# Fatty Acids Enter Cells by Simple Diffusion (43986)

DAVID ZAKIM[1]

*Division Digestive Diseases, Department of Medicine, Cornell University Medical School, New York, New York 10021*

Juxtaposed in this issue of the *Proceedings* are two short reviews on the cellular uptake of water-insoluble substances, in this case, long-chain fatty acids. These reviews reflect two different biases as to how fatty acids and similarly apolar substances enter cells: whether by simple diffusion regulated by the physical chemical properties of water, the lipid bilayer region of membranes, and fatty acids, or by a facilitated mechanism dependent on specialized binding proteins and carrier proteins. The mechanism of cellular uptake of molecules like long-chain fatty acids has broad biomedical importance. Elucidation of the mechanism has implications for pharmacokinetics, for predicting the pharmacokinetic behavior of new compounds, and potentially for directing compounds of a given structure to selected kinds of membranes. Yet the current views of the problem could not be more divergent.

For example, a spontaneous mechanism for the uptake of the nonpolar compounds by cells predicts that solubility in plasma membranes and differential partitioning between these membranes and carrier proteins in blood, which are events independent of binding to proteins in membranes, is a primary determinant of rates of uptake into cells. The key, therefore, to directing compounds to selected tissues or to changing the rate of uptake in a given tissue, if the mechanism of uptake depends only on physical chemistry, is to understand the factors that modulate the solubility of apolar compounds in lipid bilayers. By contrast, the idea that cellular uptake of compounds like fatty acids occurs by biological mechanisms will lead the field into studies of the properties of specialized proteins.

There no longer are arguments about spontaneous generation; nor does anyone dispute the idea that the internal workings of cells can be approached and understood through reductionist experimental designs that depend on the rules of the physical sciences. Nevertheless, in different guises, there is a persistent tension between those who see life processes as always singular and always dependent on biological mechanisms, albeit explained in chemical and physical terms, and those who would use general principles of the physical sciences to explain events in cells as occurring for general and usually simple physical chemical reasons independently of specialized biological mechanisms. The two opposing views about the mechanism for cellular uptake of fatty acids reflect, I believe, the biases and tension between the idea that all events in cells have a complex biological basis versus the idea that this need not be so.

I take no issue with an experimenter's initial bias, which I believe has a crucial role in experimental science. Experiments are designed to provide evidence to support the observer's bias, which typically is the starting point for all experiments. Good experiments reinforce the initial bias or force the observer to shift positions. My own impetus for pursuing the problem of the uptake of fatty acids by cells was the belief—or bias—from the start that this would be a spontaneous event requiring no biological mechanisms. I was not the first person to posit this mechanism (1, 2), but no prior attempts had been made to give it a quantitative basis.

My bias was informed by the known chemical and physical properties of lipid bilayers, water, and fatty acids. I had considerable experience in the area of membrane biochemistry and biophysics before becoming interested in the specifics of the uptake of fatty acids by cells. I had worked on the regulation of membrane-bound enzymes by interactions with their phospholipid environment. Through this work, I had experience in assaying membrane bound enzymes that metabolized water-insoluble substrates. My experience in these areas led me to believe that the uptake of fatty acids by cells would not be a biologically mediated process but would occur spontaneously simply as a consequence of the chemical and physical properties of membranes and water, as they interacted with fatty acids.

[1] To whom requests for reprints should be addressed at Division of Digestive Diseases, Department of Medicine, Cornell University Medical School, New York, NY 10021.

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The contrary bias in the accompanying review of Stremmel, i.e., the idea that specialized biochemical structures account for the entry of fatty acids and like substances into the membranes of cells and for the transmembrane transport of such substances, discounts the possibility that uptake of nonpolar compounds by cells could occur spontaneously, which is unjustified given the known physical chemical characteristics of fatty acids and membranes (see below).

## Relevant Properties of Membranes, Water, and Fatty Acids

The best-studied membrane transporters are for ions, like $Na^+$ and $K^+$, or for small, water-soluble substances like glucose. These polar substances do not dissolve in the apolar environment of lipid bilayers, which accounts for the barrier property of membranes. Additionally, avid hydration of these polar substances increases their size, which adds further to the energy for transporting them across apolar barriers. Transport of polar substances across the apolar interior of membranes depends on mechanisms that shield the polarity of the transported species from the apolar membrane environment and that dehydrate the transported species. Both processes require large amounts of energy, which is provided by binding the transported species to a polar channel that is a suitably adapted protein.

The idea that binding proteins are needed to incorporate fatty acids in biological membranes with regions of phospholipid bilayers and/or that carrier proteins to move fatty acids across membranes assumes that these processes, like the transport of polar ions or sugars, have high energy barriers. In contrast to polar ions and sugars, however, fatty acids are apolar. They are sparingly soluble in water and thus are poorly hydrated. They dissolve readily in apolar solvents like hexane. Indeed, fatty acids dissolve readily in the apolar interior of membranes (3–6), and partition avidly into lipid bilayers (or micelles) and biological membranes in preference to water (3–6). Moreover, long-chain fatty acids form bilayers readily—and spontaneously—in combination with lysophosphatidylcholines (7), which form micelles in the absence of fatty acids. A well-known example of this phenomenon is the absorption of fat in the gut, which depends on prior hydrolysis of triglycerides to fatty acids that then dissolve in mixed micelles. The latter are the vehicle for carrying fatty acids into intestinal cells (8). Once triglycerides and phospholipids have been hydrolyzed by lipases to produce fatty acids and lysophosphatides, respectively, mixed micelles form spontaneously in the gut. No proteins are needed to dissolve fatty acids in a micelle. There is hence ample evidence to show that fatty acids will enter membranes spontaneously because of the physical chemical properties of the fatty acids, the lipid regions of membranes, and water. Binding proteins for fatty acids are completely unnecessary for this process. Why then invoke specific fatty acid–binding proteins to get fatty acids into membranes?

The idea of a biological transport system for nonpolar compounds that dissolve readily in membranes makes no sense. It ignores important chemical and physical features of the systems involved, which, it seems to me, nature would not ignore. My experimental approach to determining the mechanism of cellular uptake of fatty acids was to give a quantitative basis to a mechanism of spontaneous, diffusive uptake (1, 2) by measuring the spontaneous rates of uptake of fatty acids into membranes, transbilayer movement, and then desorption of fatty acids from lipid bilayers. The rationale for this approach was simple. If the spontaneous rates of the reactions comprising the uptake process exceed (or equal) rates of uptake into tissues, cells would not need and could not use a facilitated mechanism for uptake.

## A Simple Chemical Scheme for Spontaneous Movement of Fatty Acids into and across Membranes

The overall process of trans-membrane transport is described by Steps 1 to 3 below:

$$\text{FA} - (\text{H}_2\text{O})_n + \text{Bilayer} \leftrightharpoons \text{FA} - \text{Bilayer}_o + n\text{H}_2\text{O} \quad [1]$$

$$\text{FA} - \text{Bilayer}_o \leftrightharpoons \text{FA} - \text{Bilayer}_i \quad [2]$$

$$\text{FA} - \text{Bilayer}_i\ n\text{H}_2\text{O} \leftrightharpoons \text{FA} - (\text{H}_2\text{O})_n + \text{Bilayer}. \quad [3]$$

Step 1 is the reversible transfer of fatty acid in water to a membrane, referred to as bilayer and representing the phospholipid bilayer region of a biological membrane. This is a favorable step thermodynamically, as reflected by the high preference of fatty acids for membranes in preference to water (3–6).

Step 2 is the transbilayer movement of fatty acid, which is referred to as flip-flop of fatty acids from one leaflet of a bilayer to the other. The subscripts *o* and *i* refer to the outer and inner leaflets of the bilayer respectively. The spontaneous occurrence of flip-flop is simply the diffusion of a solute (the fatty acid) in solvent (the phospholipid environment) (9), even though bilayers do not have the exact properties of bulk phase solvents (10, 11). Experience shows that energy must be expended to limit a solute to a restricted region of a solvent. Think for example of restricting NaCl to a limited region of an aqueous solution. Thus, entropic forces drive molecules of fatty acids to fill all the volume elements available. This could be prevented, be-

cause of their orientation in the membrane, by hydrogen-bonding between water at the membrane-water interface and $COO^-$ of the deprotonated acid in that flip-flop of the hydrogen-bonded fatty acid would require prior breakage of the hydrogen bond. However, not all molecules of fatty acid can be restrained in this way because a significant proportion of fatty acid molecules will be unionized (protonated) at, say, pH 7.4 (12); and since the rate of protonation/deprotonation is extremely rapid, any given molecule of fatty acid will be protonated for a substantial period of time. Finally, measured rates of diffusion of lipids in membranes are far greater than needed to support spontaneous uptake of fatty acids by tissues (13–15). So there is no reason to suppose *a priori* that flip-flop of fatty acids across a bilayer will be a slow event.

Step 3 is the same as Step 1 except for reversal of the forward direction, which in Step 3 represents spontaneous dissociation of fatty acid from the inner leaflet of the bilayer into cell water. As written, Step 1 applies to events at the outer leaflet of a bilayer, and Step 3 applies to events at the inner leaflet. We know that the concentration of fatty acids in the membranes in Step 1 to 3 will be high whereas the concentration in water will be extremely low. However, the partition coefficient for the distribution of fatty acids between membranes and water is the ratio of the rate constants in Step 1 (or Step 3) for the forward and reverse steps. The partition coefficient alone does not provide even an order of magnitude estimate of the rates of either the forward or reverse event. For example, given an observed partition coefficient of $10^6$–$10^7$ (on a mol fraction basis) for the ratio of palmitate between bilayers and water (16) and assuming a rate for the forward step in Reaction 1 that is close to the limit of diffusion for fatty acids in water, the spontaneous rate of desorption of fatty acids from a bilayer—the rate of the forward reaction in Step 3—is on the order of 10–$10^2$ $sec^{-1}$. This rate is more than adequate for supporting observed rates of uptake of fatty acids by liver (see Table I). So simple analysis of the chemical and physical features of the system provides no evidence that the overall process for the uptake of fatty acids by tissues cannot proceed spontaneously in the absence of biological mechanisms.

We assume in Step 1 that fatty acids enter membranes from water. Fatty acids are transported in the blood, however, as complexes with albumin, which overcomes the problem of bulk transport of the sparingly soluble fatty acids. The rate at which fatty acids enter membranes from the aqueous phase, as represented by Step 1 would depend, therefore, on the rate of Step 4,

$$\text{Albumin} - \text{FA} + n\text{H}_2\text{O} \leftrightharpoons \text{FA} - (\text{H}_2\text{O})_n + \text{Albumin}, \quad [4]$$

in the forward direction, in which this is the rate of dissociation of fatty acids from albumin into water.

Separate from the idea of catalyzed transport of fatty acids across membranes, it is hypothesized that fatty acids are transported directly from albumin to plasma membranes by a collisional mechanism that avoids Step 4 (17). There are no data to indicate that this is necessary for the uptake process. And, by analogy with the discussion of the rate of Step 3 in the forward direction, the high avidity of fatty acids for albumin does not mean that fatty acids do not dissociate rapidly from albumin. The high avidity of fatty acids for albumin only means that the ratio is high for the rates of binding and dissociation.

Using model systems of phospholipid bilayers, we measured the rates of all the steps depicted in Reaction 1 to 3; and using fatty acids bound to albumin, we measured the rates of the forward and reverse steps in Reaction 4. The data for the relevant rate constants are in Table I (16). Rates in Table II are the spontaneous fluxes of fatty acids from binding sites on albumin to the interior of a cell, calculated on the basis of the rate constants in Table I and physiologic concentrations of fatty acids and albumin, compared with rates of uptake of fatty acids by liver in the steady state.

It is notable in Table I that the rate of desorption of fatty acids from albumin into water (Step 4) is orders of magnitude slower than any of the membrane events (Step 1 to 3) associated with the spontaneous diffusion of fatty acids into cells. Thus, if the uptake of fatty acids by liver were limited by the rate of any spontaneous, uncatalyzed step in Step 1 to 4, it would have to be limited by the rate of dissociation of fatty acids from albumin into water (Step 4) and not by any of the membrane associated steps. We note in Table II, however, that even the relative slowness of the rate of dissociation of fatty acids from albumin does not limit the rate of uptake of fatty acid by liver in the steady state. Interestingly, too, the idea of a facilitated mechanism by which Step 4 and 1 are avoided, by direct, catalyzed transfer of fatty acids from albumin to plasma membranes, has been abandoned by the original proponents (18).

The reader may question, from the data in Table I and II, why the rate of uptake of fatty acids by liver

**Table I.** Rate Constants for Reaction 1 to 4 Measured with Palmitate as Fatty Acid

| Rate constant | Value ($sec^{-1}$) |
|---|---|
| $k_1$ | $7.4 \times 10^6$ |
| $k_2$, $k_{-2}$ | $>>>7.4$ |
| $k_3$ | 7.4 |
| $k_4$ | 0.036 |
| $k_{-4}$ | $k_{-4} >>> k_4$ |

*Note.* Data are from Refs. 4, 6, and 16, which should be consulted for experimental details.

**Table II.** Comparison of Maximal Rates of Uptake Calculated from Kinetic Constants in Table I and Measured Rates of Uptake for Fatty Acids by Perfused Liver

| Fatty acid | Calculated max rate of uptake (nmol $min^{-1}$ $g^{-1}$) | Measured rate of uptake (nmol $min^{-1}$ $g^{-1}$) |
|---|---|---|
| Myristate | 1.3 | 0.062 |
| Palmitate | 0.39 | 0.067 |
| Stearate | 0.11 | 0.063 |

*Note.* Data are from Refs. 6 and 16.

was slower than the slowest step in the sequence of transfer of fatty acids from binding sites on albumin to the interior of cells. The explanation for this is given later in some detail; but in brief, the reason why the steady state rate of uptake is slower than the slowest spontaneous step in the diffusive process of uptake is because the rate of dissociation of fatty acids from albumin is fast enough to maintain an equilibrium distribution of fatty acids between albumin and the plasma membrane of liver cells and because the rate of uptake is limited by intracellular events (6, 16). The data for the rates of Steps 1 to 4 establish, therefore, that no specialized biological mechanisms need be invoked to account for the uptake of fatty acids by the liver. No such mechanism could make the overall process of uptake go faster than it does in the absence of a facilitated mechanism(s). The data in Table II suggest that rates of uptake in the steady state will become limited by the rate of Step 4 (see Table I, for example) for fatty acids longer than C-18; but uptake rates for fatty acids longer than stearate have not been determined.

In other aspects of this work, we showed that the rate equation for the uptake of fatty acids by liver in the steady state is the following (6, 16, 19, 20):

$$\text{Uptake} = k_{met}[\text{Fatty Acids}]_{PM} \qquad [5]$$

where PM is the plasma membrane and the rate constant $k_{met}$ is for intracellular events, including metabolism. Equation 5 states that the concentration of fatty acids in the plasma membrane of a cell is the primary determinant of the rate of uptake of fatty acids by that cell in the steady state, that the rate of uptake of fatty acids by liver in the steady state is a linear function of the concentration of fatty acids in the plasma membrane of cells, and that the rate of uptake of fatty acids in cells in the steady state is the rate of metabolism of fatty acids.

Equation 5 is not a theoretical expression but is based on experimental observations that provide an internally consistent picture of the uptake process (6, 16, 19–21). Moreover, we have verified that Eq. 5 together with relevant thermodynamic data for inserting values of $[\text{Fatty Acid}]_{PM}$ and kinetic data for inserting values of $k_{met}$ predicts accurately rate of uptake of fatty acids by perfused liver (16). Since the ability to make quantitative predictions of the rate of uptake is the ultimate test of a posited mechanism, we have an important validation of the starting bias that the mechanism of uptake is diffusive.

## A Simple Explanation of the Uptake Process

Consider that the ratio of moles albumin/moles fatty acid in the albumin–fatty acid complexes in sinusoids decreases abruptly, i.e., the concentration of fatty acids in blood increases at constant concentration of albumin. What happens in this setting is that the fatty acids redistribute rapidly between albumin and plasma membranes to reach an equilibrium distribution of fatty acids in these phases. Redistribution occurs according Reaction 1 to 3. The value of $[\text{Fatty Acid}]_{PM}$ increases as the ratio of moles fatty acid/moles albumin—in sinusoids—increases. Fatty acids equilibrate extremely rapidly between the outer and inner leaflets of the plasma membrane (Table I). Then, because fatty acids partition between the bulk phases of cell water and the phospholipid bilayer region of plasma membranes, the concentration of fatty acids in cytosol adjacent to the inner leaflet of the plasma membrane increases. This increase is linearly dependent on the concentration of fatty acid in the plasma membrane and occurs independently of whether fatty acids are free in cytosol or are bound to carrier proteins. Fatty acids in cytosol, whether free or bound, diffuse to sites of metabolism in mitochondria, microbodies, and endoplasmic reticulum. The rates at which fatty acids can be metabolized are far greater, however, than the rates at which fatty acids can be delivered to sites of metabolism; additionally, the metabolic systems function at concentrations of fatty acids below values of $K_m$ for different metabolic steps (6, 16, 22). This occurs because the rate of diffusion of fatty acids to sites of metabolism is the slowest step in the overall transfer of fatty acids from blood to sites of metabolism in the cell. Thus, whereas there is an equilibrium distribution of fatty acids between albumin in sinusoids and plasma membranes, in perfused liver, the concentration of fatty acids within liver cells does not approach the equilibrium value (6, 16, 20–22). Note that $[\text{Fatty Acid}]_{PM}$ is set by the partitioning of fatty acids between albumin and plasma membrane; $[\text{Fatty Acid}]_{PM}$ cannot be higher than the concentration at equilibrium with albumin. This equilibrium can be modulated by change in the ratio of moles albumin/moles fatty acid changes, by change in the concentration of albumin, or by change in conditions that affect the equilibrium for the distribution of fatty acids be-

tween a given amount of albumin at fixed ratio of moles albumin/moles fatty acid, as for example a change in pH (23).

Obviously, the equilibrium positions of Reaction 1 and 3 will be equal when the outer and inner leaflets of a bilayer have the same compositions and the chemical properties of the surrounding water are the same for both leaflets of the membrane; but this does not apply to plasma membranes, in which the outer and inner leaflets of the bilayer have nonidentical lipid compositions (24). Nevertheless, except as just discussed, the equilibrium position of Reaction 1 (or 3) is of little consequence for driving trans-membrane flux in a living cell. Utilization of fatty acids by intracellular processes draws the flux of fatty acids across the membrane.

We expect a chemical process for uptake to be reversible and to have predictable kinetic properties under conditions in which the concentration of fatty acids in inside the cell is greater than the concentration outside the cells. "Uptake" of fatty acids by perfused liver displays reversal of flux, with appropriate rate constants and kinetic properties, when fatty acid–free albumin is perfused through livers that have attained steady state concentrations of fatty acids in cellular compartments (22).

## Extrapolating from Model Systems to Plasma Membranes

One could argue that experiments in model membranes (i.e., all experiments in which rates of Reaction 1 to 4 are measured with pure lipid bilayers) are artificial and that these results do not apply to the real problem of uptake of fatty acids by real membranes in real cells. There is no known mechanism, however, by which proteins, at concentrations in biological membranes, can inhibit interactions between small molecules like fatty acids and the phospholipid bilayer domains of the membranes. An abundance of data from studies with probes of all types, as well as calorimetric experiments, show that biological membranes have bulk lipid phases arranged as bilayers. Also, the lipid region of biological membranes are more fluid than the lipid bilayers employed in studies of model systems (see Ref. 22), which, as compared with artificial membranes used in model experiments, should enhance rates of flip-flop. Addition of proteins to phospholipid bilayers also enhances the rate of flip-flop across the bilayers (25, 26). There is sufficient knowledge about the properties of pure lipid bilayers and the affect of membrane proteins on bilayers, therefore, to support that the data on rates of Reaction 1 to 3 in model systems can be extrapolated to the problem of trans-bilayer movement of fatty acids in biological membranes.

## Other Evidence for a Chemical Mechanism of Cellular Uptake of Fatty Acids Versus a Biochemical Mechanism

The relevant data show spontaneous rates of Reaction 1 to 4 that are rapid and exceed rates of uptake of fatty acid by liver, the idea of diffusional uptake of fatty acids by cells is disputed. They show too that the rates of membrane events (Step 1 to 3) are orders of magnitude faster than the nonmembranous event (Step 4). Nevertheless, considerable data are interpreted as showing that uptake is facilitated by specific protein(s) in plasma membranes (cf., accompanying review by Stremmel). Among the lines of evidence offered to support the latter mechanism are dispute over the rates of Reaction 2, the flip-flop event (27–29); kinetic properties of the uptake system that are believed to be properties of biological mechanisms and incompatible with chemical mechanisms, as for example saturation of rates of uptake, inhibition of uptake and that uptake is energy dependent (30, 31); direct evidence for the existence of specific binding proteins in membranes (32–36); and that bacteria contain a transport system for fatty acids (37–39). We have to look therefore at this evidence to determine whether it is incompatible with a chemical mechanism for uptake of fatty acids into cells.

**Dispute about Rates of Flip-Flop.** One group of investigators has concluded that rates of flip-flop are limiting for the spontaneous transfer of fatty acids across membranes (27–29). There is considerable precedent, however, for the rapid movement of other nonpolar molecules across bilayers (40–49); and we are not the only laboratory to have shown that fatty acids move rapidly into, across, and out of membranes (41, 42, 44, 45, 47). There are, in fact, no data for Reaction 1 to 4, carried out with physiologic fatty acids, that impune the accuracy of the rate constants in Table I. Additionally, there is an important correlation between measured rates for flip-flop of protonated fatty acids (16) and deprotonated fatty acids (47).

As mentioned already, the rate of flip-flop will be retarded by forces that constrain the charged form of fatty acid to the bilayer-water interface, as for example hydrogen bonding between water and $-COO^-$. Since this hydrogen bond must be broken before flip-flop can occur, there will be a large difference in rates of flip-flop of protonated and unprotonated fatty acids. The energies of activation of flip-flop for protonated versus deprotonated fatty acid should differ by about the energy of the hydrogen-bond between $-COO^-$ and water. The lower limit to the rate of flip-flop of protonated fatty acid, which is too fast to measure directly with naturally occurring fatty acid (16, 50), can be taken as somewhat faster than the rate of hydration of

fatty acid (independent of ionization state because rates of protonation/deprotonation are extremely fast on the time-scale of flip-flop). This gives a ratio of rates of flip-flop for protonated and deprotonated fatty acid of about $10^5$, which is the difference predicted by the energy of a single, strong hydrogen bond.

We believed until recently that discrepancies in rates reported for spontaneous, diffusive flip-flop of fatty acids across bilayers reflected differences in the types of fatty acids used to study this problem. Thus, whereas our measurements indicating extremely rapid rates of flip-flop were made with physiological fatty acids, relatively slow rates for flip-flop were reported only for studies using anthroyloxy derivatives of fatty acids, which are nonphysiologic compounds (27–29, 51). The anthroyloxy group is fluorescent and thereby provides an optical probe for measuring movement of fatty acids into or out of populations of vesicles; but the anthroyloxy group is also bulky and makes the anthroyloxy fatty acids much larger in cross section than the naturally occurring, physiologic fatty acids. This difference in physical properties might account for the reported differences in transbilayer movement of fatty acids versus anthroyloxy fatty acids. We now have found, however, equally rapid rates of flip-flop for fatty acids and anthroyloxy fatty acids (50). Thus, previous work with anthroyloxy fatty acid must not have measured true rates of flip-flop (50).

**Chemistry Accounts for the "Biological" Property of Saturation of Rates of Uptake.** Whether or not the uptake of fatty acids proceeds directly from the physical chemistry of membranes, water, and fatty acids, or from a highly evolved biochemical system of binding proteins and so on, the uptake of fatty acids by liver displays properties that are considered to be uniquely biological. For example, depending on experimental conditions, uptake appears to be saturable. This property of the system, which we have validated (6), is considered incompatible with a simple chemical mechanism and thus to be evidence for a biochemical one (Fig. 1). Indeed, the linear relation between concentrations of fatty acid in plasma membranes and rates of uptake that we have reported and discussed is not what is expected for a biological process. Similarly, inhibition of the rate of uptake of fatty acids by liver in the steady state is taken as evidence for a biochemical mechanism of uptake.

A well-known tenet of biochemistry is to use extreme care in establishing mechanisms from kinetic experiments. This lesson applies very well to the present situation because a simple chemical system for uptake of fatty acids by cells will appear to be saturable under the following experimental conditions: uptake is measured as a function of an increasing concentration of the complexes albumin–fatty acid at any fixed ratio of moles albumin/moles fatty acid (6, 18).

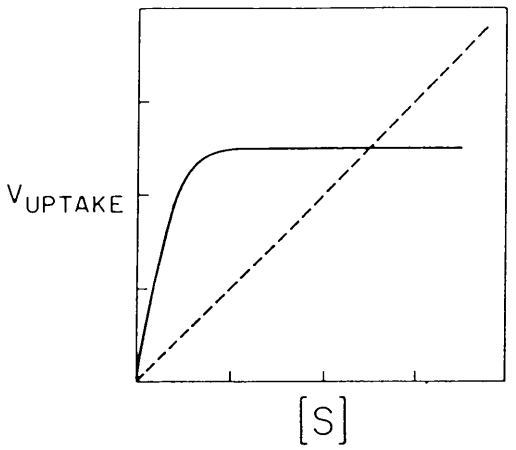


**Figure 1.** The dashed line shows a linear relationship between the rate of a process and the concentration of reactant. This relationship is thought to reflect the kinetic pattern for a simple chemical process. In the present case, the rate of the process of interest is for uptake of fatty acids in the steady state and the reactant is the fatty acid. Equation 5 in the text, the rate equation for the uptake of fatty acids by the liver, fits the kinetics of the dashed line. The solid line, by contrast, shows the rate of the process of interest reaching a finite level as the concentration of reactant becomes infinite. This kinetic pattern is typical for a reaction limited by the amount of intermediate complex that can be formed, as in the case of the binding of substrate to an enzyme or of ligand to a binding protein. The rate of uptake of fatty acids by liver as a function of variable concentrations of the complexes albumin–fatty acid for a fixed ratio of moles albumin/moles fatty acid fits the solid line (see text for explanation). (Reproduced with permission from Ref. 21.)

These are the precise conditions used to demonstrate that the rate of uptake of fatty acids, by liver for example, is saturable (6, 18). If we were dealing with a single-phase system in which all reactions occurred in water, interpretation of the data would be straightforward. The data could not be accounted for *via* a chemical mechanism. But the uptake system is multiphasic, involving water, lipids, and proteins as phases that solvate fatty acids at different points in the overall process of uptake. And, in such a system, apparent saturation of membranes with fatty acid and saturation of the rate of uptake of fatty acids by cells is a direct consequence of the physical chemical attributes of the multiphasic system in which the events of interest occur. That is, the value $[\text{Fatty Acid}]_{PM}$ will appear to reach a limiting value; and thus according to Eq. 5, the rate of uptake will reach a limiting value. Therefore, the rate of uptake will appear to be saturable if experimental conditions appear to "saturate" the plasma membrane with fatty acids.

It is easy to show that partitioning of fatty acids between membranes and albumin will appear to saturate the membranes with fatty acids as the concentration of albumin-fatty acid complexes approaches infinity, the ratio of moles albumin/moles fatty acid is constant, and the concentration of membranes is constant (Ref. 6 and Fig. 2). Hence, the presumed biochemical property of saturation of uptake of fatty acids follows

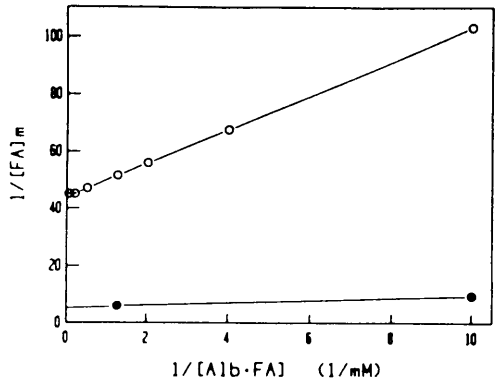


**Figure 2.** Double reciprocal plots of the concentration of fatty acids in membranes at equilibrium with fatty acids bound to albumin as a function of variable concentrations of the complexes albumin–fatty acid at a constant ratio of moles albumin/moles fatty acid at two different values of the ratio. The double reciprocal plot corresponds to the kinetic relationship given by the solid line in Figure 1. (Reproduced with permission from Ref. 6.)

simply from the chemistry of the system. Contrary, however, to the behavior of biochemical systems that really become saturated (e.g., the binding of a ligand to a specific site), the concentration of fatty acids that "saturate" membranes varies with the ratio of moles albumin/moles fatty acid in the complexes albumin–fatty acid (Fig. 3). The smaller this ratio, the higher the concentration of fatty acids in membranes that appear to be "saturated" (6). Finally, the maximum rate of uptake of fatty acids by perfused liver in the steady state, determined by extrapolation of the plot 1/rate of uptake versus 1/[albumin–fatty acid], is linearly related to the ratio moles fatty acid/moles albumin of these complexes. This property of the uptake system is again predicted by a chemical mechanism and follows directly from the physical chemistry of partitioning of fatty acids between different phases in the uptake system. The putative biochemical mechanism for uptake is not compatible with these properties of the uptake system.

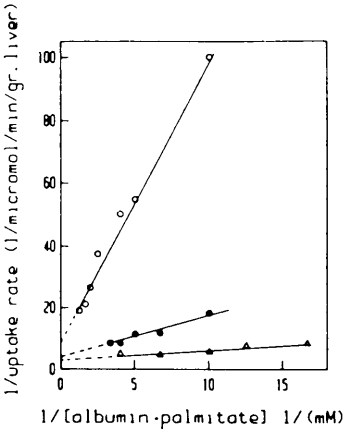


**Figure 3.** Double reciprocal plots of the rate of uptake of palmitate by perfused liver in the steady state as a function of variable concentrations of the complexes albumin–fatty acids. Each line corresponds to a different, constant ratio of moles albumin/moles fatty acid: 2/1 (○); 1/1 (●); 1/2 (△). (Reproduced with permission from Ref. 6.)

We should not overlook, however, that chemistry can provide the uptake system with other properties that appear to be "biological." The avidity of fatty acids for membranes depends on the lipid composition of the membrane because this determines how fatty acids pack with the fatty acyl chains esterified to phospholipid head groups. Variability in the lipid composition of membranes provides a biological substrate that allows for differential partitioning of fatty acid between different types of membranes. There is evidence too that the protein composition of membranes contributes nonspecifically to this effect because of the manner in which the proteins pack with the phospholipids (20). There is hence an interacting biological and physical chemical mechanism for modulating the rate of uptake of fatty acids by cells and thereby providing a basis for organ-specific differences in rates of uptake. Selectivity in rate of uptake on an organ basis, species basis, and so on then is completely compatible with diffusive, nonbiological mechanism for cellular uptake of fatty acids (see, for example, data in Ref. 20).

**Inhibition of the Rate of Uptake of Fatty Acids as a Reflection of Mechanism.** As mentioned already, the rate of fatty acid uptake by cells in the steady state can be inhibited (30, 31). The question is, Does this inhibition reflect that uptake is a biochemical process? It could. But no one has established that the rate of uptake itself can be inhibited in a biochemical context; that is, there are no experimental data showing that any of the steps in Reaction 1 to 4 can be inhibited. In fact, there are no measurements of the rates of these reactions in intact cells.

As shown already, the rate measured in cells as uptake in the steady state is the rate of metabolism of fatty acids. In other words, inhibitors of uptake in the steady state could inhibit by interfering with one or several different enzyme-catalyzed metabolic pathways of fatty acids and any inhibitor of fatty acid metabolism, whether specific or nonspecific, will appear to inhibit the uptake of fatty acids in the steady state. It is clear, too, that alterations in the equilibrium distribution of fatty acids between albumin and plasma membranes will change $[\text{Fatty Acid}]_{PM}$ in Eq. 5 and thereby appear to inhibit (or enhance) the rate of uptake of fatty acids in the steady state. There is ample evidence for concluding that all inhibitors of the uptake of fatty acids studied to date do so by inhibiting the metabolism of fatty acids and/or by perturbing the partitioning of fatty acids between membranes and albumin (21, 23). Therefore, data for the inhibition of the uptake of fatty acids cannot be used to argue for or against one or another mechanism for uptake.

**Do Fatty Acids Compete with Each Other for Uptake?** We also have looked to see whether the uptake of two fatty acids by liver would be mutually inhibitory. This could happen if different types of fatty acids competed for metabolism; or it could happen if they competed for specific binding sites on the putative membrane binding and/or carrier proteins. Mutual inhibition of uptake in the steady state does not exclude a chemical mechanism for the transfer of fatty acids from albumin to the cytosol of a cell, but inhibition of uptake *per se*, of one fatty acid by another, is not compatible with a chemical mechanism for uptake. Therefore, it is worthwhile to determine whether two different fatty acids compete for uptake. When this was done, we found that oleate did not inhibit the uptake of palmitate and vice versa in perfused liver (19).

These last experiments were criticized (52) as not appropriate for determining whether or not palmitate and oleate competed for uptake by liver because the concentrations of fatty acids used in these experiments were far lower than the $K_m$ of the uptake process (based on data in Ref. 36). Under this condition no competition would be expected, even for a facilitated process. This seems like a reasonable argument, but it is specious.

Uptake of fatty acid by cells cannot be characterized by a physically meaningful value for $K_m$. As shown already for example, whereas the kinetic *pattern* for uptake of fatty acid by liver is compatible with a facilitated process (i.e., Michaelis-Menten kinetics), but this pattern is a consequence of partitioning of fatty acid between albumin and plasma membranes (6). Moreover, a further consequence of partitioning is that, as alluded to already, what appears to be the $K_m$ for uptake varies as a function of the ratio of moles albumin/moles fatty acid, as does the apparent $V_{max}$ (6). References in the literature to a $K_m$ for the uptake process are for the concentrations of fatty acid in water. This is calculated on the basis of the binding constant for albumin-fatty acid complexes (Reaction 4), but concentration of fatty acid in water is not an independent variable. It depends on at least two other phases—albumin and the membrane. One thus could rewrite Eq. 5 on the basis of the concentration of fatty acid in water, which has a fixed relationship with the concentration in the plasma membrane. But the accuracy of $[\text{Fatty Acid}]_{PM}$ is far greater than $[FA]_{water}$.

How then do we address the choice of conditions for testing the idea that different fatty acids do or do not compete for uptake? Conditions for such experiments must be based on the empirical relationship (no mechanism is assumed) between rates of uptake of fatty acid and the concentration of the complex albumin–fatty acid, for the ratio of moles albumin/moles fatty acid used in an experiment (e.g., the concentration of the complex albumin–fatty acid at which the rate of uptake is half maximal for a given ratio of albumin/fatty acid). The experiments in Cooper *et al.* (19), for competition between oleate and palmitate for uptake by perfused liver, contained concentrations of the complex albumin–fatty acid slightly less than those needed to give half-maximal rates of uptake at the ratio of moles albumin/moles fatty acid used in the experiments. Under these conditions the uptake of two different fatty acids proceeded independently of each other.

**Binding of Fatty Acids to Membranes.** We have looked for other biochemical properties of the uptake system in efforts to explore as fully as possible the mechanism of uptake. For example, we have examined the idea that fatty acids bind to membranes by binding at selected sites (e.g., on specific proteins). We have been unable to repeat a key experiment in the literature, which is that heating membranes destroys their avidity for fatty acids (33). We find an opposite effect (19); and interestingly, proponents of a biochemical mechanism for uptake of fatty acids and bilirubin by liver also have reported that heating of liver plasma membranes enhances avidity for bilirubin, which is exactly opposite to what is expected of a biochemical mechanism and what they have reported for the binding of fatty acids (53).

We also have examined the question of binding of fatty acids to specific sites in plasma membrane by measuring the distribution of fatty acids between lipid bilayers and plasma membranes from liver. We found no evidence, over a 200-fold range of concentrations of fatty acids in plasma membranes, for binding of fatty acids to specific sites versus solvation of fatty acids by the lipid bilayer regions of the membrane. Fatty acids simply partitioned between bilayers and plasma membranes (19). This result indicates that any specific binding of fatty acids to sites in plasma membranes could account, at best, for an exceedingly small percentage of total binding and would be missed in the experiments claiming the existence of such specific binding sites.

**The Uptake of Fatty Acids by Bacteria.** The outer membrane of *Escherichia coli* contains a protein essential for normal uptake of fatty acids (37–39). Wild-type strains of *E. coli* concentrate fatty acids to a greater extent than strains containing a mutant of the fatty acid–binding protein. It is not established, however, that the rate of uptake of fatty acids, as defined by Reaction 1 to 4, is greater in wild-type than in mutant strains. Additionally, that *E. coli* have a facilitated mechanism for uptake of fatty acids in no way clarifies the problem of how fatty acids enter either *E. coli* or mammalian cells. The reasons for differences between *E. coli* and mammalian cells could be argued interminably. I consider only a simple *chemical* reason why *E. coli* has, and mammalian cells lack, a facilitated

mechanism for uptake of fatty acids. *E. coli* differ from mammalian cells not only by the lipid composition of the plasma membranes of each type of cell but also by the environments in which each must thrive. For example, the concentration of fatty acids in serum is large. Consequently, the fatty acid concentration gradient is directed inward in mammalian cells. The problem faced by a liver cell is how to avoid an overaccumulation of these potentially damaged compounds not how to accumulate them (6, 22). The problem is solved in mammalian systems by the greater avidity of albumin for fatty acids as compared with the lipid regions of plasma membranes (3, 6, 16, 19). By contrast, *E. coli* has to accumulate fatty acids from a fatty acid–poor environment and has to contend with a concentration gradient for fatty acids that is directed outward. The fatty acid–binding protein in *E. coli* hence may have nothing to do with the kinetics of the uptake process but be important for the thermodynamics of efficient uptake. This protein may subserve the role of an intracellular albumin.


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