Uptake and Metabolism of Fatty Acids by Dispersed Adult Rat Heart Myocytes

I. KINETICS OF HOMOLOGOUS FATTY ACIDS*

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An adult rat heart myocyte preparation was used to study the uptake and metabolism of the L-14C-labeled free fatty acids decanoate, laurate, myristate, palmitate, and oleate at 37°C in the absence of serum albumin. The rate of total uptake consisted of both a nonsaturable and a saturable component. The nonsaturable component corresponded to the fatty acid accumulating in the free fatty acid fraction, and the rate of this accumulation increased logarithmically as a function of chain length. The saturable component corresponded to that portion of fatty acid converted to the only detectable metabolic products: CO₂, triglyceride, and polar lipid. The reluctance product distribution did vary with that portion of fatty acid converted to the only polar lipid. The Kₘ for this process was about 1 μM and was independent of chain length. The Vₘₐₓ for the saturable component varied only slightly with chain length, from 20 ± 1 nmol/h-mg of cell protein for decanoate to 47 ± 18 nmol/h-mg of cell protein for palmitate. The relative product distribution did vary with chain length, however, ranging from primarily carbon dioxide for decanoate to approximately equal quantities of carbon dioxide, triglyceride, and polar lipid for palmitate.

Two internal pools of free fatty acid are postulated: a minor pool that equilibrates rapidly with external fatty acid and serves as the precursor for fatty acid activation, and a major pool containing most of the accumulated free acid. These two pools are interconvertible. The data support a simple diffusion or membrane-partitioning process for the accumulation of fatty acid in the second pool. The data presented in this paper are not sufficient to distinguish between a simple diffusion or a carrier-mediated process for uptake into the first pool. The saturation kinetics observed appear to represent a metabolic step such as fatty acid activation, rather than a transport carrier.

Evidence of toxicity at a higher concentration of the longer chain fatty acids limits the concentration range that can be studied in the absence of albumin. Decanoate did not appear to be toxic at concentrations up to 300 μM, but laurate at 10 μM and myristate at 5 μM appeared to uncouple respiratory control.

The metabolism of lipids by the heart is of particular importance since fatty acids, either as circulating free fatty acids or as triglyceride, are the preferred substrates for energy production (1, 2). The overall rate of free fatty acid utilization by heart muscle is determined by the supply of fatty acid to the heart and the energy requirements of the organ (2-5). Triglyceride is believed to be hydrolyzed in the capillary lumen by lipoprotein lipase, with subsequent uptake of free fatty acid (2, 6, 7).

There are conflicting reports about the mechanism of the movement of fatty acids across the plasma membranes of cells (5). It is generally believed that the first step in uptake involves an energy-independent reversible binding of the fatty acid to the cell surface (8). Whether the actual translocation across the plasma membrane involves diffusion or a carrier-mediated process is unresolved (8, 9).

The present study was undertaken to investigate the kinetic behavior of fatty acid uptake by dispersed heart myocytes in an attempt to determine the mechanism of uptake. This cell preparation, originally described by Glick et al. (10), appears to be well suited for the study of the movement of lipid molecules across plasma membranes. The majority of fatty acid that is taken up by the heart is oxidized to CO₂ and esterified to triglyceride or polar lipids, and consequently the total flux of fatty acid into the cell can easily be measured. Cell suspensions are easily manipulated. In particular, the substrate can be presented directly to the cell, thus eliminating the multiple barriers that the substrate would encounter in passing from the vascular system to the target cell in vivo or in the perfused heart.

In this paper, the saturation kinetics of four fatty acids are studied in the absence of serum albumin. The data cannot distinguish between carrier-mediated and diffusion mechanisms, and two models are presented which could explain the results. In the following paper (11), the effects of albumin, fatty acid inhibition, metabolic inhibitors, and temperature are studied, and the results of that work support the diffusion model presented in this paper.

MATERIALS AND METHODS

1Portions of this paper, including "Materials and Methods" and Figs. 1 to 6 and 8, are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 79M-2561, cite authors and include a check or money order for $2.10 per set of photocopies. Full-size photocopies are also included in the microfilm edition that is available from Waverly Press.

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Characterization of the Rat Heart Preparation—Final cell yields of approximately 15% of the ventricular weight were routinely obtained. The viability of the dispersed heart myocyte preparations ranged from 83 to 99% on the basis of trypan blue exclusion. Microscopic examination of the cell preparations revealed almost exclusively myocytes, with very few red blood cells and vascular cells. The metrizamide step eliminated mainly cellular debris and reduced the number of red blood cells and vascular cells slightly. Approximately 60% of the trypan blue-excluding cells were elongated, similar in morphology to those found in intact tissue. The remaining cells were shortened with some round cells present. In the presence of 1 mM Ca²⁺ at 37°C, the number of elongated cells dropped rapidly, but the cells were still able to exclude trypan blue.

Other investigators have also noted this phenomenon and concluded that when Ca²⁺ is maintained below 10⁻⁵ M, the myocyte preparations are suitable for metabolic studies (17, 18).

After 1 h at 37°C, the isolated cells leaked approximately 20% of the total lactate dehydrogenase activity released by sonication of the cells. Cells vortexed for 1 min could no longer exclude trypan blue or metabolize glucose or palmitate, and leaked approximately 75% of the total lactate dehydrogenase. These data suggested that membrane integrity, trypan blue exclusion, and metabolic activity are related. Consequently, all data reported for the metabolism of substrates have been normalized to 100% viable cell population.

The oxidation of glucose by the myocytes was linear for at least 30 min at a rate of approximately 60 nmol of glucose oxidized/h-mg of cell protein. This value compares favorably with the range of 25 to 45 nmol of glucose oxidized/h-mg of cell protein reported by Glick et al. (19). The rate of oxidation was saturable with a Km of 0.56 ± 0.05 mM.

The uptake of oxygen by the isolated myocytes depended on the substrate and the metabolic state of the cells. In the presence of 5 mM glucose at 37°C, oxygen uptake was 2.9 ± 1.0 nmol of oxygen/h-mg of cell protein. Addition of 50 μM dinitrophenol stimulated oxygen consumption to 300% of the control rate. Oxygen uptake in the presence of succinate (15 mM) and rotenone (0.13 mM) was approximately 0.27 nmol of oxygen/h-mg of cell protein. The addition of 6.7 μg/ml of digitonin, which disrupts the plasma membrane, caused the oxygen consumption to increase to 2.0 nmol of oxygen/h-mg of cell protein. These results indicate that succinate is excluded by the plasma membrane, and that no more than 14% of the cells were permeable to succinate, a number comparable to the per cent of trypan blue-staining cells.

Data are reported in terms of milligrams of cell protein.

There were approximately 7.1 mg of wet tissue/mg of cell protein, a factor which can be used to convert the data to a milligram of wet tissue basis for comparison with studies on intact heart.

Time Course of Fatty Acid Uptake—The time course of uptake and utilization of decanoate, laurate, myristate, palmitate, and oleate were run at various fatty acid concentrations ranging from 0.2 to 30 μM. Fig. 1A shows that the rate of uptake and utilization of decanoate at 30 μM is linear up to 30 min. This linearity in fatty acid uptake and utilization was not found at all concentrations with each of the fatty acids studied. For comparison, the uptake and utilization of oleate at 25 μM is shown in Fig. 1B. In general, the period of linear uptake was longer for the shorter chain fatty acids, and for a given fatty acid was longer at lower fatty acid concentration. It may be that this fall-off in rate is the result of a toxic effect of the fatty acids on the cells. It is known that fatty acids can uncouple oxidative phosphorylation and cause leakage of respiratory factors in mitochondria (20), affect membrane fluidity (21), cause cell and vesicle fusion (22, 23), and increase red blood cell fragility (24). To minimize the complications,
initial velocities were determined from the first 5 min of uptake, before this fall-off in rate is apparent. Even then the interpretation of kinetic data must be restricted in some cases to the lower concentration ranges, as discussed below.

Accumulation of free fatty acid, on the other hand, shows a biphasic time course. The initial rate of accumulation continues for only a few minutes, at which point either a plateau is reached (Fig. 2), or a slower rate of increase is observed (Fig. 2B). The initial rate of accumulation, as well as the plateau level, varies with the fatty acid substrate as described below. This accumulated free fatty acid fraction is that which remains with the cells after several albumin washes and is operationally defined as internalized fatty acid. Experimentally, it is not the same as the much more rapidly labeled “uptake” fraction studied by Spector et al. (8) in ascites tumor cells, a fraction that was removable by albumin washing.

No lag was observed in the rate of oxidation or esterification with the fatty acids and concentration ranges studied here, indicating that a steady state level of intermediates between external fatty acid and the final metabolic products is reached quickly. Fig. 2 illustrates that no lag in oxidation and esterification is observable even at very short time intervals (before the free fatty acid level has plateaued). This result indicates that the rates of oxidation and esterification are not functions...
of this free fatty acid pool. A short lag of about 2 min can be demonstrated with palmitate and oleate oxidation in the presence of serum albumin at external free palmitate and oleate concentrations of approximately 0.14 μM and 0.12 μM, respectively (data reported in the following paper (11)). By contrast, Samuel et al. (9) reported lag times of up to 15 min for fatty acid oxidation by cultured chick embryo heart cells. Although chick embryo heart cells have been shown to have a fully operational β-oxidation system (25), the observed difference between these two tissues may reflect subtle differences between the prenatal and adult tissue.

**Effects of Substrate Concentration on Uptake and Utilization Rates—**Fig. 3 shows the effect of substrate concentration on the rate of uptake and incorporation of decanoate (A), laurate (B), myristate (C), and palmitate (D) at 37°C. Using our assay method, there were no detectable levels of mono- or diglycerides. There are several noticeable characteristics in this series of kinetic curves. First, the CO₂ curve for myristate deviates from a simple hyperbolic saturation curve at higher concentrations. A similar but less marked deviation also occurs for laurate. This deviation is more easily seen by plotting the CO₂ production data in Eadie-Hofstee form (Fig. 4). This deviation could represent the first manifestation of the toxic effect observed at higher fatty acid concentrations mentioned above. Laurate and myristate at these concentrations could begin to uncouple oxidative phosphorylation in mitochondria, or to stimulate a plasma membrane ATPase. Either effect would relieve respiratory control and allow oxidation to proceed at a faster rate. Experiments with dinitrophenol support this conclusion and indicate that the rate of fatty acid oxidation is limited in part by respiratory control. Fig. 5 shows the effect of 25 μM dinitrophenol on myristate uptake and metabolism. Dinitrophenol increases the rate of oxidation and decreases the rate of esterification, but has little effect on the rate of free fatty acid accumulation. An Eadie-Hofstee plot of the CO₂ production data (Fig. 5B) shows that dinitrophenol affects the Vₘₐₓ, but not the Kₘ, of myristate oxidation, and eliminates most of the stimulation observed at 5 μM myristate. Hence, the stimulatory effect of higher myristate concentrations and dinitrophenol on the rate of oxidation are not additive and could therefore be working through the same mechanism.

Fig. 3 also shows an effect of fatty chain length on the relative distribution of products from the metabolism of the fatty acids. The major product from decanoate is CO₂, and the rate of triglyceride formation is low. For laurate and myristate, the rate of triglyceride formation is about one-half that of oxidation, and for palmitate the rate of triglyceride formation is about as great as the rate of oxidation. The rate of polar lipid formation is very low for all but palmitate.

The data in Fig. 3 can be treated in two ways. If the total fatty acid uptake (i.e. the sum of the CO₂, triglyceride, polar lipid, and free fatty acid values) is plotted as a function of concentration, curves similar to those found by Samuel et al. (9) are obtained, consisting of a saturable and a nonsaturable component. These curves can be fit to the function

\[ v = \frac{V_m [F_0]}{K_m + [F_0]} \]

where [F₀] is the external fatty acid concentration, P represents the first order rate constant for the nonsaturable component (interpreted under "Discussion" as the permeability coefficient of a membrane diffusion process), and Vₘₐₓ and Kₘ are the maximum velocity and the Michaelis constant for the saturable component. By assuming a value of Kₘ, this function can be reduced to the form \( f = ax + b \). The data can then be fitted to the function with a computer program by varying Kₘ until a minimum correlation value for the fit of the data is obtained. Table I lists the Kₘ and Vₘₐₓ values obtained for the saturable component by this procedure for decanoate, laurate, myristate, and palmitate.

Inspection of the data in Fig. 3, however, leads to the conclusion that the nonsaturable component of uptake corresponds to the accumulation of free fatty acid, while the saturable component consists of the products of fatty acid metabolism (i.e. CO₂, triglyceride, and polar lipid), and that one could obtain the same result more directly by treating the data separately at the outset. When total product formation is plotted as a function of external fatty acid concentration, one obtains simple hyperbolic saturation curves. These data are shown in Eadie-Hofstee plots in Fig. 6. (The 5 μM myristate
Kinetics of Fatty Acid Uptake by Rat Heart Myocytes

**Table 1**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Saturable component of total uptake (A)</th>
<th>Product formation (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_a$ (µM)</td>
<td>$V_{max}$ (nmol/h-mg protein)</td>
</tr>
<tr>
<td>Decanoate</td>
<td>0.70 ± 0.01</td>
<td>15 ± 6</td>
</tr>
<tr>
<td>Laurate</td>
<td>0.46</td>
<td>28</td>
</tr>
<tr>
<td>Myristate</td>
<td>0.64 ± 0.38</td>
<td>20 ± 11</td>
</tr>
<tr>
<td>Palmitate</td>
<td>5.1 ± 0.0</td>
<td>108 ± 2</td>
</tr>
</tbody>
</table>

and 10 µM laurate data points are omitted because the anomalous stimulation of CO₂ production at these concentrations appear unrelated to the simple saturation kinetics of uptake.) Table I also lists the $K_a$ and $V_{max}$ values obtained from several sets of product formation data for these fatty acids. Note that for decanoate, laurate, and myristate, these values are identical within experimental error to those obtained by analysis of the saturable component of total uptake. Only the $K_a$ and $V_{max}$ obtained for palmitate deviate significantly in the two methods. Fitting the palmitate total uptake data to Equation 1 is subject to the greatest error, because the nonsaturable component is so large. Therefore, one can conclude from this comparison that the saturable component of the total uptake process and the saturation observed for the metabolic product formation are one and the same. Note that the $K_a$ values for all four fatty acids are remarkably similar, and that the $V_{max}$ values vary only about 3-fold. Note also from Fig. 3 that the formation of each product saturates at similar concentrations, suggesting the saturation occurs in a step common to all products.

The rate of free fatty acid accumulation, on the other hand, is still approximately first order at fatty acid concentrations approaching saturation of product formation, corresponding to the first term in Equation 1. This term varies considerably with fatty acid chain length. Fig. 7 demonstrates a logarithmic relationship between the first order rate constant, $P$, and chain length.

**Effect of Glucose on Uptake and Utilization Rates**—Because glucose is present in these experiments, the effect of glucose on the kinetic parameters must be considered. The effect of increasing myristate concentration in the presence and absence of 5 mM glucose is shown in Fig. 8. There is a decrease in the rate of oxidation and a compensatory increase in the rate of esterification in the presence of glucose with little effect on the total rate of product formation. Interestingly, there is no change in the rate of free fatty acid accumulation. Note that the anomalous stimulation of CO₂ production by 5 µM myristate is not affected by the absence of glucose. These data are consistent with those found for adipose tissue (26), skeletal muscle (27), leukocytes (28), Erlich ascites tumor cells (8), and the perfused heart (29).

**Pulse Chase of [1-¹⁴C]Palmitate**—The accumulation of free fatty acids by myocytes shows kinetic properties very distinct from those of fatty acid product formation. To determine whether this accumulated fatty acid is available for further
metabolism, a pulse-chase experiment was carried out with [1-14C]palmitate (Fig. 9). As can be seen from Fig. 9, the radioactivity in the free fatty acid fraction decreases with time, accompanied by an increase in radioactive CO2 and triglyceride, indicating a precursor-product relationship. More than half of the free fatty acid was utilized in the 6-min portion of the experiment, indicating certainly that the bulk of this acid is available for further metabolism. The decrease in polar lipid radioactivity also indicates further metabolism of one or more components of this fraction (most likely to triglyceride).

**DISCUSSION**

The rat heart myocytes used in this study excluded trypan blue and succinate, retained lactate dehydrogenase, and exhibited respiratory control of glucose oxidation (shown by stimulation with dinitrophenol), all properties indicating that the cellular membranes remain intact, and that these cells should be a suitable system for studies with fatty acids. Such a system should more closely approximate the situation in the adult mammalian heart than previous studies with ascites tumor cells (8), or with cultured chick embryo cells (9). The study of such cell systems have been undertaken to investigate directly factors involved in the transfer of fatty acids across the plasma membrane of mammalian cells, and to exclude complications encountered in intact tissue related to the transfer from the vascular space, through the extracellular space, to the cell surface.

Previous investigations of fatty acid uptake by various mammalian cell preparations have normally been carried out in the presence of albumin, which enables the solubilization of larger quantities of fatty acid and mimics the physiological situation for free fatty acids found in serum (5). However, multiple binding of fatty acid to serum albumin complicates standard kinetic analysis of uptake by introducing an additional component into the system. In the work reported here, we have purposely omitted albumin from the incubation, even though such a situation is "nonphysiological," in order to ascertain more clearly the effects of fatty acid concentration on uptake. In the following paper, we present data describing the effects of albumin addition to the system (11), and we establish in that paper that fatty acid uptake is indeed a function of free rather than total fatty acid concentration, a conclusion just the opposite of that obtained by Samuel et al. (9) and extended by Paris et al. (30), for cultured chick cardiac cells.

In the absence of albumin we can readily see several limitations on kinetic studies with fatty acids. Experiments at the very low free fatty acid concentration obtainable in the presence of albumin are complicated by glass surface absorption effects (31), and are limited when binding to the cell surface and uptake significantly decrease the fraction of the free fatty acid present in the medium. Experiments at high fatty acid concentration have several complications. Solubility becomes a problem with longer chain fatty acids such as stearate. Micelle formation could also complicate the analysis, but the studies reported here are carried out at concentrations well below the critical micellar concentrations of all the fatty acids (32, 33). The detergent and membrane-fluidizing effects of fatty acids can have toxic effects on cellular membranes and cellular functions (20, 24). A 20 μM concentration of oleate was sufficient to cause a rapid drop in its rate of metabolism, making initial velocity measurements more difficult to obtain at these high fatty acid concentrations (Fig. 1B). Myristate and laurate at 5 μM and 10 μM concentrations, respectively, began to show anomalous substrate stimulation of the initial rate of oxidation (Fig. 3), an effect that we have interpreted as an uncoupling of respiratory control at these intermediate fatty acid concentrations. Therefore, the kinetic analysis was restricted to concentrations below which these unusual effects were observed.

The function of serum albumin as a fatty acid carrier between tissues has often been interpreted as a solubilizing one, allowing relatively high quantities of fatty acid to be transported (5). In the light of the deleterious effects that the longer chain fatty acids have on cells, even at relatively low concentrations, then one could conclude that albumin is also necessary physiologically to keep the serum concentration of free fatty acid at a level low enough to prevent toxicity to the cells.

The distribution of products of the fatty acids taken up by heart cells is simple, consisting only of free fatty acid, carbon dioxide, triglyceride, and polar lipid. Polar lipid was usually a minor component of the products, and was not further subdivided. This fraction contained at least several phospholipids, and presumably would also contain whatever levels of fatty acyl-CoA and fatty acyl carnitine accumulated in the cell. No diglycerides, monoglycerides, or sterol esters were detected. The fatty acid found in free or esterified form in the cell had not been modified by elongation or desaturation during the time of the uptake experiments.

Experiments were run in the presence of 5 mM glucose, approximating normal serum glucose concentration. Glucose is not required for the uptake and metabolism of fatty acids under the conditions of these experiments, although it does affect the proportion of fatty acid being oxidized. This slight inhibition of fatty acid oxidation by glucose may simply be another manifestation of respiratory control, since the presence of 5 μM myristate also inhibits the rate of glucose oxidation by 25% (data not shown).

There is no lag in the rate of product formation, so all intermediates between the external fatty acid and the final products must reach steady state levels very rapidly. One would expect the internalized free fatty acid to be such an
intermediate, but time course data such as that illustrated in Fig. 2 show that internal free fatty acid levels continue to increase during the early period of the incubation, and therefore the rate of product formation is not a demonstrable function of the quantity of this internal fatty acid. Glucose (Fig. 8), dinitrophenol (Fig. 5), and other metabolic inhibitors (11) affect product distribution without affecting these free fatty acid levels. Yet, the pulse chase experiment with palmitate demonstrates that the internal free fatty acid can be converted to products (Fig. 9). These seemingly contradictory observations can be resolved by assuming two pools of internal fatty acid. Fatty acid in the first pool ($F_1$), relatively small in size, reaches steady state rapidly and serves as the direct precursor for fatty acid activation and further metabolism. Fatty acid in the second pool ($F_2$) constitutes most of the internal fatty acid of the cell but occupies a much larger volume than $F_1$, so that $F_2$ concentration remains less than or equal to $F_1$ concentration. $F_1$ and $F_2$ are freely exchangeable. A candidate for pool 2, for example, might be the membrane of the sarcoplasmic reticulum or the space bounded by this membrane. Pool 1 could simply be the region at the internal surface of the plasma membrane.

Accumulation of fatty acid in pool 2, which is the nonsaturable component of uptake, displays the selectivity of fatty acid chain length expected for a simple membrane diffusion process. Diamond and Wright (34) have pointed out that selectivity patterns for diffusion of nonelectrolytes across membranes parallel those for partition into bulk lipid. The permeability coefficient $P$ is related either to the partition coefficient of the fatty acid between the aqueous and membrane phases, or to the first order rate constant of transfer of the fatty acid across the water-membrane interface, depending upon whether diffusion through the membrane or diffusion across the interface is rate-limiting (35, 36). The quantity $P$ is therefore proportional either to the free energy of transfer of fatty acid from water into the membrane (when membrane diffusion is rate-limiting), to the free energy of activation to a transition state at the water-membrane interface (when transfer across the interface is rate-limiting), or to both of these quantities (when one does not dominate the other as the rate limiting step). The variation of $P$ with fatty acid chain length, therefore, represents the incremental change in $\Delta G$ per $-$CH$_2$-$ group for one or both of these processes, and is calculated to be $-650$ cal/mol from the slope of the graph in Fig. 7. Incremental changes in $\Delta G$ per $-$CH$_2$-$ group for membrane permeation have been found to vary from $-143$ cal/mol for rabbit gall bladder (37) to $-796$ cal/mol for sarcoplasmic reticulum (38, 39). The latter value approaches that of $-860$ cal/mol for transfer of hydrocarbon from an aqueous to a hydrocarbon phase (40), indicating that similar factors govern both the permeation and the bulk-lipid partitioning processes. A major factor in each case is the decrease in solubility of fatty acids in water as the chain length is increased (34). The accumulation of fatty acid in pool 2, therefore, could involve either partitioning into, or permeation across, cellular membranes.

The saturable component of uptake, which represents the conversion of fatty acid to metabolic products, does not show much selectivity for fatty acid chain length. The $K_m$ of this process is near 1 $\mu$m for each of the four fatty acids studied (Table 1), and the $V_{max}$ shows less than a 3-fold difference between decanoate and palmitate. These data alone cannot distinguish between saturation of a transport carrier controlling entry of fatty acid into the cell, or saturation of an early metabolic step, such as fatty acid activation. That the latter is at least a reasonable possibility can be seen from the $K_m$ and $V_{max}$ values obtained for the acyl coenzyme A synthase of rat liver microsomes by Suzue and Marcel (41). Those $K_m$ values varied only slightly with chain length, and ranged from 1.3 $\mu$m for palmitate to 7.7 $\mu$m for decanoate. The $V_{max}$ values for activation of palmitate, myristate, and laurate were similar, and about 2.5 times the $V_{max}$ for activation of decanoate. Of course, later metabolic steps must have greater chain length specificity, since product distribution does vary with chain length (Fig. 3).

A number of models of varying degree of complexity could account for the kinetic data reported here. We will describe two of the simpler representative models briefly. In uptake model I, two separate transport mechanisms occur at the plasma membrane. Step 1 is the simple diffusion of fatty acid across the membrane into pool 2, with the rate given by the first term in Equation 1 (assuming $F_1$ concentration is zero or small relative to $F_0$ concentration). Step 2 is a carrier mediated transport system, delivering the fatty acid to pool 1, which is the direct precursor for fatty acid activation (step 4) and other product formation (step 5). The rate of step 2 is given by the second term in Equation 1, with the $K_m$ describing the saturation of a membrane carrier. The total rate of uptake would be the sum of the two terms of Equation 1. $F_1$ and $F_2$ are interconvertible, but $F_1$ reaches a steady state concentration earlier than $F_2$, which occupies a larger volume and hence does not exceed the concentration of $F_1$ during initial rate studies. This model is essentially like that proposed by Samuel et al. (9) to account for the saturable and nonsaturable components of uptake with chick embryo cardiac cells.

In uptake model II, step 1 represents a single membrane transport process. Step 2 represents the transfer of fatty acid from pool 1 to pool 2, a process involving simple partitioning into, or permeation across, intracellular membranes. The rate of this step is given by Equation 2.

\[ v_2 = P \cdot [F_1] \]  

(2)

during initial conditions when $[F_2]$ is very low relative to $[F_1]$. Step 3 represents the activation of fatty acid from pool 1 (or some other early metabolic step) and is governed by a simple Michaelis-Menten rate equation (Equation 3).

\[ v_3 = \frac{V_m [F_1]}{K_m + [F_1]} \]  

(3)

At steady state, then, $V_i = V_1 + V_3$, or

\[ v_i = P \cdot [F_1] + \frac{V_m [F_1]}{K_m + [F_1]} \]  

(4)
If $v_1$ and $v_3$ are rate-controlling steps in the process and step 1 is in rapid equilibrium, then

$$[r_1] = K_1 [c_1]$$

and Equation 4 reduces to the form of Equation 1, with $v$ a function of $c_0$. $K_1$ would, of course, be unity if the activity coefficient in pool 1 is the same as that in the external bulk solution.

Either model could be modified to an energy-dependent process by assuming the coupling of fatty acid uptake to a membrane electrochemical potential. In model II, such a coupling at step 1 would have the effect of making $K_1$ greater than unity. Such a coupling at step 2 would indirectly make step 1 appear to be energy-dependent, since step 3 requires ATP, and $v_1$ is, in effect, regulated by $v_2$ and $v_3$.

Uptake model II ascribes the observed saturable component of uptake to an internal metabolic process rather than to a transport carrier, and therefore the kinetic data in the paper do not address the nature of step 1. The following paper (11) reports data with various inhibitors that support model II, as well as temperature studies that indicate step 1 becomes rate-limiting at lower temperatures and exhibits the selectivity expected for a simple membrane diffusion process.

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