THE UPTAKE OF FATTY ACIDS BY THE LIVER*

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The mechanism for the transport of small compounds from the environment to the cytosol of a cell will depend on whether the compound of interest is water-soluble (e.g., salts or sugars) or water-insoluble (e.g., fatty acids or bilirubin). The reason for this division is that the plasma membranes of cells consist of a lipid bilayer, which has an apolar interior. The portions of membrane proteins that interact with this interior also are apolar. The transport of water-soluble compound across plasma membranes must be facilitated, therefore, by a mechanism that overcomes the resistance of the apolar interior to the passage of water-soluble molecules. The relevant mechanism used by cells is the synthesis of carrier proteins, as for example the Na^+-channel or the glucose transporter. By contrast, one expects water-insoluble compounds that are not amphipathic to pass readily across the apolar interior of plasma membranes (1–4). Thus, water-soluble compounds not only can be highly soluble in the apolar interior of a membrane but there is no barrier to the rapid transbilayer movement of such compounds (5–9). Transfer of such molecules from one half of the bilayer to the other, so called "flip-flop," will be facile. In the case of molecules with apolar and polar regions (amphipaths) the concentration in membranes can be high, but the passage of the polar region across the bilayer can be slow. It has not yet been shown, however, that the rate of this last process is rate determining for uptake and metabolism of an amphipath.

It was believed until relatively recently that fatty acids and other water insoluble compounds moved into cells by a simple physical process (1–4). This idea was never expressed in quantitative terms; and more recently, the problem of the uptake of fatty acids by cells has been conceptualized in the context of the problem as it applies to the uptake of water-soluble compounds (10–13). In fact, it is currently accepted by biologists interested in the problem that the movement into cells of fatty acids, and chemically related molecules, is a complex biological process involving perhaps several specialized transport proteins. The underlying experimental basis for this view is that the kinetics of the uptake process

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have characteristics, such as saturability, that are believed to be incompatible with physical or chemical as compared with biological mechanisms. The accepted paradigm for uptake by liver, as it applies to fatty acids, is depicted in Figure 1, along with the chemical reactions the putative transport proteins are alleged to catalyze. The scheme in the left side of Figure 1 was not derived from direct experimental observations of the rates of the reactions in the right side of the Figure. The experimental issue raised by the scheme in Figure 1, therefore, is this: are the rates of the spontaneous reactions in the right side of the Figure faster or slower than rates of uptake of fatty acids by liver? If they are all faster, then uptake *per se* cannot be facilitated by a biological mechanism. If one or several steps are slower than uptake, then these steps must be facilitated. It is important to remember, however, that whether or not the reactions in Figure 1 need to be facilitated *in vivo* does not influence the observation that membranes will concentrate fatty acids for simple chemical reasons. Receptor proteins are not needed for this purpose.

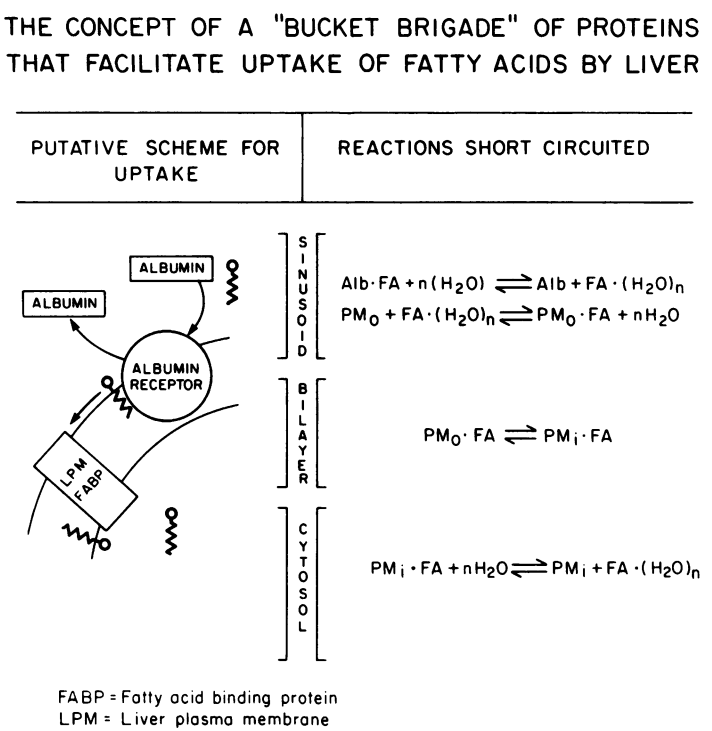

FIG. 1. The concept of a "bucket brigade" of proteins that facilitate the uptake of fatty acids by liver. The scheme on the left side of the figure assumes that the reactions on the right side are too slow to account for actual rates of uptake. In addition, the scheme is derived in part to keep fatty acids from interacting with water, and to provide a biological mechanism that can be saturated and inhibited.

Listed in Table 1 are the rate constants for the reactions in Figure 1, with palmitate as the fatty acid. The data were obtained in model systems, but the rate constants for the same processes in natural membranes will be equal to or greater than those in the Table (14–17). The main point of the data is that the slowest step in the transfer process in Figure 1 is the rate of hydration of palmitate bound to albumin. The next slowest step is approximately 200-fold faster. An exact numerical value for the rate constant for transbilayer movement of palmitate (flipflop) cannot be given because of methodologic limitations, but this step is at least as fast as that for release of palmitate from membranes into water.

The logical next question to consider is the quantitative relationships between the rate of reaction [1] (the slowest step in Figure 1)

$$\text{Alb}\cdot\text{Fa} + n\text{H}_2\text{O} \rightleftharpoons \text{Alb} + \text{FA}\cdot(\text{H}_2\text{O})_n \qquad [1]$$

and rates of uptake of palmitate by the liver. From the known rate constant for reaction [1], and given the concentrations of albumin and fatty acid in liver sinusoids, the flow rate and the mean transit time for blood flow in the liver, one can calculate the maximum amount of fatty acids that can be transferred spontaneously from albumin to plasma membranes in perfusion experiments with conditions of flow and mean transit time approaching those in an intact animal (15). The data bearing on this question are in Table 2 for 3 different fatty acids. These data show that the rate of reaction [1] cannot limit the uptake of fatty acids by liver. In addition, the data indicate that the distribution of a fatty acid between albumin in liver sinusoids and hepatocyte plasma membranes will be at equilibrium *in situ*. This is true under all conditions, as for example different fatty acids, ratios of albumin/fatty acids and

TABLE 1

Rate constants for the spontaneous transfer of palmitate from its binding site on albumin to the inside of a membrane vesicle. Data are from references (14) and (15), which should be consulted for experimental details

Description of reaction	Rate constant/sec
Release of palmitate from albumin	0.036
Uptake of solvated palmitate by membranes	$\sim 10^7$
Transbilayer movement of palmitate (flip/flop)	≥ 7.4
Release of palmitate from membrane into cytosol	7.4

TABLE 2

Comparison of the uptake of fatty acids by perfused liver with the maximal uptake allowed if reaction [1] were rate limiting (calculated maximum uptake). The data are from (15).

Fatty acid	Cal'd Max uptake (nmol/min/g)	Observed uptake (nmol/min g)
Myristate	1.3	0.062
Palmitate	0.39	0.067
Stearate	0.11	0.063

concentrations of the complex albumin-fatty acid (14, 15). It seems clear, therefore, that the transfer of fatty acids from binding sites on albumin to cytosol of the liver is a physical process, not a biological one. Although the chemistry of the system is quite consistent with this conclusion, this apparently physical-chemical mechanism of uptake must explain the apparent biological properties of the uptake process.

The first problem is to explain how a system of passive uptake becomes saturated at infinite concentrations of substrate (10, 15). The second problem is to explain how a passive physical system of uptake can be inhibited.

The reason why fatty acid uptake by liver can be saturated, even though uptake is a passive, physical process, is shown by the data in Figure 2. What are plotted in this Figure (in double reciprocal form) are the concentrations of fatty acid in hepatocyte plasma membranes as a function of the concentrations of the complexes albumin·fatty acid for 2 different ratios of albumin/fatty acid. Because the system consists of 2 phases (albumin and membranes) and the amount of only one of the phases (albumin) varies under the condition of the kinetic experiment, the concentration of fatty acids in the membranes reaches a finite limit at less than infinite concentrations of "substrate." This causes the rate of uptake to reach a limiting value because the rate of uptake of fatty acids by liver, under steady state conditions, is determined by the concentration of fatty acids in the hepatocyte plasma membranes (15,

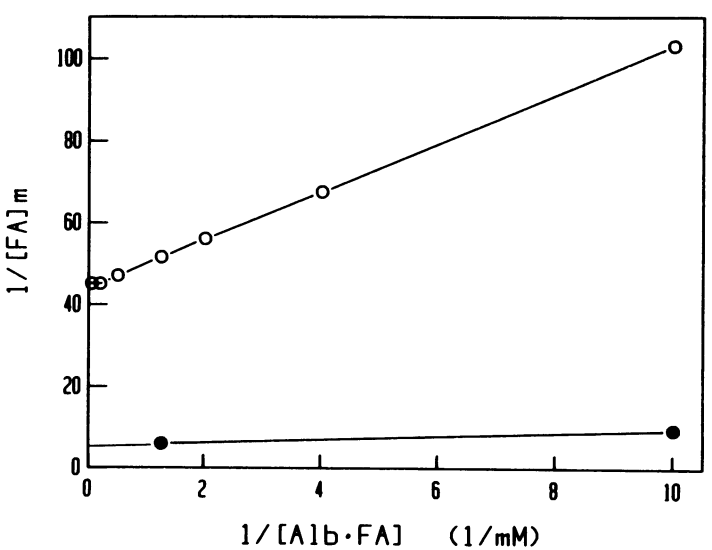

FIG. 2. Calculated concentrations of palmitate in lipid bilayers at various concentrations of the complexes albumin·palmitate at fixed ratios of palmitate/albumin (nmol/mol) of 0.5 (○) or (●). The data are in double reciprocal form. Reprinted with permission from *Biochemistry* 1985; *25*: 2013.

16). Thus, the uptake of fatty acids by the liver is analogous with charging a capacitor. The capacitor in this case is the plasma membrane. The voltage of the capacitor, which drives diffusion across the cell and hence determines uptake, has the form $V = kQ$. In terms of the uptake of fatty acids, Q is the equilibrium concentration of fatty acids in the plasma membrane, i.e. the distribution of fatty acids between albumin and membranes. The rate constant k is the rate constant for the release of fatty acid from membranes (Table 1). Therefore, the simple, physical system of fatty acid uptake can be shown to be saturable in kinetic experiments because the system consists of 2 phases. In the kinetic experiment, the size of the membrane phase is fixed; but the size of the albumin phase is varied purposely. For this reason the concentration of fatty acids in the membranes appears to reach a finite limit.

The uptake of fatty acids by the liver appears to be subject to inhibition (17–19). But this in fact is not the case because one cannot measure the actual rate of the uptake process, i.e., the initial rate at which fatty acid moves from albumin to plasma membranes.

The measurement of the rate of uptake of fatty acids by the liver, under steady state conditions, is in reality a measurement of the rate of metabolism of fatty acids (15, 16). This rate, as mentioned already, is "set" by the equilibrium distribution of fatty acids between albumin and plasma membranes. However, inhibitors of fatty acid metabolism will appear to be inhibitors of the uptake of fatty acids, if the experiment is carried out under steady state conditions. For this reason, inhibition of the rate of uptake of fatty acids by any compound must be shown not to affect rates of hepatic fatty acid metabolism before it can be considered to influence uptake *per se*. This has not been determined for any of the compounds that are alleged to inhibit the rate of uptake of fatty acids by the liver.

REFERENCES

1. Spector AA, Steinberg D, Tanaka A. Uptake of free fatty acids by Erlich ascites tumor cells. *J Biol Chem* 1965; 240: 1032.
2. Spector AA, Steinberg D. The utilization of unesterified palmitate by Erlich ascites tumor cells. *J Biol Chem* 1965; 240: 3747.
3. DeGrella RF, Light RJ. Uptake and metabolism of fatty acids by dispersed adult rat heart myocytes II. Inhibition by albumin and fatty acid homologues, and the effect of temperature and metabolic reagents. *J Biol Chem* 1980; 255: 9731.
4. Schwartz SE, Dietsdry JM Permeability characteristics of muscle membrane. *Biochim Biophys Acta* 1980; 600: 961.
5. Deuticke B. Properties and structural basis of simple diffusion pathways in the erythrocyte membrane. *Rev Physiol Biochem Pharmacol* 1977; 78: 1.
6. Doody MC, Pownall HJ, Kao YJ, et al. Mechanisms and kinetics of transfer of a fluorescent fatty acid between single-walled phosphatidylcholine vesicles. *Biochemistry* 1980; 19: 108.
7. Oldendorf WH. Lipid solubility and drug penetration of the blood brain barrier. *Proc Soc Exp Biol Med* 1974; 147: 813.

8. Eibl H-J. Phospholipids as functional constituents of biomembranes. *Angew Chem* 1984; 23: 257.
9. Honig BH, Hubbel W, Flewelling, RF. Electrostatic interactions in membranes and proteins. *Ann Rev Biophys Chem* 1986; 15: 163.
10. 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* 1978; 211: 1048.
11. Abumrad NA, Park JH, Park CR. Permeation of long-chain fatty acid into adipocytes. Kinetics, specificity, and evidence for involvement of a membrane protein. *J Biol Chem* 1984; 259: 8945.
12. Stremmel W, Strohmeyer G, Borchard F, et al. Isolation and partial characterization of fatty acid binding protein in rat liver plasma membranes. *Proc Nat Acad Sci USA* 1985; 82: 4.
13. Stremmel W, Berk PD. Hepatocellular uptake of sulfobromophthalien (BSP) and bilirubin is selectively inhibited by an antibody to the liver plasma membrane BSP/bilirubin binding protein. *J Clin Invest* 1986; 78: 822.
14. 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.
15. Noy N, Donnelly TM, Zakim D. Physical-chemical model for the entry of water-insoluble compounds into cells. Studies of fatty acid uptake by the liver. *Biochemistry* 1986; 25: 2013.
16. Cooper RB, Noy N, Zakim D. A physical-chemical model for cellular uptake of fatty acids: prediction of intracellular pool sizes. *Biochemistry* 1987; 26: 5890.
17. Van der Steen ATM, DeJong WAC, DeKruijff B, et al. Lipid dependence of glycophorin-induced transbilayer movement of lysophosphatidylcholine in large unilamellar vesicles. *Biochim Biophys Acta* 1981; 647: 63.
18. Berk PD, Potter BJ, Stremmel W. Role of plasma membrane ligand-binding proteins in the hepatocellular uptake of albumin-bound organic anions. *Hepatology* 1987; 7: 165.
19. Stremmel W. Translocation of fatty acids across the basolateral rat liver plasma membrane is driven by an active potential-sensitive Sodium-dependent transport system. *J Biol Chem* 1987; 262: 6284.

DISCUSSION

Schreiner (Washington, DC): as you know, one of the fascinating things for a Nephrologist is the formation of the cholesterol ester, cholesterol-palmitate, which forms doubly refractile crystals (DRFB's) in the urine. The cholesterol comes from the epithelial cells of the nephron. No one really knows why this happens. Could you guess or speculate from your experiments whether the presence of a low concentrate of albumin could facilitate the availability of cholesterol esters, or whether it's the high level of cholesterol that could facilitate the crystal formation.

Zakim: I would not want to speculate. It's a system we have not looked at. You're discussing a variety of factors that no one has looked at – such as what determines the rate of esterification of cholesterol, which is probably an event that occurs in the plane of the membrane. One point I would like to make from the data I presented is that nature is economical and will take advantage of whatever there is to take advantage of. One can explain with very simple chemistry what appears to be biological behavior in the uptake of fatty acids by cells. Another important point is one needs to know the quantitative behavior of the system you are studying. Once you have this you can calculate how the system will behave in a variety of different situations because it's simply chemical. With fatty acids and albumin and bilirubin we know from making very simple test tube experiments that we can predict how the system will behave quantitatively in an intact animal.