C_A C_F + k_{-1} C_{AF} - G_1 C_F, \quad (2)$$

$$\frac{\partial C_{AF}}{\partial t} = D_{AF} \frac{\partial^2 C_{AF}}{\partial x^2} + k_{+1} C_A C_F - k_{-1} C_{AF}, \quad (3)$$

where D_A , D_F , and D_{AF} are the diffusion coefficients of A , F , and AF , respectively; k_{+1}, k_{-1} are the association and dissociation rate constants. Equation (2) accounts for the linear, first-order metabolic consumption of F in the slab, with G_1 denoting the metabolic rate constant. Parameter values follow those of Weisiger *et al.*⁴⁰ and are listed in Table 1.

Note that if we assume, as has been assumed in the past,^{11,15,27-29,40,43} that $D_A = D_{AF}$ then the total albumin concentration satisfies the diffusion equation; given the proper boundary conditions we can prove that the total albumin concentration is constant throughout the whole region of solution, thus allowing an analytical simplification of the system of equations, but this cannot be done in the general case presented by Eqs. (1)–(3). However, by existence and uniqueness theorems, the system of Eqs. (1)–(3) has a unique solution that can be obtained by numerical procedures once the three initial conditions and six boundary conditions are provided.

Boundary Conditions

At $x=0$, i.e., at the interface with the bulk fluid which serves as a source for the various constituents, a steady supply of protein and fatty acid coexist in an equilibrium state, and the concentrations are constant:

$$C_F(0, t) + C_{AF}(0, t) = C_{FT}, \quad (4)$$

$$C_A(0, t) + C_{AF}(0, t) = C_{AT}, \quad (5)$$

$$\frac{C_A(0, t) C_F(0, t)}{C_{AF}(0, t)} = k_{d1} = \frac{k_{-1}}{k_{+1}}, \quad (6)$$

where C_{FT} and C_{AT} are the total concentrations of fatty acid and of albumin at the source, which is determined by the experimental conditions.

Equations (4)–(6) uniquely determine the concentrations at the source [actually, they result in a quadratic equation for $C_{AF}(0, t)$ but since $C_{AF} \leq C_{AT}$, the alternative root is excluded].

At $x=x_1$, the membrane is impermeable to both of the proteins, A and AF , and thus each of them has zero flux into or out of the membrane. Following Weisiger *et al.*⁴⁰ we call these the “no flux” boundary conditions, meaning that there is no gradient for diffusional flux at the boundary:

$$D_A \frac{\partial C_A}{\partial x} \Big|_{x=x_1} = 0, \quad (7)$$

$$D_{AF} \frac{\partial C_{AF}}{\partial x} \Big|_{x=x_1} = 0. \quad (8)$$

Note that the above two conditions imply that the products of the chemical reactions taking place at the membrane face are being “carried” by diffusional processes yielding no net protein flux.

For the transport of F we consider two optional cases:

Case 1: A finite leak of F at $x=x_1$, hence

$$D_F \frac{\partial C_F}{\partial x} \Big|_{x=x_1} = -P C_F(x_1, t). \quad (9)$$

Case 2: A complete sink of F at $x=x_1$, hence

$$C_F(x_1, t) = 0, \quad (10)$$

where P is the permeability constant. Obviously, Case 2 is a special solution of Case 1 when $P \rightarrow \infty$.

Initial Conditions

At $t=0$:

$$C_A(x, 0) = C_A(0, 0), \quad (11)$$

$$C_{AF}(x, 0) = C_{AF}(0, 0), \quad (12)$$

$$C_F(x, 0) = C_F(0, 0) [1 - x/x_1]. \quad (13)$$

These are arbitrary, given that at $x=0$ Eqs. (4) to (6) are satisfied, and could just as well be replaced by using zero concentrations throughout the slab. The choice of the linear distribution in Eq. (13) does not need justification since the steady-state solution is not affected by the initial distribution of the constituents but only by the conditions at the boundaries (see discussion in the Sensitivity Analysis section).

Metabolic Consumption of the Substrate

Kolkka and Salathé¹⁶ have assumed a zero-order constant metabolic consumption across the slab. This assumption can introduce a mathematical difficulty when the substrate concentration is very low. Actually, a consumption function $G(C_F)$, which is $G_1 \cdot C_F$ in Eq. (2), might have one of the following forms:

1. Zero order, $G(C_F)=G_0$.
2. First-order rate constant, $G(C_F)=G_1 \cdot C_F$.
3. First-order Michaelis–Menten type reactions, $G(C_F)=V_{\max} \cdot C_F/(K_m+C_F)$. At very high C_F/K_m the Michaelis–Menten form is zero order; at very low C_F/K_m it is first order.
4. A more complicated option is to replace the above irreversible reaction with a reversible one of the following type:

where E and M are the enzyme and the metabolite. This requires accounting for E and EF at each position, and the original system of three equations is extended to five equations by adding the following equations:

$$\frac{\partial C_E}{\partial t} = (k_{-1}^- + k_{-2}^-) C_{EF} - k_{+1}^- C_E C_F,$$

$$\frac{\partial C_{EF}}{\partial t} = k_{+1}^- C_E C_F - (k_{-1}^- + k_{-2}^-) C_{EF},$$

and $G_1 \cdot C_F$ in Eq. (2) is replaced with

$$-k_{-1}^- C_{EF} + k_{+1}^- C_E C_F.$$

Note that two assumptions are associated with the above equations. First, the diffusion of E and EF is negligible. Second, the dissociation of EF to M and E is irreversible.

When $\tilde{k}_{+1}$ and $\tilde{k}_{-1}$ are both fast compared to $\tilde{k}_{-2}$, there is effectively equilibrium dissociation for the binding of F to E to form EF and $\tilde{k}_{-2}$ is the rate-limiting step. Thus, this option reduces to the above Michaelis–Menten reaction with $V_{\max}=E_T \cdot \tilde{k}_{-2}/2$ and $K_m=\tilde{k}_{-1}/\tilde{k}_{+1}$.

Numerical Scheme

We replace the spatial derivatives with finite differences (central, second-order differences) and then apply standard routines to solve the resulting first-order, initial-value problem. The interval $[0, x_1]$ is divided into N subintervals, each of width $\Delta x = x_1/N$ and Eqs. (1)–(3) are

converted to $3N$ ordinary differential equations with $3N$ unknowns: $\{C_{A_i}, C_{F_i}, C_{AF_i}\}_{i=1}^N$

$\{C_{A_0}, C_{F_0}, C_{AF_0}\}$ are needed for the solution of the ordinary differential equations for the first subinterval, and are determined by the concentrations at the source. $\{C_{A_{N+1}}, C_{F_{N+1}}, C_{AF_{N+1}}\}$ are needed for the solution at the membrane face ($i=N$) and are given by writing Eqs. (7)–(10) in a finite difference form:

$$\left\{ \begin{array}{l} C_{A_{N+1}} = C_{A_{N-1}} \\ C_{F_N} = 0 \quad \text{for the sink case} \\ \text{or: } C_{F_{N+1}} = C_{F_{N-1}} - \frac{2\Delta x P}{D_F} C_{F_N} \\ \quad \text{for the finite permeability case} \\ C_{AF_{N+1}} = C_{AF_{N-1}} \end{array} \right\} \quad (15)$$

The equality $C_{A_{N+1}} = C_{A_{N-1}}$ means the boundary is impermeable, or reflecting, and that the gradient for protein is zero.

The first-order stiff initial value problem is solved using `LSODE` or `LSODA` FORTRAN packages which implement backward differentiation formulas, and the resulting linear systems are solved by a direct method (LU factorization).

RESULTS

The steady-state concentration profiles for the sets of parameters in Table 1 are given in Figs. 1 and 2. All the figures demonstrate numerical results that are accurate within a relative error of 10^{-4} for 30 segments and 10^{-5} for 100 segments. Good agreement between results was obtained by using different routines (`LSODE`, `LSODA`, which are freely available Livermore solvers for ODE, and `DIVPAG`, purchasable from `IMSL` library).

The general shape of the concentration profiles is similar for the two types of boundary conditions at the membrane and all initial concentrations: a monotonic increase of C_A when propagating from $x=0$ to $x=x_1$, and a monotonic decrease of C_F and C_{AF} ; C_A and C_{AF} change linearly (except for a narrow layer near but not quite at x_1 , $x=x_1^-$, where their slope is flattened due to the choice of the boundary conditions for A and AF at $x=x_1^-$, zero gradients, to represent the impermeability of the membrane), keeping their weighted sum constant. But the slope of C_F is characterized by a steep decrease near the membrane (as a result of the permeation through the membrane high F fluxes are created). The exact shape of the graphs is a function of the boundary conditions and the parameter values as discussed in the following section.

The steady-state results given in Figs. 1 and 2 were obtained with the numerical scheme written for the transient case. In order to determine *a priori* the time required to reach a steady state, we have estimated the time constants of the various mechanisms involved. These are the maximum of x_1^2/D (the time constant of the diffusion), $1/G_1$ (where G_1 is the consumption of fatty acid when it exists), and the time constant of the reaction terms which is given by

$$\frac{1}{k_{-1}} \max \left[\left(1 - \frac{C_{AF}}{C_A C_F} k_{d1} \right), \left(\frac{C_F}{C_{AF}} - \frac{k_{d1}}{C_A} \right), \left(\frac{C_A}{C_{AF}} - \frac{k_{d1}}{C_F} \right) \right].$$

With the set of parameters given in Table 1 most of the change occurs within the first 30 s. The numerical results verify the estimation of the time constant. This is demonstrated in Fig. 3, where we show the time dependence of C_F for a case of high initial concentrations of all three constituents, which filled the region and were in an equilibrium state until $t=0$, when a sink was presented in the far end. When leakage at the membrane is finite, $P < \infty$, the profiles of all concentrations are less steep because spatial and temporal changes are more moderate. Consequently, the system reaches its steady state sooner than in the sink case.

SENSITIVITY ANALYSIS FOR A SINGLE-REGION SYSTEM

The difficulties involved in performing experiments and obtaining reliable values for the parameters are compounded by the fact that the parameter values are highly dependent on the experimental conditions and may in fact change *in vivo*. Consequently, one must view the accuracy of the parameter values with caution. As Schwab and Goresky²⁸ observed, parameter values estimated by fitting model solutions to data are also dependent on the details of the models. The comparison between the concentration profiles computed by this model and observed physiological concentrations should be done in the light of the following sensitivity analysis.

Dissociation and Association Rate Constants

The effects of these rate constants on the concentrations are estimated while holding constant the value of k_{d1} (the equilibrium constant). By Eq. (6), a change of k_{d1} implies a change of the boundary concentrations at the source [$C_A(0), C_F(0), C_{AF}(0)$] and the sensitivity to these parameters is discussed in Paragraph E below. When k_{d1} is fixed, the boundary (source) concentrations remain the same but the relative contribution of the reaction term varies with the values of k_{-1} and k_{+1} ; as the reactions speed up (as k_{-1} and k_{+1} increase) more AF dissociates near the membrane to “compensate” for the depletion in F by permeation, thus higher values of $C_F(x_1)$ are obtained and the steep gradient in F diminishes, while the gradients for both proteins become steeper. Conversely, for very low reaction rates F is lost at $x = x_1$ but less F is supplied by the AF dissociation, leading to very low $F(x_1)$ values. However, in contrast to the membrane region where the concentrations are governed by the F leakage, near the source the concentrations are determined by the interplay between the diffusion mechanism and the chemical reactions. Therefore, near the source, slower reactions lead to shallower gradients in protein concentrations, and lower net flux of F . These phenomena as well as the high sensitivity of the concentrations with respect to k_{-1} and k_{+1} are demonstrated in Fig. 4.

Diffusion Coefficients

The importance of the diffusion mechanism for the transport of F is reflected by the high sensitivity of the concentrations (and the efficiency of the transport) with respect to the diffusion coefficients of A and AF (Fig. 5). Increasing D 's reduces gradients, while decreasing D 's leads to steeper gradients. This tendency is of course limited, because the concentrations are practically constant for D 's which are 100 times their original values, and further increasing the D 's would not affect the concentrations.

Boundary Conditions at the Membrane ($x=x_1$)

By comparing the continuous versus the dashed curves in Figs. 1 and 2 (moderate P versus infinite P , which is a sink) one deduces that the rate of transport at the membrane affects the shape of the concentration profiles throughout the region, with the largest effects observed near the membrane.

Increasing P above 5 cm s^{-1} results in almost no change in the solution because the permeability is large enough that the membrane behaves like a sink for F and the flux is then limited by the rate of dissociation from $AF \rightarrow F$ (see Table 2). Decreasing P means restricting the loss of F , so the profile of $C_F(x)$ is flatter and $C_F(x)$ is closer to $C_F(0)$. Obviously, the dependence of the solution on P is weak at very low or very high values of P , but is strong for intermediate values, e.g., $0.01 < P < 0.1 \text{ cm/s}$, when flux by diffusion and by permeation are comparable, and when dissociation of F from AF is not rate limiting. For these P values, doubling P gives a reduction in $C_F(x_1)$ to 60%–75% of the previous level, depending on P . When P is very high, so that the permeation is not limiting, then doubling P halves $C_F(x_1)$, so that J_F is not greatly changed and the diffusion of AF becomes the limiting factor.

Initial Conditions

The steady-state solution was found to be insensitive to the choice of the initial conditions, as long as they do not contradict the boundary conditions. When the linear distribution of C_F [Eq. (13)] was replaced by a quadrature (parabolic dependence of C_F on x) the differences became minor and insignificant within less than 5 s. This reflects the tendency of the solution to reach an equilibrium within a short time. Perhaps a more “natural” choice of initial conditions is the following: assume that there is a region that contains only albumin (with C_{AT} concentration) and at a certain moment ($t=0$) we attach this region to the source, i.e., at $x=0$ this region has C_A , C_F , C_{AF} values that are identical to the source equilibrium values, but the rest of the region ($x>0$) is still unaffected, specifically,

$$C_A(x, 0) = \begin{cases} C_A(0, 0) & x=0 \\ C_{AT} & x>0 \end{cases}, \quad (16)$$

$$C_F(x, 0) = \begin{cases} C_F(0, 0) & x=0 \\ 0 & x>0 \end{cases}, \quad (17)$$

$$C_{AF}(x, 0) = \begin{cases} C_{AF}(0, 0) & x=0 \\ 0 & x>0 \end{cases}, \quad (18)$$

where the source concentrations are uniquely determined by Eqs. (4)–(6). Using these initial conditions results in the same steady-state concentration profiles as in Figs. 1-2, the ones computed with Eqs. (11)–(13). The only difference is that now it takes longer to reach the steady state. This could be expected since applying linear C_F decrease at $t=0$ resembles more the final, steady-state situation than Eqs. (16)–(18), which are of the delta function type. Applying Eqs. (16)–(18) for the case of initial high albumin and finite leakage at $x=x_1$ necessitates $T=80$ s for obtaining steady-state concentrations, doubling the time it takes when using Eqs. (11)–(13). For the low albumin case much shorter times are needed.

In conclusion, one can use any initial conditions that satisfy the boundary conditions at $x=0$ and $x=x_1$. The only effect would be on the rate of the convergence of the numerical, transient solution to the steady-state one.

The steady-state solution was reached here in about 35 s, whereas Schwab and Goresky²⁷ found that it took less than 10 s to reach a steady state. This discrepancy between their findings and ours is explained by the strong linkage between the stability of the solution and the values of the parameters: they have used a permeability constant which is 5–6 times smaller than the one we used. Therefore, all changes in their model are less pronounced and steady state is achieved more quickly.

Concentrations at $x=0$

The concentration curves are highly dependent on the concentrations at $x=0$ as is evident from a comparison of Figs. 1 and 2. It is most interesting to determine the effect of the source concentrations on the profiles within the region, because such determinations reveal the extent of the diffusional facilitation by a mobile binding protein. The relative efficiency of the transport of F we define as the fraction of the F supplied at $x=0$ that is transported at x_1 , and for any given P . Figure 6 presents those C_F ratios as a function of C_{AT}/C_{FT*} , demonstrating:

1. A highly nonlinear dependence of the F flux on the protein concentrations. At very low protein concentrations there is practically no facilitation and $C_F(x_1)$ is determined by the diffusion rate of F alone and the permeability of the membrane. Thus, at low protein concentrations when P is very low most of the F is confined within the region yielding high $C_F(x_1)/C_F(0)$, while high P gives high clearance of F and low $C_F(x_1)/C_F(0)$. At high protein concentrations it is more likely that the rate-limiting mechanism is the finite permeability of the membrane, and thus increases of C_{AT}/C_{FT} above 2 have little effect on $C_F(x_1)$. However, for intermediate values of protein concentrations near $C_{AT}/C_{FT} = 1$ the facilitation of the transport of F by the proteins is pronounced. This saturation-like dependence is typical for any system that involves several mechanisms where for each range of parameter values a different mechanism is rate limiting.
2. Because of the nonlinearity of Eq. (6), keeping C_{AT}/C_{FT} constant and raising all concentrations accordingly involves higher $C_{AF}(0)$ at the expense of $C_A(0)$ and $C_F(0)$ and in general, we find that although AF cannot leak through the membrane (therefore its flux there equals zero), the higher C_{AF} is, the more efficient is the F transport. This is shown in Fig. 6, where raising C_{FT} (and C_{AT} in proportion) from 10^{-5} to 5×10^{-4} M results in higher ratios of $C_F(x_1)/C_F(0)$, except for a small range of $C_{AT}/C_{FT} < 1.0$ where the albumin binding reduces the relative efficiency of transport.
3. Facilitation via diffusion of AF is greatest when each F that is dissociated from AF may permeate through the membrane.

Note that physiological systems are often characterized by values of C_{AT}/C_{FT} just greater than 1.0, where facilitation is highly significant.

Metabolic Consumption

Just as for the relative concentrations, the interplay between the various mechanisms determines whether the profiles are highly sensitive with respect to the rate of consumption G_1 within the unstirred layer. From inspection of Eq. (2), whenever $k_{+1} \cdot C_A \gg G_1$, then G_1 has little influence on the flux. For the fatty acid case, k_{+1} is of the order of $10^{10} \text{ moles}^{-1} \text{ ml s}^{-1}$, and at only very low concentrations of A will a small G_1 be a major factor. Consumption of F in the unstirred plasma layer or space of dissociation in the liver is certainly small, but not necessarily zero, for there is uptake into micelles. Thus, for the physiological range of parameters, the consumption is secondary compared to the dissociation mechanisms, and to the permeation process. The sensitivity of the solution to G_1 in the range of $0.05\text{--}0.10 \text{ s}^{-1}$ is small. When the consumption is so high that half of the available F is consumed, there is only an increase of 16% in the slopes $\partial C_A/\partial x$ and $\partial C_{AF}/\partial x$ for the high albumin case. The result at the boundary is that $C_A(x_1)/C_A(0) = 1.159$ instead of 1.137 when there is no consumption; the difference is small because there is enhanced AF dissociation when F is reduced by metabolic consumption.

FORMULATION OF THE TWO-REGION MODEL

The System of Equations

The field of interest $[0, x_2]$, where $x_2 = x_1 + \delta x$ and δx is the thickness of Region II, is partitioned by a membrane at $x = x_1$, and we define Region I to be from the source to x_1 (to the left of the membrane), and Region II, $x_1 < x \leq x_2$ (to the right of the midmembrane). There are different F binding proteins, A in Region I and B in Region II. Free F can permeate the membrane at x_1 in both directions, and can permeate the membrane at x_2 to allow unidirectional loss. Neither A and its complex AF , nor B and its complex BF can cross the membrane.

Equations (1)–(3) describe events in Region I, while in Region II there is a similar set:

$$\frac{\partial C_B}{\partial t} = D_B \frac{\partial^2 C_B}{\partial x^2} - k_{+1} C_B C_F + k_{-1} C_{BF}, \quad (19)$$

$$\frac{\partial C_F}{\partial t} = D_{F_2} \frac{\partial^2 C_F}{\partial x^2} - k_{+1} C_B C_F + k_{-1} C_{BF} - G_2 C_F, \quad (20)$$

$$\frac{\partial C_{BF}}{\partial t} = D_{BF} \frac{\partial^2 C_{BF}}{\partial x^2} + k_{+1} C_B C_F - k_{-1} C_{BF}, \quad (21)$$

where D_B , D_{F_2} , and D_{BF} are the diffusion coefficients of B , F , and BF respectively; K_{+1} , K_{-1} are the association and dissociation rate constants for BF . The term $G_2 C_F$ indicates first-order metabolic consumption of F in Region II.

Boundary and Initial Conditions

At the source end, Eqs. (4)–(6) apply. At $x=x_1$ we have to consider the two faces of the membrane.

At $x=x_1^-$ (the inner side of the midmembrane): A and AF are confined to Region I, thus Eqs. (7) and (8) still hold. However, Eq. (9) or (10) is replaced by

$$D_F \frac{\partial C_F}{\partial x} \Big|_{x=x_1^-} = -P_1 C_F(x_1^-, t) + P_2 C_F(x_1^+, t). \quad (22)$$

For purely passive transmembrane permeation P_2 will ordinarily be identical to P_1 , but these may differ for many reasons, for example, differences between the pH or potential within the regions. Similarly, at $x=x_1^+$ (outer side of the midmembrane), the impermeability with respect to the proteins is expressed by

$$D_B \frac{\partial C_B}{\partial x} \Big|_{x=x_1^+} = 0, \quad (23)$$

$$D_{BF} \frac{\partial C_{BF}}{\partial x} \Big|_{x=x_1^+} = 0, \quad (24)$$

and the permeation of F through the membrane at x_1 is given by

$$D_{F_2} \frac{\partial C_F}{\partial x} \Big|_{x=x_1^+} = -P_1 C_F(x_1^-, t) + P_2 C_F(x_1^+, t). \quad (25)$$

At $x=x_2$ (far end of Region II) we write

$$D_B \frac{\partial C_B}{\partial x} \Big|_{x=x_2} = 0, \quad (26)$$

$$D_{BF} \frac{\partial C_{BF}}{\partial x} \Big|_{x=x_2} = 0. \quad (27)$$

Assuming a linear permeability of F through the x_2 membrane, and considering that no F exists behind the membrane so that there is no return flux, yields

$$D_{F_2} \frac{\partial C_F}{\partial x} \Big|_{x=x_2} = -P_3 C_F(x_2). \quad (28)$$

Initial conditions at $t=0$. As was true for the one-region model, the steady-state situation is insensitive with respect to the initial distribution of the constituents in the two-region case as well. One option is to assume that before the diffusion process started we had two regions; each region contained a different type of protein with given concentration (C_{AT} and C_{BT}). Then, at $t=0$, we attached Region I to a source where fatty acids and albumin are in equilibrium state. Thus the initial distribution of the various constituents is

$$\begin{aligned} C_A(x, 0) &= \begin{cases} C_A(0, 0) & x=0 \\ C_{AT} & 0 < x < x_1 \\ 0 & x > x_1 \end{cases} \\ C_F(x, 0) &= \begin{cases} C_F(0, 0) & x=0 \\ 0 & x > 0 \end{cases} \\ C_{AF}(x, 0) &= \begin{cases} C_{AF}(0, 0) & x=0 \\ 0 & x > 0 \end{cases} \\ C_B(x, 0) &= \begin{cases} C_{BT}(0, 0) & x_1 < x < x_2 \\ 0 & x < x_1 \end{cases} \\ C_{BF}(x, 0) &= 0. \end{aligned} \quad (29)$$

The Numerical Scheme

The system of Eqs. (1–3), (19–21) is solved simultaneously using Eq. (29) for initial conditions and Eqs. (4–8), and (22–28) as boundary conditions. The numerical procedure follows the lines described in the Numerical Scheme section, the system of equations is converted to an initial value problem by using central differences instead of spatial derivatives. The initial value problem is solved efficiently by the LSODE routine (a routine that implements backward differentiation formulas). Since there is bidirectional flux of F across the first membrane the systems of equations in the two regions are coupled and must be solved simultaneously.

RESULTS

The numerical results are obtained by using the parameter values listed in Table 1 for Region I and those listed in Table 3 for Region II. The C_{AT} and C_{FT} values chosen were for the high albumin case. Here the transient period is longer than the one found for the one-region model, and the steady state is not reached until after a few minutes, confirming the need for the numerical time-dependent solution. Transients occur with changes in flow (not considered

here) or metabolism or changes in the source concentration. This extension of the transient period is expected: the concentrations in Regions I and II cannot reach steady-state level until both $C_F(x_{1-})$ and $C_F(x_{1+})$ stabilize. The duration of the transient period is governed mainly by the thickness of regions, by P_1 , P_2 , and P_3 and by the diffusion and dissociation rates.

When one simulates the transport of F for capillary–tissue exchange within the myocardium the second stagnant region is the endothelial cell, with a typical δx length of $0.4 \mu\text{m}$ (lower boundary of δx in Table 3) for endothelial cells and more than $30 \mu\text{m}$ for some muscle and fat cells. When Region II is thin the gradients in protein concentrations are negligible (Table 4) but are substantial when its thickness is comparable to x_1 (Table 5). Gradients in F are determined mainly by the rate of transport at the two membranes. C_F changes considerably within a region, no matter how narrow it is.

In general, each region is characterized by a monotonic, linear increase of the unbounded protein and monotonic decrease of both free and bounded fatty acids. A discontinuity in C_F occurs at the membrane at x_1 , as in Fig. 7; this discontinuity is always a decrease when $P_1=P_2$ but can be an increase if a concentrating process such that $P_1<P_2$, i.e., if there is asymmetric permeation with the rate from Region II to Region I being less than that from I to II.

The accuracy of the numerical method is evaluated by determining whether or not the fluxes in steady state are independent of x when there is only loss at x_2 (no metabolic consumption).

The total F flux from left to right (or toward higher x) is given by:

$$J_F(x) = \begin{cases} \left| D_F \frac{\partial C_F}{\partial x} + D_{AF} \frac{\partial C_{AF}}{\partial x} \right| & 0 < x < x_1 \\ -P_2 C_F(x_1^+) + P_1 C_F(x_1^-) & x = x_1 \\ \left| D_{F_2} \frac{\partial C_F}{\partial x} + D_{BF} \frac{\partial C_{BF}}{\partial x} \right| & x_1 < x < x_2 \\ P_3 C_F(x_2^-) & x = x_2 \end{cases} \quad (30)$$

The ratio $[\max J_F(x) - \min J_F(x)]/J_F(x_2)$ is a measure of the accuracy of our numerical solutions. We found that the ratio of flux variation to the mean flux of $J_F(x)$ is 10^{-4} at most, meaning that the flux of F is virtually constant (and therefore accurate) and that steady state was reached.

SENSITIVITY ANALYSIS FOR THE TWO-REGION SYSTEM

The conclusions drawn in Section 4 regarding the sensitivity of the concentrations with respect to the reaction rate constants, coefficients of diffusion, the initial conditions and the consumption reactions, all remain valid here as long as the thickness of the regions is comparable to that considered there ($50 \mu\text{m}$) and the sensitivity of the concentrations is similar to that found for Region I where a change of parameters of one region affects the other region as well. When we put $x_2 - x_1$ at $0.1 \mu\text{m}$, as for the thinnest part of the endothelial cell, the sensitivity with respect to reaction rates, diffusion coefficients and metabolic consumption within Region II is diminished.

Sensitivity with Respect to P_1

Increasing P_1 means that more F is transferred from Region I to Region II. The asymmetry, $P_1 > P_2$, will lead to a situation where C_F is higher in Region II than in Region I, as seen for example in column three of Table 4, unless losses by G_2 or P_3 compensate. In general, increasing P_1 makes the gradients of all concentrations in both regions steeper; more F permeates the membrane at x_1 , and the gradient of C_F increases, thus more of $C_F(x_1^-)$ is lost,

and this causes an enhanced AF dissociation that leads to steeper gradients of both C_A and C_{AF} . Much more F is available for Region II, which means higher C_{BF} and lower C_B (due to the high affinity of the fatty acids and the binding protein: when there is higher C_F the protein tends to be in a bound form). However, when δx for Region II is large (e.g., $40\ \mu\text{m}$), then raising P_1 leads to steeper gradients of C_B and C_{BF} as well. As for the one-region case, when P_1 is sufficiently high the sensitivity with respect to it diminishes but within the physiological range of parameters there is a very high sensitivity with respect to P_1 . For example, increasing P_1 tenfold causes four-times steeper gradients within Region I, almost five times higher values of C_F at Region II and a 60% increase in the fraction of bound protein concentrations within Region II ($C_{BF}/C_{BT}=84\%$ for the high P_1 case compared to $C_{BF}/C_{BT}=53\%$).

Sensitivity with Respect to P_2

Increasing P_2 allows more F to return to Region I from Region II, thus reducing the gradients of all concentrations. The reason is straightforward: when there is less net flux of F from I to II, C_F in Region I is higher and $\partial C_A/\partial x$ is smaller and the gradients in A and A_F are likewise reduced. In Region II, since C_F is relatively low, most of the binding protein B remains in its unbound form. As was the case with P_1 , the high sensitivity with respect to P_2 also depends on the range of parameter values, and saturation occurs when P_2 is above a certain level ($P_2 \cong 1\ \text{cm/s}$ and $P_1 = P_3 = 0.00833\ \text{cm/s}$ means that almost all F moles that are transferred to Region II return to Region I, and a further increase of P_2 would not affect the concentrations).

Sensitivity with Respect to P_3

Increasing P_3 increase the net flux of F from left to right at all positions, x , thus increasing gradients of all concentrations. The three permeability parameters have different effects. Increasing P_2 or P_3 lowers C_F values in Region II (because the leakage of F from Region II is enhanced). While high P_2 enhances flux from II to I and reduces the gradients within Region I, raising P_3 has the opposite effect, because depletion of F within Region II increases the flux of F from I to II causing steeper gradients in Region I. In this sense P_3 plays the same role as P_1 does (but to a lesser extent because of the barrier resistance at x_1). Within Region II, P_3 and P_1 have opposite effects: increasing P_3 leads to a higher degree of BF dissociation (in order to replenish the F that is “lost” at x_2), and thus reduces C_{BF} . Increasing P_1 leads to higher values of C_{BF} because more F enters Region II.

DISCUSSION

We have presented a simulation of mass transfer by facilitated diffusion mechanisms in one and two-region systems. This model represents an extension of the one region, two variable, no metabolic consumption, steady-state model of facilitated diffusion (e.g., Refs. 28 and 40). We have, however, not extended the model as they did to account for intracapillary axial gradients from inflow to outflow but focused here on the local radial gradients. The multiregion model may also have to account for receptors and carriers within the membranes instead of the simple passive transport at the membrane that is modeled here. Ichikawa *et al.*¹⁴ obtained experimental data showing that if the membrane permeability to the ligand is low, the albumin contribution to the flux is negligible. Such might be the case for fatty acid, for which the myocardial endothelial permeability appears modest,³⁵ leading us, unlike Ichikawa *et al.*, to believe that there may be an additional mechanism at the membrane for releasing the fatty acid from the albumin.

We believe that the way to elaborate the more complex phenomenon is by formulating a series of models of increasing complexity, starting with this one, because:

1. One can achieve valuable information and understanding from studying the simpler models. For example, the dependence of the concentrations on the reaction rates or

on the coefficients of diffusion found for the one region, simple case remains valid for the two-region case and is expected to be true also in cases where more complicated, carrier-mediated transport occurs at the membrane.

2. Comparing one model variation with another reveals the importance of the factors that are accounted for in one model and ignored by the other one. Thus, the relative importance of the various mechanisms and simplifying assumptions can be elucidated.
3. The analysis demonstrates that a simple model can serve as a building block for a more complicated case. Thus, a straightforward extension of the system of differential equations enables the numerical solution for the n -region problem. Likewise, replacing $G \cdot C_F$ with any other function of C_F enables us to apply any consumption reactions, replacing the boundary condition with another that accounts for carriers enables more accurate simulation of the transport within the membrane. All this is accomplished by using the same methodology.

In addition, applications to other than fatty acid facilitated transport systems are through finding the appropriate parameters. Sensitivity analysis can help determine *a priori* how the system under consideration will react and in what ways it will be different from the free F “study case.” It might also help in designing experiments because it gives us guidance as to which are the most important parameters, e.g., from our analysis it is clear that determination of the diffusion coefficients within Region I is crucial, but as long as Region II is very narrow only a rough estimate of the diffusion coefficients within Region II will do. The insensitivity of the model with respect to the low rates of metabolic consumption means that error in the estimates of the consumption reactions has little effect, and that it is much more important to have accurate measures of the dissociation rate constants.

The parameter values selected for the conditions described here are taken mainly from those considered by Weisiger *et al.*⁴⁰ for the low albumin example case and of Van der Vusse *et al.*³⁵ for the high albumin case. The latter case, with $C_{FT}/C_{AT}=0.9$, is in the normal physiological range. Our analyses differ from those of Weisiger *et al.*^{40,41} in the way C_F is calculated; they used an approximation suitable for equilibrium at low molar ratios only, $C_F/(C_{AF}+C_F)=1/(1+C_{AT}/k_d)$, while we account for an exact equilibrium in the source solution for single-site binding. However, we know that Spector *et al.*³³ demonstrated that there were several binding sites for long-chain fatty acids on albumin, raising the question of the adequacy of our single-site assumption. Bojesen and Bojesen⁶ found that they could account well for the first three binding sites, which is quite adequate for the physiological range up to $C_{FT}/C_{AT}=1.5$. They give $C_F=v \cdot k_d/(3-v)$ where $v=C_{FT}/C_{AT}$, the k_d is the reciprocal of their association constant $k_a=2.3 \times 10^8 \text{ M}^{-1}$ or $k_d=4.35 \times 10^{-9} \text{ M}$ or about one-tenth of the value we used in Table 1, and the 3 is for three binding sites. Their estimate at 37°C was $k_d=34 \text{ nM}$, using the three sites. At very low palmitate/albumin ratios it would be better to use their expression $C_F=v \cdot k_d/3$. Likewise, Richieri *et al.*²³ observed experimentally tight binding with an apparent k_d of 30 nM at 37 °C when translated from their data using the expression of Bojesen and Bojesen while accounting for the three sites. The k_d estimated by Richieri *et al.*²³ and Bojesen and Bojesen's⁶ expression for C_F accounting for the first three fatty-acid binding sites on albumin are probably the values to use, i.e., 34 nM at 37 °C, 15 nM at 23 °C, 8 nM at 10 °C, and 4 nM at 0°C.

Our results suggest that:

1. Within each region the concentration of the uncomplexed protein increases monotonically toward the site of loss of F , while the concentrations of fatty acid in both its free and complexed forms decrease.

2. The concentrations of proteins vary linearly except for a narrow layer near the membranes, where they tend toward zero gradients. The sum of the proteins weighted by the diffusion coefficients remains constant with time and with location.
3. The gradient of C_F changes considerably when F approaches the membrane, going from moderate to steep.
4. A steady state is achieved within 0.5 to 3 min depending on the initial conditions and on the parameter values. With two regions of comparable thickness there is a long transient period. Having a time-dependent method of solution is important for dynamic situations.
5. Our steady-state solutions are not sensitive to the initial conditions at all. They are less sensitive to low consumption rates than to reaction rates, diffusion coefficients, permeability constants at the membrane, and concentrations at the source. In a multiregion situation, parameters of the thicker region have a significant effect on the other region, but a very thin region has only a negligible effect on its adjacent region.
6. The interplay among all the parameters determines the overall sensitivity of the concentrations, e.g., the threshold level that designates the saturation with respect to the permeability constant changes with the change of the source concentrations, or the sensitivity with respect to the diffusion coefficients diminishes when the length of the region is shortened etc.
7. Facilitation of fatty acid flux by diffusion of AF increases the flux of F by 50–300 times while maintaining low concentrations of free fatty acid in order to prevent damage to cells.

The most natural extension for these models is one that simulates more accurately the transport across the membrane. We feel from the data of Van der Vusse *et al.*³⁵ that it is most likely that there are albumin receptors on the membranes which facilitate the release of F from AF there. There is abundant discussion and debate on this point, nicely reviewed by Weisiger⁴² and extended by Schwab and Goresky.²⁸ Expressing a viewpoint opposite to ours, Morgan *et al.*²¹ show that the flux of ligand across an inert membrane between two compartments (mixing chambers without diffusion gradients) was constant over a wide range of total ligand concentrations at constant ligand/albumin ratio, and that the fraction of ligand permeating diminishes as the albumin concentration is increased. Our present model will give these results by setting the diffusion coefficients and k_{d1} to high values. While these analyses give results directionally similar to the observations of Weisiger *et al.*⁴⁰ and to those of Van der Vusse *et al.*³⁵ the absolute fluxes are quantitatively inadequate if the observations of Van der Vusse *et al.*³⁵ on the values of P are correct. The nature of the linkage between the carriers and the receptors or transporters is still unknown. Do the receptors carry F through the membrane or do they simply strip the F from AF , raising the local concentration above the equilibrium level? A future investigation of these issues is critical to the understanding of fatty acid transport.

Note added in proof: To obtain the equilibrium concentrations at the source, given C_{AT} , C_{FT} , and k_{d1} , one solves a quadratic. Defining $a = -(C_{AT} + C_{FT} + k_{d1})$ and $b = C_{AT} \cdot C_{FT}$, then at

equilibrium, $C_{AF} = 0.5 \left(-a + \sqrt{a^2 - 4b} \right)$; but if $C_{AF} > C_{AT}$ or $C_{AF} > C_{FT}$, then $C_{AF} = 0.5 \left(-a - \sqrt{a^2 - 4b} \right)$. Then $C_A = C_{AT} - C_{AF}$ and $C_F = C_{FT} - C_{AF}$.

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REFERENCES

1. Antohe F, Popov D, Radulescu L, Simionescu N, Borchers T, Spener F, Simionescu M. Heart microvessels and aortic endothelial cells express the 15 kDa heart-type fatty acid-binding proteins. *Eur. J. Cell Biol* 1998;76:102–109. [PubMed: 9696349]
2. Ballard FB, Danforth WH, Naegle S, Bing RJ. Myocardial metabolism of fatty acids. *J. Clin. Invest* 1960;39:717–723. [PubMed: 13796251]
3. Bass, L.; Pond, SM. The puzzle of rates of cellular uptake of protein-bound ligands. In: Pecile, A.; Rescigno, A., editors. *Pharmacokinetics*. Plenum; New York: 1988. p. 245-269.
4. Bassingthwaighte JB, Noodleman L, van der Vusse GJ, Glatz JFC. Modeling of palmitate transport in the heart. *Mol. Cell. Biochem* 1989;88:51–58. [PubMed: 2674667]
5. Bassingthwaighte JB, Wang CY, Chan IS. Blood-tissue exchange via transport and transformation by endothelial cells. *Circ. Res* 1989;65:997–1020. [PubMed: 2791233]
6. Bojesen IN, Bojesen E. Water-phase palmitate concentrations in equilibrium with albumin-bound palmitate in a biological system. *J. Lipid Res* 1992;33:1327–1334. [PubMed: 1402400]
7. Daniels C, Noy N, Zakim D. Rates of hydration of fatty acids bound to unilamellar vesicles of phosphatidylcholine or to albumin. *Biochemistry* 1985;24:3286–3292. [PubMed: 3839682]
8. Dole VP. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest* 1956;35:150–154. [PubMed: 13286333]
9. Fleischer AB, Shurmantine WO, Luxon BA, Forker EL. Palmitate uptake by hepatocyte monolayers. Effect of albumin binding. *J. Clin. Invest* 1986;77:964–970. [PubMed: 3949983]
10. Ghitescu L, Fixman A, Simionescu M, Simionescu N. Specific binding sites for albumin restricted to plasmalemmal vesicles of continuous capillary endothelium: Receptor-mediated transcytosis. *J. Cell Biol* 1986;102:1304–1311. [PubMed: 3007533]
11. Gonzalez-Fernandez JM, Atta SE. Transport of oxygen in solutions of hemoglobin and myoglobin. *Math. Biosci* 1981;54:265–290.
12. Goresky CA, Stremmel W, Rose CP, Guirguis S, Schwab AJ, Diede HE, Ibrahim E. The capillary transport system for free fatty acids in the heart. *Circ. Res* 1994;74:1015–1026. [PubMed: 8187271]
13. Gutknecht J, Bruner LJ, Tosteson DC. The permeability of thin lipid membranes to bromide and bromine. *J. Gen. Physiol* 1972;59:486–502. [PubMed: 5063846]
14. Ichikawa M, Tsao S, Lin T, Miyauchi S, Sawada Y, Iga T, Hanano M, Sugiyama Y. Albumin-mediated transport phenomenon observed for ligands with high membrane permeability. Effect of the unstirred water layer in the Disse's space of rat liver. *J. Hepatol* 1992;16:38–49. [PubMed: 1484165]
15. Jacquez JA. The physiological role of myoglobin: More than a problem in reaction-diffusion kinetics. *Math. Biosci* 1984;68:57–97.
16. Kolkka RW, Salathé EP. A mathematical analysis of carrier-facilitated diffusion. *Math. Biosci* 1984;71:147–179.
17. Lassers BW, Wahlqvist ML, Kaijser L, Carlson LA. Effect of nicotinic acid on myocardial metabolism in man at rest and during exercise. *J. Appl. Physiol* 1972;33:72–80. [PubMed: 5037413]
18. Linssen MC, Vork MM, de Jong YF, Glatz JF, van der Vusse GJ. Fatty acid oxidation capacity and fatty acid-binding protein content of different cell types isolated from rat heart. *Mol. Cell. Biochem* 1990;98:19–25. [PubMed: 2176272]
19. Massouye I, Hagens G, van Kuppeveldt TH, Madson P, Saurat JH, Veerkamp JH, Pepper MS, Siegenthaler G. Endothelial cells of the human microvasculature express epidermal fatty acid-binding proteins. *Circ. Res* 1997;81:297–303. [PubMed: 9285630]
20. Mitchell PJ, Murray JD. Facilitated diffusion: The problem of boundary conditions. *Biophysik* 1973;9:177–190. [PubMed: 4790858]
21. Morgan D, Stead C, Smallwood R. Kinetic assessment of apparent facilitation by albumin of cellular uptake of unbound ligands. *J. Pharmacokinet. Biopharm*: 1990;18:121–135. [PubMed: 2348379]
22. Murray JD. On the molecular mechanism of facilitated oxygen diffusion by haemoglobin and myoglobin. *Proc. R. Soc. London Biol* 1971;178:95–110. [PubMed: 4397269]

23. Richieri GV, Anel A, Kleinfeld AM. Interactions of long-chain fatty acids and albumin: Determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* 1993;32:7574–7580. [PubMed: 8338853]
24. Rose CP, Goresky CA. Constraints on the uptake of labeled palmitate by the heart: The barriers at the capillary and sarcolemmal surfaces and the control of intracellular sequestration. *Circ. Res* 1977;41:534–545. [PubMed: 902359]
25. Sage H, Johnson C, Bomstein P. Characterization of a novel serum albumin-binding glycoprotein secreted by endothelial cells in culture. *J. Biol. Chem* 1984;259:3993–4007. [PubMed: 6368555]
26. Schnitzer JE, Oh P. Albondin-mediated capillary permeability to albumin. Differential role of receptors in endothelial transcytosis and endocytosis of native and modified albumins. *J. Biol. Chem* 1994;269:6072–6082. [PubMed: 8119952]
27. Schwab AJ, Goresky CA. Free fatty acid uptake by polyethylene: What can one learn from this? *Am. J. Physiol. (Gastrointest. Liver Physiol. 24)* 1991;261:G896–G906.
28. Schwab AJ, Goresky CA. Hepatic uptake of protein-bound ligands: Effect of an unstirred Disse space. *Am. J. Physiol* 1996;270:G869–G880. [PubMed: 8967500]
29. Sen AK. An analysis of carrier-facilitated transport through thin films of hemoglobin and myoglobin. *Math. Biosci* 1988;89:1–7.
30. Smith KA, Meldon JH, Colton CK. An analysis of carrier-facilitated transport. *AICHE. J* 1973;19:102–111.
31. Sorrentino D, Stump D, Potter BJ, Robinson RB, White R, Kiang CL, Berk PD. Oleate uptake by cardiac myocytes is carrier mediated and involves a 40-kD plasma membrane fatty acid binding protein similar to that in liver, adipose tissue, and gut. *J. Clin. Invest* 1988;82:928–935. [PubMed: 3417874]
32. Sorrentino D, Robinson RB, Kiang CL, Berk PD. At physiologic albumin/oleate concentrations oleate uptake by isolated hepatocytes, cardiac myocytes, and adipocytes is a saturable function of the unbound oleate concentration. Uptake kinetics are consistent with the conventional theory. *J. Clin. Invest* 1989;84:1325–1333. [PubMed: 2794064]
33. Spector AA, Fletcher JE, Ashbrook JD. Analysis of long-chain free fatty acid binding to bovine serum: Albumin by determination of stepwise equilibrium constants. *Biochemistry* 1971;10:3229–3232. [PubMed: 5165844]
34. Svenson A, Holmer E, Anderson LO. A new method for the measurement of dissociation rates for complexes between small ligands and proteins as applied to the palmitate and bilirubin complexes with serum albumin. *Biochim. Biophys. Acta* 1974;342:54–59. [PubMed: 4824927]
35. Van der Vusse GJ, Little SE, Reneman RS, Bassingthwaite JB. Transfer of fatty acids across myocardial endothelium. *Am. J. Physiol* 1999;000 (Heart Circ. Physiol. 00).
36. Van der Vusse, GJ.; Glatz, JFC.; Van Nieuwenhoven, FA.; Reneman, RS.; Bassingthwaite, JB. Transport of long-chain fatty acids across the muscular endothelium. In: Richter, EA.; Galbo, H.; Kiens, B.; Saltin, B., editors. *Skeletal Muscle Metabolism in Exercise and Diabetes*. Adv. Exp. Med. Biol. Vol. 441. Plenum; New York: 1998. p. 181-191.
37. Ward WJ III. Analytical and experimental studies of facilitated transport. *AICHE. J* 1970;16:405–410.
38. Weisiger R, Gollan J, Ockner R. Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science* 1981;211:1048–1051. [PubMed: 6258226]
39. Weisiger RA, Ma WL. Uptake of oleate from albumin solutions by rat liver. Failure to detect catalysis of the dissociation of oleate from albumin by an albumin receptor. *J. Clin. Invest* 1987;79:1070–1077. [PubMed: 3031131]
40. Weisiger RA, Pond SM, Bass L. Albumin enhances unidirectional fluxes of fatty acid across a lipid-water interface: Theory and experiments. *Am. J. Physiol* 1989;257:G904–G916. (Gastrointest. Liver. Physiol. 20). [PubMed: 2610260]
41. Weisiger RA, Pond S, Bass L. Hepatic uptake of protein-bound ligands: Extended sinusoidal perfusion model. *Am. J. Physiol. (Gastrointest. Liver. Physiol. 24)* 1991;261:G872–G884.

42. Weisiger, RA. The role of albumin binding in hepatic organic anion transport. In: Tavaloni, N.; Berk, PD., editors. *Hepatic Transport and Bile Secretion: Physiology and Pathophysiology*. Raven; New York: 1993. p. 171-196.
43. Wittenberg BA, Wittenberg JB, Caldwell PRB. Role of myoglobin in the oxygen supply to red skeletal muscle. *J. Biol. Chem* 1975;250:9038–9043. [PubMed: 1194276]
44. Zakim D. Fatty acids enter cells by simple diffusion. *Proc. Soc. Exp. Biol. Med* 1996;212:5–14. [PubMed: 8618951]

FIGURE 1.

Variation of the steady-state concentrations of A , F , and AF for a source with high albumin concentration. Case 1 (solid lines): a membrane at $\tilde{x} = 1$ with finite permeability. Case 2 (dashed lines): a sink at $\tilde{x} = 1$. Values of parameters are given in Table 1.

FIGURE 2.

Variation of the steady-state concentrations of A , F , and AF for a source with low albumin concentration. Case 1 (solid lines): a membrane $\tilde{x}=1$ with finite permeability at the far end. Case 2 (dashed lines): a sink at $\tilde{x}=1$. Values of parameters are given in Table 1. At $\tilde{x}=0.5$ the ratio of fluxes of AF to free F is about 300.

FIGURE 3.

C_F in cases of high albumin concentration at the source and an infinite sink at the far end, for various times. Initial condition was equilibration over $0 \leq x \leq x_1$ with the source, with membrane changed from $P=0$ to $P \rightarrow \infty$ at $t=0$.

FIGURE 4.

C_F in steady state for cases with high albumin concentration at the source and $P=0.833 \times 10^{-2} \text{ cm s}^{-1}$ at the membrane at x_1 for various dissociation rate constants (k_{-1}). k_{d1} is kept constant.

FIGURE 5.

Effect of diffusion coefficients for A and F (when $D_A = D_{AF}$) on steady-state profiles of C_A for cases with high albumin concentration at the source and a finite leakage at x_1 . D refers to the values of the diffusion coefficients for both D_A and D_F specified in Table 1. Other parameters are as in Table 1. Boundary conditions are as in Eqs. (4)-(9).

FIGURE 6.

Transmembrane fatty acid flux as a function of albumin concentration (modeling one ligand binding site per albumin molecule) for two levels of total fatty acid at the source C_{F_T} and two different membrane permeabilities. Other parameters as in Table 1.

FIGURE 7.

Steady-state, normalized concentrations of and the proteins in the C_F two regions of comparable thickness ($x_1=50\mu\text{m}$, $x_2=40\mu\text{m}$). The affinity of F for A is higher, $k_{d1}=3\times 10^{-11}$ moles/ml, than that for protein B , $K_{d1}=10^{-10}$ moles/ml. The permeabilities are $P_1=P_2=P_3=0.008\ 333\ \text{cm/s}$. Concentrations at the boundaries are given in Table 5.

TABLE 1

The parameters used in the present analysis.

Parameter	Comments
$x_1 = 5 \times 10^{-3}$ cm	$x_0 = 0$
$T = 60-100$ s	$t \in [0, T]$
$D_A = 9.35 \times 10^{-7}$ cm ² s ⁻¹	Diffusion coefficient for albumin
$D_{AF} \approx D_A$	Diffusion coefficient for albumin-fatty acid complex
$D_F = 5 \times 10^{-6}$ cm ² s ⁻¹	Diffusion coefficient for unbound fatty acid
$P = 0.0083$ cm s ⁻¹	Permeability for a cell membrane to fatty acid
$k_{-1} = 0.142$ s ⁻¹	Dissociation rate constant for fatty acid from albumin site 1
$k_{d1} = k_{-1}/k_{+1} = 3 \times 10^{-11}$ moles/ml=30 nM	Equilibrium dissociation constant for albumin-fatty acid (giving $k_{+1} = 4.7 \times 10^{-9}$ ml mole ⁻¹ s ⁻¹)
$C_{AT} = C_A(0) + C_{AF}(0) = 1.5 \times 10^{-9}$ moles/ml	Low albumin concentrations
$C_{FT} = C_F(0) + C_{AF}(0) = 1.0 \times 10^{-10}$ moles/ml	Low albumin concentrations
$C_{AT} = C_A(0) + C_{AF}(0) = 6.0 \times 10^{-7}$ moles/ml	High albumin concentrations
$C_{FT} = C_F(0) + C_{AF}(0) = 5.4 \times 10^{-7}$ moles/ml	High albumin concentrations
$G_1 = 0.0-0.1$ cm s ⁻¹	First order metabolic rate constant

Dependence of concentrations on the permeability coefficient, P.High albumin case. All concentrations are normalized with respect to $C_A(0,0)$.

TABLE 2

$P(\text{cm/s})$	0.000 833	0.008 33	0.05	0.5	5	Infinity (sink)
$C_A(x_1)$	1.018	1.137	1.379	1.601	1.643	1.648
$C_F(x_1)$	0.004 28	0.003 22	0.001 48	0.000 23	0.000 025	0
$C_{AF}(x_1)$	8.937	8.819	8.576	8.354	8.312	8.307

TABLE 3

The parameters used for Region II.

Parameter	Comments
$\delta\lambda=4\times10^{-5}-4\times10^{-3}$ cm	$x_2=x_1+\delta\lambda$: Endothelial or muscle cells
$T=100-2000$ s	$t\in[0,T]$
$D_B=4.6\times10^{-7}$ cm ² /s	Diffusion coefficient for binding protein
$D_{F2}=1.5\times10^{-6}$ cm ² /s	Diffusion coefficient for free fatty acid
$D_{BF}\cong D_B$	Diffusion coefficient for <i>BF</i> complex
$G_2=0.05$ s ⁻¹	Metabolic consumption rate
$K_{-1}=0.1$ s ⁻¹	Dissociation rate constant
$K_{d1}=K_{-1}+K_{+1}=10^{-10}$ moles/ml	Equilibrium dissociation constant
$P_2\geq0.008$ 333 cm/s	Permeability of membrane at x_1^+ (outerface)
$P_3\geq0.008$ 333 cm/s	Permeability of membrane at x_2
$C_{BF}=3\times10^{-7}$ moles/ml	Total binding protein concentration in $x_1<x<x_2$

Steady-state concentrations [normalized with respect to $C_A(0)$] in the two regions and their dependence on the permeability constants: $x_1=50\text{ }\mu\text{m}$; $\delta x=0.4\mu\text{m}$, $P=0.008\text{ }333\text{cm/s}$. High albumin case.

TABLE 4

Permeability constants	$P_1=P_2=P_3=P$	$P_1=P_2=P_3=10^*P$	$P_1=10^*P, P_2=P_3=P$	$P_2=10^*P, P_1=P_3=P$	$P_3=10^*P, P_1=P_2=P$
<i>Region I</i>	$0 - x_1^-$	$0 - x_1^-$	$0 - x_1^-$	$0 - x_1^-$	$0 - x_1^-$
C_A :	1-1.072	1-1.245	1-1.341	1-1.014	1-1.111
$C_P \times 10^3$:	4.45-3.78	4.45-2.41	4.45-1.77	4.45-4.32	4.45-3.45
C_{Af} :	8.955-8.883	8.955-8.710	8.955-8.615	8.955-8.942	8.955-8.844
<i>Region II</i>	$x_1^+ - x_2$	$x_1^+ - x_2$	$x_1^+ - x_2$	$x_1^+ - x_2$	$x_1^+ - x_2$
C_B :	2.327	2.884	0.786	4.083	3.745
$C_P \times 10^3$:	2.08-1.70	1.83-0.575	9.73-7.97	0.399-0.328	0.831-0.261
C_{Bf} :	2.651	2.094	4.192	0.895	1.233

TABLE 5

Steady-state concentrations [normalized with respect to $C_A(0)$] in the two regions: $x_1=50\text{ }\mu\text{m}$; $\delta x=40\text{ }\mu\text{m}$,
 $P_1=P_2=P_3=0.008\text{ }333\text{cm/s}$.

	Region I	Region II
	$0 - x_1^-$	$x_1^+ - x_2$
C_A :	1–1.052	C_B : 2.235–2.318
$C_F \times 10^3$:	4.45–3.96	C_F : 2.74–1.22
C_{AF} :	8.955–8.904	C_{BF} : 2.743–2.663