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A Physical–Chemical Model for Cellular Uptake of Fatty Acids: Prediction of Intracellular Pool Sizes†

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ABSTRACT: If the uptake of fatty acids by liver is a physical, not a biological, process, then the size and location of the intrahepatic pool of fatty acids can be predicted from uptake rates and thermodynamic data. The purpose of the experiments in this paper was to test the accuracy of this idea. Rat livers were perfused with palmitate bound to albumin, and the total amounts of palmitate removed from the perfusate were measured at 3-s intervals. The intrahepatic pools of palmitate calculated from these data were 13.8 and 23.0 nmol/g of liver at ratios of palmitate/albumin (mol/mol) (afferent side) of 2/1 and 4/1, respectively, in the steady state. The intrahepatic pools of palmitate calculated from the distributions of palmitate between membranes, H_2O, albumin, and fatty acid binding protein and the measured first-order rate constants for acyl-CoA ligases in mitochondria and microsomes were 12.1 and 34.6 nmol/g for perfusate ratios of palmitate/albumin of 2/1 and 4/1, in the steady state. Intrahepatic pools of palmitate measured after establishment of a steady-state rate of uptake were 15.0 and 31.8 nmol/g for these ratios of palmitate/albumin of 2/1 and 4/1.

Many water-insoluble compounds, such as long-chain fatty acids, bilirubin, and a number of drugs, circulate in blood bound tightly to albumin (Spector & Fletcher, 1978; Kamisaka et al., 1974). The mechanism for transfer of such compounds from albumin to the interiors of cells has been studied extensively (Spector et al., 1965; Spector & Steinberg, 1965; Mahadevan & Sauer, 1971, 1974; Samuel et al., 1976; DeGrella & Light, 1980; Abumrad et al., 1981, 1984; Stremmel et al., 1985; Weisiger et al., 1981a,b), but no consistent proposal for the mechanism has emerged. Results from early studies suggested that transfer of ligands from albumin to cellular membranes proceeded by simple diffusion (Spector et al., 1965; Spector & Steinberg, 1965). However, observations that uptake of fatty acids could be saturated (Abumrad et al., 1981, 1984; Stremmel et al., 1985; Weisiger et al., 1981a) and inhibited (Madhadevan & Sauer, 1971, 1974) were thought to be incompatible with a physical mechanism for uptake. This misconception (Noy et al., 1986) has led to the idea, now widely accepted, that uptake depends on a series of protein receptors (Abumrad et al., 1981, 1984; Madhadevan & Sauer, 1971, 1974; Samuel et al., 1976; Stremmel et al., 1985, 1986; Weisiger et al., 1981a). Moreover, the concept that hepatic uptake of compounds like fatty acids is mediated by a series of proteins fails to consider that these compounds are soluble in lipid bilayers, distribute readily from albumin to bilayers (Daniels et al., 1985), and rapidly traverse the latter (Deuticke, 1977; Doody et al., 1980; Eibl, 1984; Oldendorf, 1974; Noy & Zakim, 1985a; Noy et al., 1986).

As a first step in clarifying factors controlling the distribution of water-insoluble compounds in tissues and rates of uptake of these compounds into cells, we measured the spontaneous rates of the individual steps in the process of transfer of fatty acids from albumin to the interior of liver cells (Daniels et al., 1985; Noy & Zakim, 1985a; Noy et al., 1986). These observations provided a quantitative verification that the uptake of fatty acids, at least by liver, was a physical process (Noy et al., 1986). The purpose of the experiments in this paper was to test quantitative predictions about the size and intracellular locations of the intrahepatic pool of fatty acids, on the basis of the idea that transfer of fatty acids from al-

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bumin in plasma to the interior of live cells is a physicochemical process.

Materials and Methods

Uptake of Palmitate by Perfused Rat Liver. Male Wistar rats (Charles River Breeding Labs) weighing 350 g and fed a standard diet were used for the liver perfusion experiments in situ as described by Noy et al. (1986). The rats were anesthetized with methoxyflurane and placed on a warming blanket. The hepatic circulation was isolated by cannulation of the portal vein with a 16-gauge catheter (Deseret Angiocath) and cannulation of the inferior vena cava, through the right atrium, with a 16-gauge catheter. The abdominal inferior vena cava was tied off. In selected experiments, the common bile duct was cannulated. The perfusate was circulated by a peristaltic pump (LKB Multiperpex). After cannulation of the portal vein, the liver was flushed with 700 USP units of heparin and the perfusion begun at a rate of 8 mL/min. Following complete isolation of the liver, the flow rate was increased to 20 mL/min and equilibrated over 5 min with oxygenated Krebs–Henseleit buffer containing 5 mM glucose, 5 mM glutamate, 5 mM pyruvate, and 10 mM *N*-(2-hydroxymethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.4. The temperature at the outflow during perfusion was 35–38 °C. The viability of the livers was assessed by monitoring bile flow, which averaged 4 μL/min.

Perfusate solutions were prepared with 0.2 mM [^{14}C]albumin (New England Nuclear) to which was complexed either 0.4 or 0.8 mM [^{3}H]palmitate (New England Nuclear). After equilibration with 0.2 mM albumin (Sigma, bovine, essentially fatty acid free) in oxygenated Krebs–Henseleit buffer, test solutions were perfused in a single pass. Fractions of effluent were collected at 3-s intervals for 9 min. Aliquots of each fraction were counted in a liquid scintillation counter using Liquiscint (National Diagnostics). Channels for [^{14}C]albumin and [^{3}H]palmitate were used simultaneously. The test solution in the albumin washout experiments was replaced with 0.2 mM albumin, once the steady state was reached.

Analysis of Liver Lipids. To determine the content of free fatty acids and metabolic products in the liver, livers were perfused until a steady-state rate of uptake was established, which required 3–4 min. A portion of liver then was frozen rapidly during the perfusion using an aluminum block clamp (Small Parts, Inc.) precooled to the temperature of liquid nitrogen. Blocks of liver frozen in this way were stored at −80 °C. Frozen blocks of liver were homogenized, after addition of the extraction mixture (Kates, 1972), with a Polytron homogenizer. Classes of lipids, in the extracted fractions, were separated by thin-layer chromatography (Kates, 1972). Plates were chromatographed to a front of 135 cm using ethyl ether/benzene/ethanol/acetic acid, 40/50/2/0.2 (v/v). After the plates were dried, they were rechromatographed in the same direction using ethyl ether/hexane, 6/94 (v/v). The final chromatography was at 90° to the original direction using petroleum ether/ethyl ether/acetic acid, 90/15/1.5 (v/v). Test plates were scraped according to R_f's determined on control plates for authentic standards of palmitate, dipalmitin, tripalmitin, cholesterol palmitate, and dipalmitoylphosphatidylcholine (Sigma). Control plates were sprayed with sulfuric acid/dichromate solution (Kates, 1972) and charred to determine R_f's. Portions of rapidly frozen liver also were homogenized and counted for [^{14}C]albumin to correct for palmitate bound to albumin in the vascular space.

Activity of Long-Chain Fatty-Acyl-CoA Ligase. Rat liver microsomal fatty-acyl-CoA ligase was assayed as described in Noy and Zakim (1985a). The values of V_{max}^{app} were determined graphically from assays of activity at variable concentrations of palmitate and constant concentrations of ATP and CoA, which are believed to be present in the cytosol of liver cells in situ (Siess et al., 1978).

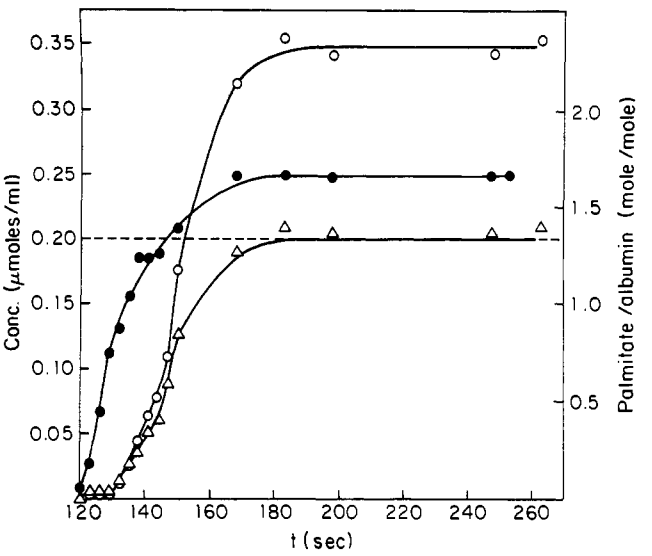

FIGURE 1: Uptake of palmitate by perfused rat liver. Concentrations (millimolar) of [^{3}H]palmitate (○) and [^{14}C]albumin (△) in the effluent are shown for the initial rise and steady state. The concentration of albumin was 0.2 mM throughout, (---) with the ratio of palmitate to albumin (●, right scale) becoming constant in the steady state. The liver in this experiment was perfused with Krebs–Henseleit buffer containing 0.2 mM albumin. At $t = 0$, this solution was exchanged for one containing 0.2 mM [^{14}C]albumin complexed with 0.4 mM [^{3}H]palmitate. Fractions of the effluent were collected at 3-s intervals and analyzed for radioactivity.

Results

Amount of Free Palmitate in the Liver at Steady State Can Be Calculated from Uptake Data. The model proposed by Noy et al. (1986) predicts that the pool of free fatty acids in liver cells can be calculated from uptake data alone. This is so because there is a quantitative relationship between the equilibrium distribution of fatty acid between albumin and plasma membranes (K_{eq}) and the amount of fatty acid (FA) metabolized at any instant (Noy et al., 1986). Consider the following analysis. The liver contains no labeled palmitate at the onset of a perfusion experiment, but it is equilibrated with albumin. The time period from the onset of perfusion with the albumin–FA complex to establishment of a steady rate of uptake may be characterized as the time it takes for the albumin–FA complex, in the perfusate, to mix completely and evenly in the hepatic vascular bed. There will be, in the pre-steady-state period, a decreasing proportion of palmitate removed from albumin as one gets closer to the steady state. Thus, the palmitate/albumin ratio in the effluent collected will increase to a constant value, which is reached at the onset of the steady state. The concentration of albumin in the effluent is constant through the entire experiment. If [^{3}H]palmitate is bound to [^{14}C]albumin, the changing ratio of fatty acid/albumin, during the mixing period, can be monitored in the effluent as well as the amounts of palmitate taken up by the liver at each moment during the period of mixing. Results of a typical experiment are shown in Figure 1.

The liver in Figure 1 was perfused with a solution containing 0.2 mM albumin but not palmitate. At time zero, the perfusate was switched to a mixture containing [^{14}C]albumin and [^{3}H]palmitate. The ratio of palmitate to albumin (mol/mol) was 2 in the input side of the perfusate and was constant over the course of the whole experiment. The data in Figure 1 allow

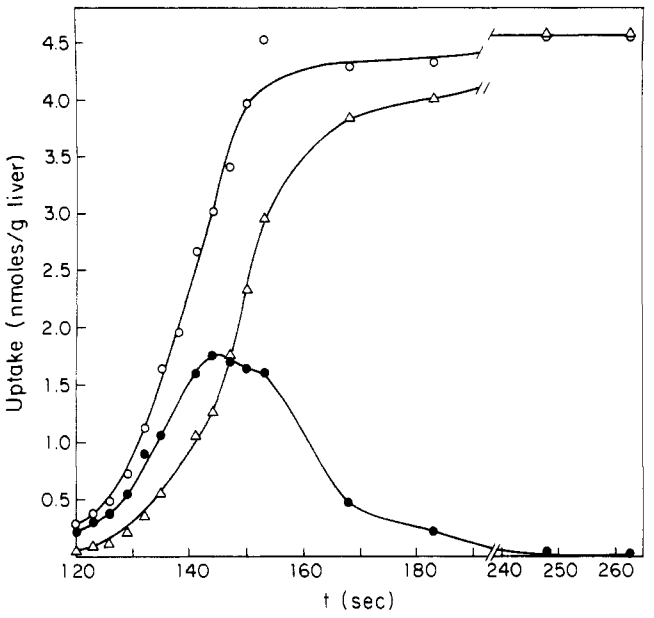

FIGURE 2: Uptake of palmitate in components of total uptake. Total uptake of palmitate at each time point (○) was measured by analysis of effluent fractions of the experiment depicted in Figure 1. Uptake due to metabolism (△) was obtained from uptake rates observed under steady-state uptake conditions at varying palmitate/albumin ratios (see text). Accumulation of palmitate during the rise to steady state (●) was calculated by subtracting the uptake due to metabolism from total uptake.

one to calculate the total amount of palmitate that was taken up by the liver. The problem is to determine, during the pre-steady-state period, how much of the fatty acid was metabolized and how much was accumulated by the liver. This is done by using the linear relationship between values for K_{eq}, the equilibrium distribution of palmitate between albumin and plasma membrane, and rates of metabolism of palmitate (Noy et al., 1986).

Since the distribution of palmitate between albumin and plasma membranes will be at equilibrium at each moment during the period of mixing (Noy et al., 1986), the pre steady state can be considered to represent a series of instantly changing steady states, in which the metabolism of fatty acid is determined, at each instant, by the "equilibrium"concentration of palmitate in plasma membranes. It follows that the amount of palmitate accumulating in the liver as free palmitate in the pre-steady-state period can be calculated from eq 1.

$$(\text{palmitate})_{\text{accumulated}} = \sum_{1}^{T/\Delta t} [(\text{uptake})_t - (\text{uptake})_m]\Delta t \quad (1)$$

T in eq 1 is the mixing period until uncomplexed albumin is cleared from the liver, and Δt is the collection time for each fraction; $(\text{uptake})_t$ is the total amount of palmitate that was taken up by the liver; $(\text{uptake})_m$ is the sum of the amounts of palmitate metabolized in each fractional time period during the pre steady state, and $(\text{uptake})_m$, at each instant, can be calculated from a plot of the linear relationship between total uptake per minute per gram in the steady state for perfusion of livers with varying ratios of palmitate/albumin (Noy et al., 1986). This relationship was determined separately for the livers used in the present experiments. In addition, we determined in separate experiments that once the steady state was achieved, there was no further accumulation of palmitate as free palmitate. These results validate that $(\text{uptake})_t = (\text{uptake})_m$ in the steady state.

Table I: Uptake of Palmitate by Perfused Livers during Approach to Steady State[a]

palmitate/albumin ratio (mol/mol)	total uptake	amount metabolized	amount accumulated
2	70.97 ± 5.6 (7)	57.13 ± 5.6	13.80
4	133.26 ± 13.2 (6)	110.32 ± 13.7	23.00

[a] Livers were perfused with solutions of 0.2 mM (^{14}C]albumin complexed with 0.4 or 0.8 mM [^{3}H]palmitate. Uptake and the palmitate/albumin ratio in the effluent were measured every 3 s until a steady-state rate of uptake was achieved. The amount metabolized at each point during the approach to equilibrium was calculated from steady-state values for the quantitative relationship between metabolism of palmitate and the equilibrium distribution of palmitate between plasma membranes and albumin at variable molar ratios of palmitate/albumin, as described in the text. Values are given as nanomoles per gram of liver (mean ± SEM) for 6 or 7 livers (in parentheses).

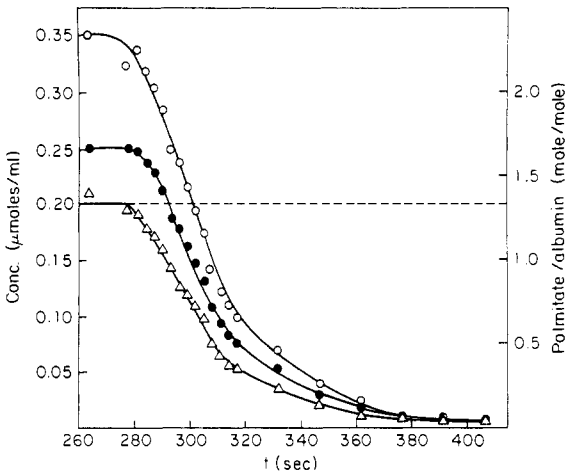

FIGURE 3: Perfusion with unlabeled 0.2 mM albumin during the steady state. The liver was perfused with a solution containing 0.2 mM [^{14}C]albumin complexed with 0.4 mM [^{3}H]palmitate until a steady-state uptake rate was established. The perfusion solution then was exchanged for one containing 0.2 mM unlabeled albumin. No palmitate was added. Fractions of the effluent were collected at 3-s intervals until all [^{14}C]albumin was washed out of the liver. Concentrations (millimolar) of [^{3}H]palmitate (○) and [^{14}C]albumin (△) in the effluent were shown for the period during which they are depleted from the liver by perfusing with unlabeled albumin. The concentration of albumin (---) was 0.2 mM throughout. The ratio of palmitate to albumin (●, right scale) decreases from a constant value in the steady state.

The amounts of palmitate taken up by liver at 3-s intervals during the pre-steady-state period are plotted in Figure 2. The calculated amounts of palmitate metabolized at each 3-s interval are shown too, as are the amounts of palmitate accumulating as free palmitate in the liver. The integrated amounts for uptake, metabolism, and accumulation are shown in Table I. Data are shown for several experiments under two different conditions: palmitate/albumin ratios in the perfusate of 2 and 4.

A second method for predicting the size of the intrahepatic pool of palmitate from uptake data is to do an experiment that is the mirror image of that in Figures 1 and 2. Rat livers were perfused with solutions of [^{14}C]albumin containing [^{3}H]palmitate at mole ratios of 2 or 4. After a steady-state rate of uptake was established (4–5 min), the perfusate was exchanged for a solution containing albumin but no palmitate. Perfusion with this solution was continued until all [^{14}C]albumin and [^{3}H]palmitate were cleared from the liver. The same considerations apply to this experiment as to the one in Figure 1. Measuring the amounts of both [^{14}C]albumin and [^{3}H]-palmitate in fractions at the outflow gives the continuously

Table II: Uptake of Palmitate by Perfused Livers during Albumin Washout[a]

palmitate/albumin ratio (mol/mol)	total uptake	amount metabolized	amount stored as palmitate
2	20.27 ± 5.9	33.15 ± 7	−12.81
4	90.0 ± 26	112.6 ± 30	−22.5

[a] Livers were perfused with [^{14}C]albumin–palmitate complexes containing either 2 or 4 mol of [^{3}H]palmitate per mol of albumin. The concentration of albumin was 0.2 mM. After the steady state was achieved, in terms of constant rate for the uptake of palmitate, the perfusate was switched to unlabeled albumin free of palmitate. Samples of the effluent were collected at intervals of 3 s; the zero time for the data shown was when the perfusate was switched to unlabeled albumin. Uptake was measured from analysis of effluent samples and compared to data for steady-state values at each point of the washout, as discussed in the text. Values are given as nanomoles per gram of liver (mean ± SEM) for four different livers perfused with palmitate/albumin ratio of 2/1 and three livers perfused with palmitate/albumin ratios of 4/1.

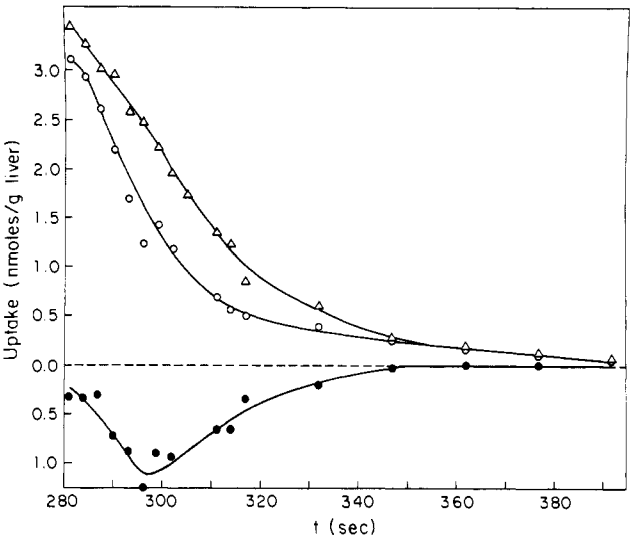

FIGURE 4: Palmitate uptake during albumin washout. Total palmitate uptake at each time point (○) was measured by analysis of effluent fractions from the experiment depicted in Figure 3. Uptake due to metabolism (△) was obtained from uptake rates observed under steady-state uptake conditions at varying palmitate/albumin ratios (see text). Subtraction of uptake due to metabolism from total uptake (●) shows "negative" uptake, reflecting depletion of a fatty acid pool during the washout period.

decreasing value of the palmitate/albumin ratio during the washout period. These data are shown in Figure 3. The amount of palmitate taken up by the liver during the washout period can be determined from the data in Figure 3. Subtracting the uptake due to metabolism at the various mole ratios of palmitate/albumin along the curve allows for the quantitation of palmitate that is being depleted from the storage pool in the liver during the washout period. These data are shown in Figure 4. The second component is found to be constituted of "negative" values, as expected. The physical significance of the negative sign is to indicate that the storage compartment decreased. Presented in Table II is the total amount of stored palmitate that was calculated to be metabolized during the washout period, calculated by using eq 1.

Amount of Free Palmitate in Liver. In order to measure the amount of a fatty acid in liver during steady-state rates of uptake, rat livers were perfused with solutions containing 0.20 mM [^{14}C]albumin to which was complexed [^{3}H]palmitate at ratios of palmitate/albumin (mol/mol) of 2 or 4. The livers were perfused until steady-state rates of uptake were established (3–4 min), as determined by monitoring [^{3}H]palmitate in the effluent of the perfusate. They then were frozen rapidly as described under Materials and Methods. Lipids were extracted from weighed amounts of each liver and separated by class by thin-layer chromatography (see Materials and Methods). Zones corresponding to authentic lipids were scraped into scintillation vials and counted for [^{3}H]palmitate. To correct for the amount of palmitate that was bound to albumin in the vascular space in the liver, a weighed amount of liver was homogenized and counted for [^{14}C]albumin. From the known ratio of palmitate to albumin in the perfusate, on the effluent side, the amount of palmitate in the vascular space was obtained. This was subtracted from the total amount of free palmitate found in the extracts.

Shown in Table III are the amounts of free palmitate and esterified products in liver shortly after steady-state rates of uptake were established. The pool sizes of free palmitate were 15.0 and 31.8 nmol/g of liver in livers perfused with solutions containing palmitate–albumin complexes at mole ratios of 2 and 4, respectively. These measured values are quite close to values of the pool of fatty acids estimated by the analyses in Figures 1 and 2. The correspondence of the measured and predicted values validates the utility of the model for uptake that was proposed previously (Noy et al., 1986). Not only does the model predict the amount of free palmitate in liver under steady-state conditions for uptake but also it shows that this amount depends on the ratio of fatty acid to albumin in perfusates. The ratio of the pools of free fatty acids in livers perfused with 2 or 4 mol of fatty acid per mole of albumin is essentially what is predicted from the physical model.

Location and Size of Intracellular Pools of Palmitate. The intracellular pool of free palmitate will be located in the plasma membrane of the liver cells, the internal membranes such as endoplasmic reticulum and mitochondria, free in cytosol, and in cytosol bound to proteins. The sizes of these pools can be estimated, by using the model, as follows. The amount of palmitate in the plasma membranes can be calculated from the K_{eq} for the distribution of palmitate between albumin and plasma membranes as a function of the ratio in the steady state of palmitate/albumin in the effluent of the perfusate. These values were 1.7 and 3.6 for perfusion with palmitate/albumin ratios of 2 and 4, respectively. The partitioning of fatty acids into plasma membranes is due only to the lipids of the membrane (Cooper et al., submitted for publication). Calculation of the total amount of palmitate in plasma membranes is based

Table III: Concentrations of Palmitate and Its Metabolites in Perfused Liver[a]

palmitate/albumin ratio (mol/mol)	phospholipid	di-/triglycerides	cholesteryl palmitate	palmitate	total palmitate uptake
2	29 ± 2.2	16.8 ± 2.8	2.9 ± 0.6	15 ± 1.5	160 ± 25
4	64.5 ± 10.6	22.6 ± 5.1	3.6 ± 0.6	31.8 ± 2.8	262 ± 28

[a] Livers were perfused with solutions containing [^{3}H]palmitate and [^{14}C]albumin at molar ratios of 2 or 4, as described under Materials and Methods. The concentration of albumin was 0.2 mM. When steady-state rates of uptake were reached, the livers were frozen rapidly by clamping with aluminum blocks cooled in liquid nitrogen. The lipid content in frozen samples was analyzed as described under Materials and Methods. Values are nanomoles per gram of liver and are means ± SEM (N = 4). The differences between total uptake of palmitate and amounts found in the liver are attributed to oxidation.

Table IV: Concentrations of Palmitate in Intracellular Membranes and Hepatic Cytosol[a]

	palmitate/albumin ratio (mol/mol)			
	2/1		4/1	
hepatic fraction	nmol/g of liver	mol %	nmol/g of liver	mol %
plasma membranes	12.1	0.38	33.7	1.05
mitochondria	0.357	0.0025	0.485	0.0035
microsomes	0.214	0.0011	0.290	0.0014
free palmitate in cytosol	0.003		0.008	
protein-bound palmitate in cytosol	0.030		0.080	
total calcd	12.8		34.6	
total obsd	15.0		31.8	

[a] The concentrations of palmitate in the membranes of endoplasmic reticulum and mitochondria were calculated from steady-state rates of uptake and the kinetic constants for palmitoyl-CoA ligase assayed with physiologic concentrations of ATP and CoA as described under Materials and Methods. The concentration of palmitate in plasma membranes was calculated from the known equilibrium distribution for palmitate between plasma membranes and albumin for the given ratios of albumin/palmitate. Palmitate in cytosol was calculated from the known equilibrium distributions of palmitate between microsomes and H_2O and between plasma membranes and H_2O (see text). The concentrations for palmitate are given as nanomoles per gram and as mole percent (moles of palmitate per mole of membrane phospholipid).

on the total amount of phospholipids in plasma membranes since the amount of cholesterol in plasma membrane has a constant relationship to the amount of phospholipids. It was estimated that 50% and about 8% of total phospholipids were associated with endoplasmic reticulum and with the plasma membranes of the hepatocyte, respectively (Cook, 1958). The amount of phospholipids in the endoplasmic reticulum membranes was estimated to be 15 mg/g of liver (DePierre & Dallner, 1975). From these values, one can calculate that there are about 2.4 mg or 3200 nmol of phospholipid in plasma membranes per gram of liver. The amounts of palmitate calculated to be in plasma membranes are shown in Table IV.

The amounts of palmitate in the endoplasmic reticulum (ER) and mitochondria (mito) were calculated as follows. Under the condition of steady-state uptake of palmitate by the liver, the uptake of fatty acids must equal flux through the acyl-CoA ligase catalyzed reaction. The flux of fatty acids through the ligase-catalyzed reactions can be used, therefore, to assess the concentrations of palmitate involved in the following binding steps:

$$\text{ligase}_{ER} + FA \rightarrow \text{ligase}_{ER}\text{–}FA$$

$$\text{ligase}_{mito} + FA \rightarrow \text{ligase}_{mito}\text{–}FA$$

This is done by solving for [S] in the expression $v = V_{max}/K_m[S]$. Values of $V_{max}^{apparent}$ and $K_m^{palmitate}$ were determined for microsomes and mitochondria by using concentrations of ATP and CoA believed to occur in situ (Siess et al., 1978). The data in Table III show that about 33% of the total flux of fatty acids is accounted for by esterification. We attributed this fraction of metabolism to be due to flux through the ligase in endoplasmic reticulum. The remainder of the flux was attributed to metabolism by ligase in mitochondria. The values of v used to calculate [S] were $0.33(\text{uptake})_t$ and $0.67(\text{uptake})_t$ in endoplasmic reticulum and mitochondria, respectively.

As for plasma membranes, only the phospholipids of mitochondria and microsomes are important for partitioning of fatty acids into these organelles (Cooper et al., unpublished results). The amounts of phospholipid in endoplasmic reticulum and mitochondria per gram of liver were taken as 20 and 14 μmol, respectively (Cook, 1958; DePierre & Dallner, 1975). Calculated values of palmitate in these intracellular compartments are given in Table IV. The equilibrium distribution (ratio of mole fractions) of palmitate was 0.81 between mitochondrial outer membranes and plasma membranes, 0.94 between endoplasmic reticulum and plasma membranes, and 1.16 between endoplasmic reticulum and mitochondrial outer membranes.

The concentration of palmitate in cytosol adjacent to the plasma membrane was calculated on the basis of the partition coefficient for palmitate between lipid bilayers and water (Noy & Zakim, 1985b). Similarly, cytosolic concentrations for palmitate were calculated for the region adjacent to internal membranes. A linear gradient was assumed for the intracellular distribution of palmitate across a distance of 0.5×10^{-3} cm, which is the radius of the hepatocyte. The total free palmitate was found by integration. The palmitate bound to intracellular fatty acid binding protein was calculated on the basis of a K_d of 10^{-5}, a concentration of the protein of 10^{-4} M (Ockner et al., 1982), and the gradient of fatty acid concentrations given above. We assumed that the fatty acid binding protein was distributed uniformly in cytosol. This may not be the case (Fournier & Rahim, 1985), but the assumption facilitates giving an order of magnitude to the upper limit of fatty acids bound to this protein. The calculated amounts of palmitate in cytosol are given in Table IV.

The total calculated amounts of palmitate in liver at the steady state compare favorably with the measured amounts at palmitate/albumin ratios of 2 and 4. The closeness of calculated and observed amounts of intrahepatic palmitate validated the analyses used for assessing the concentrations of palmitate in mitochondria and endoplasmic reticulum.

Discussion

Transfer of Fatty Acids from Plasma to Cells. Experimental evidence in relevant model systems indicates that there is no basis for supposing that uptake of hydrophobic substances at the plasma membranes of cells will be a "biological process". It has been demonstrated, by using a variety of systems, that water-insoluble compounds, whether charged or not, move rapidly into lipid bilayers. Organic anions also move spontaneously across lipid bilayers at rates that are fast as compared with rates of intracellular metabolism (Almgren & Swarup, 1982; Benz et al., 1976; Honig & Hubbel, 1986; Jordan & Stark, 1979; Doody et al., 1980; Van Dail & Centricky, 1984; Wolkowicz et al., 1984). Accordingly, we have undertaken in this and prior publications (Daniels et al., 1985; Noy et al., 1986) a systematic study of whether the physical–chemical properties of fatty acids in mixed lipid/water systems could account for the observed rates of uptake in perfused livers. The data in Noy et al. (1986) show directly that the rate of uptake of fatty acids by perfused liver can be accounted for by the physical–chemical properties of fatty acids in model systems and that this process need not depend on specialized biological mechanisms in plasma membranes or the cytosol of cells. The present experiments provide further evidence that a physical–chemical model for the uptake of palmitate by the liver can predict the rate of uptake, the disposition of uptake between metabolism and storage, and the size and location of the intrahepatic store of free palmitate. Admittedly, several assumptions were made in order to calculate the disposition and concentrations of palmitate in different intracellular locations. We believe, however, that these are reasonable. Thus, the calculations of the intracellular location of palmitate are based primarily on the assumptions that (i) the thermodynamic properties of cellular membranes are the same in the cell as for the isolated membranes and that

(ii) the kinetic properties of acyl-CoA ligase are the same in endoplasmic reticulum or mitochondria in cells and in freshly isolated microsomes or mitochondria, respectively. There is no reason to believe that either of these assumptions can be questioned. It is highly unlikely, therefore, that the close correspondence between predicted and observed values for storage of palmitate in perfused livers is coincidental. The amounts of phospholipids in different cellular membranes are not known exactly. In addition, the concentrations of ATP and CoA used to obtain V_{max}^{app} and $K_m^{palmitate}$ may not be the exact concentrations occurring in the perfused liver. Uncertainties in these quantities do not alter the major implications of the data, however.

Our view of the problem of uptake of organic compounds, as it relates to fatty acids, is that the liver cell really has no biological mechanism for the uptake of fatty acids. Fatty acids dissolve readily in lipid bilayers, and because a large fraction are protonated at physiologic pH (Small et al., 1984), fatty acids will "flip-flop" readily from one half of a bilayer to another. We think it is important to keep in mind that by contrast with the uptake of polar substances, the problem faced by the cell is not how to get apolar compounds in but how to keep them out of the plasma membrane and the interior of the cell. Therefore, to ensure the viability of the cell, there must be a mechanism to minimize the fatty acid content of plasma membranes (Pande & Mead, 1986; Wojtczak, 1979). This mechanism is provided by the high avidity of albumin for fatty acids.

The distribution of fatty acids between outer and inner halves of the plasma membrane may depend to a small extent on how membrane-bound proteins penetrate into the apolar regions of a bilayer since proteins, at least in plasma membranes, decrease by about 15–20% the extent to which fatty acids will partition into the lipids of the membrane (Cooper et al., submitted for publication). This effect of proteins on the equilibrium distribution of fatty acids in biological membranes vs. lipid bilayers of the same lipid composition will not alter significantly the rate of flux of fatty acids from the outer to the inner half of the plasma membrane and the subsequent spontaneous rate of hydration of fatty acids in cytosol.

Problem of Diffusion of Fatty Acids between Intracellular Compartments. It has been proposed that transport of fatty acids in the cytoplasm is facilitated (Ockner et al., 1982). We dismissed this possibility in an earlier publication because of a gross error in calculating the amount of fatty acids bound to the fatty acid binding protein in cytosol (Noy et al., 1986). The value calculated in Noy et al. (1986) for the concentration of fatty acids in cytosol bound to fatty acid binding protein is really the fraction of this protein with bound fatty acid. This error is corrected by the data in Table IV, which show that almost all the fatty acids in cytosol are bound to the so-called "fatty acid binding protein". The physiologic significance of this bound fraction of fatty acids is unclear, however, because there remains a major difficulty in explaining the intracellular movement of fatty acids. The data in Table IV, for example, indicate that the metabolism of palmitate in liver is diffusion-limited, and since there is no certain way to calculate the distribution of fatty acid binding protein in the cell (Fournier & Rahim, 1985), it is difficult to decide the contribution of complexes between fatty acid and binding protein to flux across the cytoplasm. However, this is only a part of the problem of understanding how a compound like palmitate moves across the cytosol. For example, the endoplasmic reticulum nearly touches the plasma membrane at many sites (Jones & Fawcett, 1966), which provides a short path for transfer of fatty acid, via cytosol, from plasma membrane. In addition, diffusion of fatty acid in endoplasmic reticulum should be fast because of the reduction of diffusion from three to two dimensions (Adam & Delbruck, 1968; Eigen, 1974) and the relatively high concentration of fatty acid in these membranes (about 0.05 M). However, the data for the amount of fatty acids inside the cell are not compatible with the idea that fatty acids "flow" through the endoplasmic reticulum. If this were the case, the livers would have contained vastly greater amounts of free palmitate in the intracellular compartments. Whatever the explanation for the apparent limitation on diffusion in the cells, we think the data in this paper suggest that the question of diffusion of fatty acids inside cells, like that for the uptake process per se, should be cast in a new context. The problem of understanding the intracellular diffusion of fatty acids (and perhaps similar compounds) has been considered until now to be finding a pathway for this process. The real problem may be to account for why diffusion is as slow as it is.

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Registry No. Palmitic acid, 57-10-3; cholesteryl palmitate, 601-34-3.

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Modulation of Monomer-Polymer Equilibrium of Phosphorylated Smooth Muscle Myosin: Effects on Actin Activation†

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ABSTRACT: Actin activation of the adenosinetriphosphatase (ATPase) of phosphorylated gizzard myosin at low (2 mM) free Mg^{2+} concentration and 50 mM total ionic strength continues to increase on raising the free Ca^{2+} concentration near pCa 3. Similar levels of activity can be obtained by increasing the free Mg^{2+} concentration to a higher (in excess of 4 mM free) concentration. In the presence of micromolar concentrations of free Ca^{2+} and low free Mg^{2+} concentration, the actin-activated adenosine 5'-triphosphate (ATP) hydrolysis exhibits an initial rapid rate which progressively slows to a final, lower but more linear rate. In the presence of high divalent cation concentrations, the fast rate of ATP hydrolysis is maintained during the entire ATPase assay. The ionic conditions which favor the slow rate of ATP hydrolysis are correlated with increased proportions of folded myosin monomers while higher rates of ATP hydrolysis are correlated with increased levels of aggregated myosin. Elevating the thin filament proteins to saturating concentrations does not abolish the change in ATPase rate or the final distribution of myosin aggregates and monomers; however, the stability of the myosin aggregates is enhanced by the presence of thin filament proteins in low divalent cation conditions. The nonlinear profile of the actin-activated ATP hydrolysis in low divalent cation concentrations is eliminated by utilizing nonfilamentous, phosphorylated heavy meromyosin. The data presented indicate that Ca^{2+} and Mg^{2+} alter monomer-polymer equilibrium of stably phosphorylated myosin. The alteration of monomer-polymer equilibrium by Ca^{2+} at low Mg^{2+} concentration modulates ATPase rates.

Actin activation of the Mg·ATPase[1] activity of smooth muscle myosin requires the phosphorylation of the 20 000-dalton light chains of myosin by myosin light chain kinase (Gorecka et al., 1976; Chacko et al., 1976; Sobieszek & Small, 1977). Studies using purified phosphorylated myosin demonstrate that the maximal activation of Mg·ATPase by actin requires Ca^{2+} and tropomyosin (Chacko et al., 1977; Rees & Frederickson, 1977; Chacko & Rosenfeld, 1982; Nag & Seidel, 1983). The level of Ca^{2+} concentration required for actin in the presence of tropomyosin to maximally activate the Mg·ATPase of phosphorylated myosin (Chacko et al., 1986) is higher than that required for myosin light chain kinase activity

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[1] Abbreviations: ATP, adenosine 5'-triphosphate; ATPase, adenosinetriphosphatase; EDTA, (ethylenedinitrilo)tetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; MOPS, 3-(*N*-morpholino)propanesulfonic acid; HMM, heavy meromyosin; PMSF, phenylmethanesulfonyl fluoride; FPLC, fast protein liquid chromatography; P_i, inorganic phosphate; *s*, sedimentation coefficient; TCA, trichloroacetic acid.

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