Constraints on the Uptake of Labeled Palmitate by the Heart

The Barriers at the Capillary and Sarcolemmal Surfaces and the Control of Intracellular Sequestration

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SUMMARY While long chain free fatty acids (FFA) are known to be a major fuel of the mammalian myocardium, little is known about the processes which limit their uptake in the intact heart. Conventional biochemical techniques necessitate the destruction of membrane barriers which could be significant in vivo. Steady state extraction techniques yield information only on net extraction. The necessary information for the demonstration of rate-limiting steps can only be obtained by a technique that preserves the structure of the organ and yet is sensitive to processes with rate constants on the order of tenths of a second. The multiple indicator dilution technique is ideal for this type of investigation. Using a perfused in situ dog heart, we made simultaneous injections of \(^{14}C\)-albumin (intravascular reference), \(^{14}C\)-sucrose (capillary surface and interstitial space reference), and \(^3H\)-palmitate (a representative of the long chain free fatty acids) into the left coronary artery and collected samples rapidly from the coronary sinus. The activity of each isotope in the venous samples was divided by the activity per milliliter in the injection to give the outflow fraction per milliliter and plotted vs. time to give the normalized coronary sinus concentration-time curve for each tracer. The curves were analyzed by a two-barrier model, which includes both the constraints at the capillary and muscle cell surfaces and an irreversible sequestration reaction inside the myocyte for palmitate. The capillary permeability of palmitate was only 2- to 3-fold greater than that for sucrose; the palmitate entered the myocyte, whereas the sucrose did not; and the intracellular sequestration of palmitate was found to take place at a rate inversely proportional to the arterial lactate concentration. Normally some palmitate returns to the interstitial space and capillary after entering the cell. Toxic doses of flavaspidic acid or dinitrophenol changed this. They caused virtually complete sequestration of all the palmitate that entered the cell. The data indicate that the capillary endothelium is a major barrier to the extraction of FFA, that the activity of the reaction catalyzed by long chain fatty acyl thiokinase is controlled by the blood lactate concentration, and that flavaspidic acid and the uncoupler dinitrophenol in some way accelerate FFA activation.

LONG CHAIN free fatty acids (FFA) are a major fuel of the mammalian myocardium, particularly when the animal is in a fasting state. Although the FFA are very lipid soluble, the heart normally extracts only 40-50% of labeled palmitate in a single transit time. This constraint on extraction could be due either to limited capillary permeability, as predicted by Issekutz et al., or to a limited intracellular metabolizing capacity, as proposed by Oram et al. The heart also exhibits the phenomenon of substrate competition: increased blood lactate levels will reduce the extraction and oxidation of FFA and, concomitantly, the utilization of lactate will increase. This competition could occur at the level of the capillary or the sarcolemmal membranes, or at the level of an intracellular metabolic control site.

In order to study the relative importance of these possible constraints on FFA extraction, it is essential to preserve the structure of the organ, to maintain it as close to a physiological state as possible, and yet, at the same time, to measure the kinetics of processes with rate constants of the order of tenths of a second. Those conventional biochemical techniques which necessitate the destruction of the capillary and cell membrane barriers are clearly not appropriate. The approach of measuring the steady state levels of metabolite or its tracer in the blood entering and leaving the organ, on the other hand, while preserving the barriers and structure of the organ intact, will provide only estimates of net extraction. It will not detect how much of the metabolite has actually entered the cell unless the tissue concentration of the metabolite is measured later by a destructive technique.

The single injection, multiple indicator dilution technique has been used successfully in the liver for studying the rate-limiting steps in glucose and galactose uptake. We therefore decided to adapt it to the study of metabolite extraction in heart, since it has previously been shown to be useful in understanding nonmetabolite transport in this organ. Although interpretation of data obtained by this technique is dependent upon the use of a correct model of the system, it is unsurpassed as a nondestructive tool for the study of blood-tissue exchange. Once the
validity of the model has been established in animals, the approach will then be directly applicable to humans, since it is, in essence, nondestructive.

Using a two-barrier model which we previously developed to describe the distribution of labeled water across the capillary and sarcolemmal barriers in the heart, we have been able to analyze the transient blood-tissue exchange and uptake of labeled palmitate and to outline the major rate-limiting steps. We have found that, normally, the capillary endothelium is the major barrier to the movement of FFA molecules but that, with elevated blood lactate levels, the rate of intracellular sequestration becomes markedly reduced, so that this process also becomes a significant rate-limiting factor. Since the sequestration reaction is also inhibited by α-bromopalmitate, a known competitive inhibitor of the activating enzyme, long chain fatty acyl thiokinase, we conclude that lactate may be a possible allosteric inhibitor of this enzyme. We have also discovered that, in toxic doses, flavaspidic acid and the uncoupler dinitrophenol have an anomalous effect on activation, in that no palmitate returns to the capillary from the tissue cell.

Methods

APPARATUS

The experimental apparatus was identical to that described previously. Briefly, the main stem or branch of the left coronary artery of a pentobarbital-anesthetized mongrel dog was cannulated via a carotid artery and perfused via an extracorporeal circuit with blood from a femoral artery. The coronary sinus was catheterized via the right jugular vein. Perfusion pressure, flow rate, rectal temperature, intraventricular or aortic pressure, and lead 2 of the electrocardiogram were monitored continuously. The animal was ventilated by a constant volume respirator, even though the chest had been securely closed after the cannulation.

INJECTION MIXTURE

Three radioactive tracers were added to 6 or 7 ml of blood, adjusted to the same hematocrit as the animal: palmitic-9,10-3H(N) acid [0.5 mCi, saponified with KOH (New England Nuclear)], sucrose-14C(U) [0.1 mCi (New England Nuclear)], and 125I-albumin [0.05 mCi (C. E. Frosst)]. Dog albumin (Fraction V powder; Miles Laboratories) was added to compensate for plasma protein dilution, after addition of the tracers.

EXPERIMENTAL PROTOCOL

When the preparation was in a steady state with respect to systemic pressure, heart rate, and coronary resistance, a sudden injection of 0.3-0.5 ml of the injection mixture was made into the perfusion tubing. Simultaneously, a collection rack was started and samples were collected from the coronary sinus at a rate of 0.5-0.8 sec/sample. Immediately following the run, samples were collected from the aorta for determinations of blood gas tensions, pH, and free fatty acid and lactate concentrations. Homologous blood was transfused as necessary.

In order to demonstrate the superimposition of the lactate effect in a preparation, we increased the lactate concentration by the intravenous infusion of the sodium lactate (Sigma) at a rate of about 0.1 g/min for at least 15 minutes before the second run. In later experiments, we used a loop cannula in the femoral artery. In a few of our early experiments, where end cannulas had been placed in the femoral artery, baseline lactate levels were found to be already elevated, presumably due to hindlimb ischemia. α-Bromopalmitate (Eastman Chemicals) was prepared by saponification with KOH. A total volume of about 30 ml, containing 1 g of α-bromopalmitate, resulted. The soap could only be solubilized by adding 1 g of dog albumin (Fraction V powder; Miles Laboratories) and heating to about 40°C. Where this was part of the experimental protocol, the mixture was infused intra-arterially into the perfusion tubing at a rate of about 0.2-0.4 ml/min or until vasodilation was observed. At a blood flow rate of about 100 ml/min, this infusion would give input plasma α-bromopalmitate concentrations of between 0.4 and 1.0 μEq/ml.

In the final set of experiments, 1 g of 2,4-dinitrophenol was dissolved in 30 ml of 95% ethanol and infused intra-arterially into the coronary cannula at a rate of about 0.02-0.05 ml/min or until marked S-T elevation was noted on the electrocardiogram. Alternately, 1.5 g of flavaspidic acid β-methyl glucoside were dissolved in 30 ml of saline and infused into the cannula at a rate of about 0.5 ml/min or until S-T elevation was observed. In these and the preceding experiments, flow was maintained constant in the face of changes in vascular resistance.

ANALYSIS OF SAMPLES

Next, 0.1 ml of each sample was diluted in 1.5 ml of normal saline, pipetted into a test tube, and then analyzed in a gamma ray spectrometer for 3H activity. The protein was then precipitated with 0.2 ml of a 25% solution of trichloroacetic acid, and 0.2 ml of this supernatant was pipetted into Aquasol (New England Nuclear) scintillation cocktail and analyzed for 14C activity. The original samples were then centrifuged, and 0.1 ml of supernatant plasma was extracted by the Dole method. A sample of the heptane upper phase containing the free fatty acids was then pipetted into the scintillation cocktail and analyzed for 3H activity. Standards prepared from the injection mixture were analyzed for the three isotopic activities by the same methods. The activity for each isotopic species in each venous sample was divided by the corresponding activity injected and plotted against the time after injection to give a normalized coronary sinus outflow fraction per milliliter vs. time curve, for each tracer.

Arterial blood samples were analyzed for total free fatty acids by the method of Dole, modified only by the use of an alcoholic Nile Blue indicator; and for lactate, by use of the enzyme lactate dehydrogenase (Boehringer Mannheim).

Form of the Data

The upper panels of Figure 1 show the two sets of normalized outflow fraction per milliliter vs. time curves from experiment 3, both before and after the infusion of lactate (the numerical data underlying the curves in this
The relationship between the curves is interpreted qualitatively in the following way. All tracers are dispersed by the heterogeneity of transit times in the coronary circulation. $^{125}$I-Albumin can be assumed to be confined to the intravascular space in a single transit, and therefore its outflow is determined only by the intravascular transit times. The $^4$C-sucrose and $^3$H-palmitate are also distributed in the spaces outside the capillary. In the early samples the sucrose curve is lower than that of albumin, due to the permeation of some of the sucrose molecules through either junctional gaps between the capillary endothelial cells or vesicular channels perforating these cells. Later in time, after most of the albumin has traversed the capillaries, the outflow fraction per milliliter for the sucrose tracer rises above that of albumin, as the sucrose returns to the capillary from the interstitial space about the capillary. If the collection is continued for long enough, all of the sucrose label is recovered at the outflow. The total area under the sucrose curve is then equal to that under the albumin curve. It is appropriate to note that, in the heart, sucrose is a more appropriate extracellular space reference than it is in skeletal muscle. In skeletal muscle, on exposure to hypertonic sucrose, the volume of endoplasmic reticulum increases, indicating penetration of sucrose into this compartment of the muscle. In mammalian heart muscle, no swelling of the sarcoplasmic reticulum occurs at all. Sucrose appears to be excluded from all compartments of the heart muscle and, thus, to be an ideal extracellular space reference.

FFA molecules, represented by $^3$H-palmitate, behave like sucrose in the initial samples except that, in this experiment, the capillary permeability is somewhat higher. Later in time the labeled palmitate outflow fraction per milliliter becomes very reduced with respect to the sucrose because a proportion of the palmitate which has crossed the sarcolemma is sequestered inside the cardiac muscle cell, and fails to return to the circulation. When the arterial blood lactate concentration is raised, a larger proportion of the labeled palmitate which has left the capillary returns to it, and the downslope of the palmitate curve actually crosses over the albumin curve in much the same manner as the sucrose curve. Qualitatively, from the change in shape of the curves, one would infer that elevation in the blood lactate has reduced the uptake of palmitate by the myocyte but has not apparently affected the initial efflux of labeled palmitate through the capillary wall.

The log ratio-time plots in the lower panels of Figure 1 display the relations between the vascular reference and diffusible tracer curves in a more compact form. The initial linear parts of these plots represent the initial permeation of the diffusible tracer through the capillary endothelium. In the absence of return of tracer from the extravascular space, there would be no change from this initial slope. When important return of tracer to the capillary begins, the log ratio-deviates downward and eventually crosses zero in the case of the nonsequestered or partially sequestered tracer.

While we can determine immediately from the initial part of the outflow curves the relative capillary permeability of palmitate, return of tracer to the capillary as a function of time is determined by the size of the apparent interstitial space and the cardiac muscle cell volumes, the permeability of the sarcolemmal membrane, and the rate of sequestration inside the myocyte. These factors can be dealt with only by means of an explicit model of the exchanging element of the microcirculation, the capillary and its associated tissue.

**Analysis of Data**

**PARAMETERS FROM THE SUCROSE DATA**

Since sucrose does not enter mammalian cells, its outflow pattern is unaffected by sarcolemmal permeability or intracellular events. It is affected especially by transcapillary exchange, hence, by the capillary permeability. If we assume that this is a characteristic biological constant, then the measured capillary permeability-surface product for sucrose, arising in any experiment, is proportional to the total area of capillaries perfused. In the present work, this value serves as an index against which the permeability-surface product for palmitate may be compared, to ascer-
tain whether its capillary permeability changes independently of surface area.

We have previously analyzed the characteristic phenomena leading to the shape of labeled sucrose outflow curves in the heart and have shown that, in addition to the intrinsic permeability of the capillary, the distribution of capillary transit times exerts a major and dominating influence, and that autoregulatory responses in the coronary vasodilation. We have shown that, from the interrelations between the labeled sucrose curve and the labeled albumin curve (the appropriate intravascular reference parameter, there is no way to obtain the absolute values. The absolute values are given by

\[ a_s = a'_s / (k_s) \]

Since \( k_s \) cannot be optimized as a separate parameter, there is no way to obtain the absolute values. When \( b_s = 0 \), the capillary transit times are constant and, when \( b_s = 1 \), there is maximal heterogeneity of capillary transit times (the dispersion of the intravascular reference is only due to dispersion in the capillaries, and large vessel transit times are constant).

At any given instant in time, these four parameters provide information not only on the sucrose permeability but also on the plasma flow and degree of heterogeneity of the coronary circulation. Characteristically, if the system is held at constant flow, all four parameters will change with vasodilation.11

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maximum heterogeneity of the transit times, the model "fitted." Again, it provided an unreasonable physiological inference. It provided a set of minimum capillary transit times which were physically impossible.11 This, in turn, enabled us to make appropriate choices in continuing our model analysis. While one cannot establish a general proposition from two examples, it would seem to be a useful rule of thumb that the optimum parameters predicted from a model that "fits" the data should be checked by another technique, if possible.

The two-barrier model is found to be indeterminate with respect to the relative size of the interstitial space, when finite intracellular sequestration is included. A predetermined estimate of the space can be found, however, which can then be used in the modeling.18 The extracellular space of distribution accessible to labeled sucrose corresponds to the interstitial volume, and the value for \( y_i \), the ratio of the interstitial space to the capillary plasma space, corresponds to the ratio of the space sizes.19 Palmitate is virtually completely bound to albumin, and the ratio of the interstitial space available to palmitate to its capillary plasma space, \( y_s \), will depend directly on the corresponding albumin contents. The gel-like character of the extracellular interstitial space in the heart exerts an excluded volume effect on albumin. From data on the extravascular distribution of labeled albumin, Dewey20 has estimated the equilibrium albumin concentration in the extracellular space to be 0.33 times that in the plasma. Thus we can equate \( y_i/y_s = 0.33 \). We have used this value for the relative interstitial space accessible to labeled palmitate for all experiments, realizing that it may vary slightly from one preparation to another.

The final model for palmitate uptake is identical to the model we developed to analyze labeled water distribution in the heart, except for the inclusion of intracellular sequestration (which had already been considered in the mathematical development, in anticipation of this possibility) and the use of a fixed value for the ratio of the accessible interstitial space to the plasma space. Using the structural parameters \( a_1 \) and \( b_1 \) from the fit to the sucrose curve and the same albumin curve as the intravascular reference, and assuming that transport across both capillary and sarcolemmal membranes is equilibrative, we can obtain four unambiguous parameters:

\[
\begin{align*}
&k_c = \text{capillary permeability-surface product per unit interstitial space for palmitate (equal to both } k_1 \text{ and } k_2 \text{ in our original model),} \\
&k_m = \text{sarcolemmal permeability-surface product per unit interstitial space for palmitate (equal to both } k_3 \text{ and } k_4 \text{ in our original model),} \\
&\gamma_i/\theta_i = \text{ratio of apparent interstitial to intracellular spaces for palmitate, where } \gamma_i = \text{ratio of interstitial to plasma space (assumed to be 0.33 } y_i \text{) and } \theta_i = \text{ratio of intracellular to plasma space for palmitate, and} \\
&k_{seq} = \text{rate constant for the irreversible sequestration reaction per unit intracellular space for palmitate (equal to } k_3 \text{ of the model).}
\end{align*}
\]

Since we have assumed that \( \gamma_i/y_s = 0.33 \), due to the lower concentration of albumin in the interstitial space compared with the plasma space, \( k_c \) and \( k_m \) must be

TABLE 2  Steady State Activities of Labeled Palmitate in Arterial and Coronary Sinus Plasma and Cardiac Lymph

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Arterial</th>
<th>Venous</th>
<th>Lymph</th>
<th>Ratio (lymph/arterial)</th>
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<td>1</td>
<td>1983</td>
<td></td>
<td>393</td>
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\*3H-palmitate was infused at a constant rate intravenously for 90 minutes. A 1-hour collection of cardiac lymph was made, beginning 30 minutes after the infusion was begun. The plasma values were obtained 1 hour after the infusion was started. The low lymph/arterial activity ratio can be explained by decreased binding sites for palmitate in the lymph since the lymph/arterial albumin ratio is about 0.74.18
multiplied by 0.33 in order to provide a set of parameters which, on direct comparison with values for other substances that are distributed into the whole interstitial space, will provide relative permeabilities, at least at the capillary level. These corrected parameters, denoted \( k_s \) and \( k_m \), are later reported in Table 4.

\( \gamma_l/\theta_l \) is only an apparent ratio of space sizes. It will reflect primarily the contents of proteins binding FFA in the two spaces and their affinities. At the level of the cell, the FFA are known to be tightly bound both by sites on the mitochondrial membrane and sarcoplasmic reticulum\(^{21,22} \) and by an acceptor Z protein.\(^{23} \) Thus the value for \( \gamma_l/\theta_l \) will be proportional to the ratio of the number of occupied binding sites in the interstitium to those inside the cell. This parameter is important since it will affect, with the cellular entry coefficient, the rate of exposure of the FFA to the sequestration process. As the value \( \gamma_l/\theta_l \) diminishes, a larger proportion of the space accessible to palmitate, outside the capillary, will come to be located inside the myocyte.

**OPTIMIZATION PROCEDURE**

In order to evaluate the integral in the expression for the returning component of the model, it is necessary to interpolate between experimental points on the intravascular reference curve. This was done by passing a spline fit through the experimental points and then using the spline coefficients, as required. Parameters were optimized by a steepest descent technique. The model was stored as a Fortran program and run on an IBM 360 computer from a remote terminal. Optimization of the four parameters of the palmitate model was greatly aided by the use of a Tektronix 4012 terminal, which permitted immediate graphical display of the fit. Thirty to 60 minutes of computer processing unit time were generally required to optimize one palmitate curve.

**Results**

Table 3 shows the hemodynamic data and the lactate and total FFA arterial concentrations for the experiments analyzed. As previously reported,\(^{11,12} \) infusions of both flavaspidic acid and dinitrophenol result in coronary vasodilation.

Figure 2 shows the results achieved by fitting the model to the experimental data shown in Figure 1. The total palmitate outflow curve is made up of two components: the throughput or nonexchanging first component, and the returning or second component. Only the second component of the model-derived sucrose curve is shown.

With the increase in blood lactate concentration, the later part of the palmitate-returning component is found to be greatly increased, although the throughput component is unchanged. The changed form is due to a reduction in the rate of intracellular sequestration.

### Table 3 Hemodynamic Parameters and Substrate Concentrations

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Run</th>
<th>Perfused heart weight (g)</th>
<th>Flow (ml/min)</th>
<th>Heart rate (min(^{-1}))</th>
<th>Systolic pressure (torr)</th>
<th>Perfusion pressure (torr)</th>
<th>Resistance (torr - min/ ml)</th>
<th>Lactate concentration (mg/dl)</th>
<th>Arterial FFA concentration (mm/liter)</th>
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<td>30</td>
<td>0.31</td>
<td>27.7</td>
<td>0.242</td>
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* L-Lactate infusion.
† a-Bromopalmitate infusion.
‡ Flavaspidic acid infusion.
after the lactate infusion, but the later part of the returning or second component is increased. The increase appears to be due to reduced sequestration of label by the myocyte. For comparison, the returning component of the sucrose curve is also shown. The returning component of the palmitate curve appears sooner than that for the sucrose curve both because of the palmitate’s higher capillary permeability and its smaller space of distribution in the interstitium.

Table 4 presents the optimized values obtained for model parameters by fitting the sucrose and palmitate curves from 15 technologically satisfactory experiments. On the average, the relative palmitate capillary permeability is 2 or 3 times that for sucrose. The value for the corrected parameter \( k_{eq} \), the palmitate sarcolemmal permeability-surface product per unit sucrose interstitial space, \( \times 0.33 \) averages 0.52. The values show no correlation with the arterial lactate concentration which is independent of heart rate, systolic pressure, or blood pH (Fig. 3). Infusion of \( \alpha \)-bromopalmitate results in no discernible effect on this parameter. In contrast, \( k_{eq} \), the rate of intracellular sequestration per unit intracellular space, shows a significant (\( P < 0.01 \)) negative linear regression with arterial lactate concentration which is independent of heart rate, systolic pressure, or blood pH (Fig. 3).

During flavaspidic acid or dinitrophenol infusions, the palmitate curve appears to come to be composed only of throughput material. The implication of this is that the sequestration constant \( k_{eq} \) has become exceedingly large. The fitting program did not converge satisfactorily under these circumstances. In all, three runs were carried out during the infusion of flavaspidic acid and three during the infusion of dinitrophenol. The results were similar in all instances. Because of our difficulty in obtaining an optimized numerical fit, only one of these runs, the one carried out during flavaspidic acid infusion, is listed in Tables 3 and 4. A characteristic change is found in the form of the log ratio-time plot of data required during the infusion of either of these substances (Fig. 4). In the baseline or control run, the effect of the returning material is portrayed by the shaded areas. Infusion of flavaspidic acid has produced not only vasodilation, with decreases in the characteristic initial slopes of the plots, in the case of the palmitate curve, it has also resulted in the virtually complete disappearance of the returning component.

**Discussion**

**VALIDITY OF THE ASSUMPTIONS OF THE MODEL**

The major assumption of the model is that, in an organ such as the heart with a high density of capillaries, there exists no substantial concentration gradient radial to the capillaries during an infusion experiment.
capillary axis in the interstitial or cellular spaces. In other words, the resistance offered by the capillary and sarcolemmal membranes to the movement of molecules is very much larger than that of the aqueous spaces bounded by them. The possibility nevertheless exists that albumin (MW = 68,000) could be significantly limited in its diffusion in the interstitial space relative to sucrose. Bassingthwaighte has quantitatively analyzed the error in assuming instantaneous radial equilibration in a well perfused organ like the heart with intercapillary distances of the order of 10 μm and found it to be negligible even for low diffusion coefficients (1 \times 10^{-6} \text{ cm}^2/\text{sec}) and high capillary permeabilities. If the diffusion coefficient for sucrose at 37°C is about 1 \times 10^{-6} \text{ cm}^2/\text{sec}, then that for albumin will be about 1 \times 10^{-6} \times \sqrt{342/68,000} = 0.7 \times 10^{-6} \text{ cm}^2/\text{sec}. Thus, even for albumin with negligible capillary permeability relative to sucrose, the assumption still appears valid, even allowing for some restriction of diffusion due to the presence of mucopolysaccharides in the interstitial space.

It is also appropriate to point out that these experiments also uphold some of the other less crucial assumptions implicit in the modeling. We have assumed that the separation of tracers of different molecular weights due to diffusion across the velocity gradients in laminar flow in the larger vessels (Taylor effects) is negligible. The labeled palmitate is largely bound to albumin, while the labeled sucrose is not protein-bound. If sucrose is being significantly retarded in its transit along the large vessels, relative to albumin, due to faster diffusion across the laminar velocity gradients, the effect will be expected to be seen as an increased early extraction of sucrose relative to palmitate. No perceptible effect of this sort is found. We have also assumed that the heterogeneity of transit times is such that the capillary transit time \( \tau_c(t) \) increases as time passes. From this assumption we would expect that, early in time, before the return of exchanging material:

\[
\ln \frac{T C(t)_{\text{alb}}}{C(t)_{\text{suc}}} = k_{\text{suc}} \tau_c(t) = \frac{2P_s}{r(1 - \text{Hct})} \tau_c(t)
\]

\[
\ln \frac{T C(t)_{\text{alb}}}{C(t)_{\text{palmitate}}} = k_{\text{palmitate}} \tau_c(t) = \frac{2P_{\text{palmitate}}}{r(1 - \text{Hct})} \tau_c(t)
\]

where \( P_s \) and \( P_{\text{palmitate}} \) are the capillary permeabilities for sucrose and palmitate, respectively, \( r \) is the capillary radius, and \( \text{Hct} \) is the hematocrit. From this

\[
\ln \frac{T C(t)_{\text{alb}}}{C(t)_{\text{suc}}} \ln \frac{T C(t)_{\text{alb}}}{C(t)_{\text{palmitate}}} = \frac{P_s}{P_{\text{palmitate}}}
\]

for any time, \( t \), after the appearance time.

Figure 5 shows that, early in time, each of the logarithmic ratios increases with time, in a preparation with a well maintained heterogeneity of transit times, so that \( \tau_c(t) \) increases with time. The ratio of the values of the two logarithmic ratios is constant over early time as expected. The findings tend to bolster our assumption of the presence of parallel large vessel-capillary microcirculatory

![Figure 3](image1)

**Figure 3** Plot of the sequestration rate constant, \( k_{\text{seq}} \), as a function of the arterial blood lactate concentration. The linear regression relating the two variables is highly significant (\( P < 0.01 \)). Ninety-five percent confidence limits are shown as dashed lines. The infusion of \( \alpha \)-bromopalmitate reduces \( k_{\text{seq}} \), independent of the lactate concentration.

![Figure 4](image2)

**Figure 4** Log ratio-time plots before and after flavaspidic acid infusion (the data are from experiment 10). The dashed lines indicate what would be expected in the absence of tracer returning to the capillary, and the shaded area emphasizes the amount of returning material. During the infusion of flavaspidic acid, the returning component disappears. The capillary wall appears to become effectively the only barrier to the extraction of palmitate.
partition functions derived from the fit of the model to the data. The slopes of the lines are proportional to the permeability of the instance, the plasma space). It is expected to be the same for tracers with finite capillary permeability, which are distributed in the same space in the blood (in this instance, the plasma space).

![Figure 5](image)

**Figure 5** Representative log ratio-time plots (the data are from run 2 of experiment 3). The dashed lines are the transit time partition functions derived from the fit of the model to the data. The slopes of the lines are proportional to the permeability of the capillary for each species. The intercept on the abscissa will be expected to be the same for tracers with finite capillary permeability, which are distributed in the same space in the blood (in this instance, the plasma space).

The intercept of these lines with the time axis also deserves comment. When there is a high degree of vasoconstriction in the coronary arterial tree and if the dispersion of intravascular tracer results only from the dispersion of capillary transit times and the large vessel transit times are constant (b = 1), then the time between the intercept and the appearance time is equal to the minimum capillary transit time. With vasodilation, this is no longer true; b decreases. As b approaches zero, the intercept falls earlier in time, until when b = 0, it is at t = -∞. We would expect, however, the intercept on the time axis for two tracers of different permeabilities to occur at the same point, regardless of the degree of vasoconstriction. Unfortunately, only when b = 1 will this time have any physical meaning. This equivalence of intercepts then becomes a simple practical test for the propriety of the intravascular reference substances for different tracers.

We have also assumed that there is no significant diffusion shunting between capillary networks with differing transit times. If this were not the case, it would be impossible to completely extract all the material that has left the capillary, since some of it would immediately return via capillaries with shorter transit times. Figure 4 (bottom panel) shows complete removal of all of the FFA that has left the capillary. The assumption is again upheld. The figure also shows that the linear transit time partition function, which is the basis of the organ modeling, holds true for an indefinitely long time. In the presence of returning tracer, which masks this function later in time, we could previously only assume that it held true for all experimental time. We now have an objective demonstration of the validity of this assumption.

**EFFECT OF LACTATE ON INTRACELLULAR SEQUESTRATION**

While it has previously been known that increase in the blood lactate concentration results in a reduction of the extraction of long chain free fatty acids, both in the whole animal and in the heart, independent of their plasma concentration, the site at which this effect is mediated and its mechanism have not been elucidated. Our present studies show that the decrease in extraction is due only to a reduced rate of intracellular sequestration. There is no change in capillary or sarcolemmal membrane permeabilities with change in the blood lactate concentration. It appears that the reaction whose relative rate constant is represented by k_{sec} is the activation reaction catalyzed by long chain fatty acid thiolkinase,

\[
RCOOH + ATP + CoASH \rightleftharpoons R - C - SCoA + AMP + P_i + O
\]

While this reaction by itself is potentially reversible in vivo, it is effectively irreversible, due to the presence of inorganic pyrophosphatase which catalyzes the breakdown of the pyrophosphate,

\[
P_i + H_2O \rightarrow 2P_i.
\]

This activation reaction effectively sequesters the fatty acid molecules, since the coenzyme A derivatives do not penetrate the cell membrane. The brominated compound α-bromopalmitate is known to competitively inhibit this reaction. Our observation that k_{sec} is reduced by α-bromopalmitate infusion, independent of the lactate concentration, thus conforms to expectations.

The problem now reduces to one of how lactate inhibits the activation reaction. Oram et al. have suggested that, in vivo, the speed of this reaction may be limited by the availability of CoASH or accumulation of the acyl-CoA products. If lactate inhibits the reaction by reducing CoASH and, possibly, increasing the level of acyl-CoA derivatives, this hypothesis can be easily tested simply by measuring tissue CoASH and acyl-CoA levels after lactate infusion. However, there is already evidence that this mechanism is unlikely. We know that moderate increases in the concentrations of FFA, glucose, pyruvate, and acetate have no inhibiting effect on FFA extraction. Thus it is more likely that lactate and possibly ketone bodies are allosteric inhibitors of long-chain fatty acyl thiolkinase. Whatever the regulatory mechanism, this reaction is a committed step. Once the FFA molecule has entered this reaction, it must proceed to be completely removed from the cell. The brominated compound, represented by k_{sec} is the activation reaction catalyzed by long chain fatty acid thiolkinase, which is the basis of the organ modeling, holds true for an indefinitely long time. In the presence of returning tracer, which masks this function later in time, we could previously only assume that it held true for all experimental time. We now have an objective demonstration of the validity of this assumption.

**COMPARISON WITH STEADY STATE TECHNIQUES**

The measurement of the steady state extraction of a metabolite or its labeled tracer across an organ yields information only on the net uptake of material. In order to
LABELED FREE FATTY ACID UPTAKE IN THE HEART/Rose and Goresky

Figure 6 Comparison of actual palmitate outflow curves (the data are again from experiment 3) for low lactate concentration (upper panels) and high lactate concentration (lower panels) with those predicted theoretically for \( k_{seq} = 0 \) and \( k_{seq} = \infty \). The panels on the lefthand side show the differences between actual and theoretical total outflow curves; and those on the right, between actual and theoretical returning components. In all cases, the throughput component (material which has never left the capillary) is shown by dotted shading. When \( k_{seq} = 0 \), all of the material injected would eventually be detected in the outflow, and the area under the palmitate curve would eventually equal the area under the albumin curve. Thus the lined shading indicates the material that has been removed by sequestration. The difference between the throughput and the line for \( k_{seq} = \infty \) in the lefthand panels is material which has returned to the capillary from the interstitial space without entering the myocyte.

measure the kinetics of uptake (the rate-limiting steps in the passage from blood to tissue), it is necessary to use a transient technique. This distinction is crucial in the case of FFA uptake. At normal flow rates a large fraction, 40-50% of the FFA initially present in arterial blood, never leaves the capillary. This is the dotted shading in Figure 6. Of the fraction that leaves the capillary, some returns from the interstitial space without entering the tissue cell. Even if the sequestration rate constant were infinite (and intracellular FFA concentrations were zero), there would still be some returning material. This is shown in Figure 6 as a dashed line (\( k_{seq} = \infty \)). The difference between the dotted shading and the dashed line in the lefthand panels is material that has returned to the extracellular space without entering the cell. Normally, when \( k_{seq} \) is finite, some of the material that has entered the cell returns to the extracellular space. At low lactate levels (upper panels, Fig. 6), little FFA leaves the cells and the experimental outflow tends to approach but does not reach the theoretical outflow profile for an infinite rate of sequestration. At high lactate levels (lower panels, Fig. 6), the material returning from the cell increases greatly until it approaches the theoretical outflow profile for a zero rate of sequestration. Thus, because of the large amount of material that never enters the cell, inferences drawn from a steady state measurement will lead to an underestimate of the actual metabolic change inside the cell, unless capillary and tissue cell membrane permeabilities are known and are taken into consideration.

Because of the barriers at the capillary and cell walls, the concentrations of FFA in the three compartments will be very different. Figure 7 shows the theoretical steady state concentrations of labeled palmitate in the three compartments using typical values for \( k_{seq} \), \( k_{inj} \), \( \gamma_1 \), \( \gamma_2 \), and \( \theta_1 \) for high and low values of \( k_{seq} \). Measured values of total unlabeled FFA concentrations may be higher due to lipolysis of triglycerides. Despite a 5-fold change in \( k_{seq} \), there is only a small change in the end-capillary concentration relative to arterial concentration. Interstitial concentrations are about 25% of arterial and show a larger change. Intracellular concentrations are only about 6% of arterial and show a 2-3-fold increase with the decrease in \( k_{seq} \). This probably explains why Olson29 found a large increase in the cardiac concentration of FFA after inhibition of FFA uptake by acetocetate or very high levels of pyruvate.

One could entertain the possibility that lactate inhibited the FFA permeability of the sarcolemmal membrane. From our previous exploration of the steady state case,19

Figure 7 Predicted steady state concentrations parallel to the capillary axis in the intravascular, interstitial, and intracellular spaces for low and high values of \( k_{seq} \), the rate constant for irreversible intracellular sequestration per unit intracellular space. In this example \( k_{inj} = 0.1 \) sec\(^{-1}\), \( k_{inj} = 0.33 \) sec\(^{-1}\), \( \gamma_1 = 1.2 \), \( \tau_c = 3 \) sec, and \( \gamma_1 \theta_1 = 0.1 \). The heavy lines represent the normal state; and the dashed lines, the situation after lactate inhibition of FFA uptake.
the outflow plasma concentration in response to a steady input concentration \( u(0, t) \) is

\[
u(L, \infty) = \frac{\frac{1}{\gamma_1 k_{11} k_{12} k_{\text{seq}}}}{\gamma_2/\theta_1 k_{12} + \frac{1}{k_{\text{seq}}}}\exp\left(-\frac{\gamma_2/\theta_1 k_{12} + \frac{1}{k_{\text{seq}}}}{\gamma_1 k_{11} k_{12} k_{\text{seq}}}t\right)
\]

where \( L \) is the length of the capillary and \( W \) is the velocity of capillary flow. From this, for a given net extraction and capillary permeability, the following relation between possible values for \( k_{\text{seq}} \) and \( k_\alpha \) must hold (the subscripts 1 and 2 will be used for the two equivalent states):

\[k_{\text{seq}} = \frac{k_{11}}{1 + \left[\frac{\gamma_1}{\theta_1}\right]k_{11} - \frac{1}{k_{\text{seq}}} - \frac{1}{k_{\text{seq}} 2}}\]

If, on raising the lactate concentration, the sarcolemmal permeability had decreased and \( k_\alpha \) had remained unchanged, then the outflow curve would have changed in the manner shown in Figure 8. There would have been more material returning earlier in time and less later in time due to the decreased sarcolemmal permeability. Thus it can be seen that, once an explicit model has been developed and tested, it is possible to determine qualitatively, from inspection of the data, at which steps an intervention has affected the handling of a metabolite. At this point in time we have not discovered anything that reduces the sarcolemmal transport of palmitate.

**PARAMETERS ARISING FROM THE MODELING: CAPILLARY AND SARCOLEMMA PERMEABILITIES, AND RATES OF INTRACELLULAR SEQUESTRATION**

The analysis we have applied to the present in vivo data has provided for the first time estimates for a metabolically useful substrate of both capillary and sarcolemmal membrane permeabilities and intracellular utilization rates. The potential utility of this approach in the consideration of other substrates is obvious. We have also previously been able to utilize this approach to show that the movement of labeled water is restricted by barriers at the capillary endothelium and the sarcolemma.12

For a very lipid-soluble molecule, palmitate exhibits a surprisingly low capillary permeability—no more than 2 or 3 times the permeability of sucrose molecules which pass through either vesicular channels or interendothelial slits, occupying perhaps 1/2,000 of the total area. Since the capillary permeability of sucrose is about \( 3 \times 10^{-5} \) cm/sec,11 that for palmitate is about \( 10^{-4} \) cm/sec. A number of possible explanations for this limited permeability could be advanced. One of the major factors limiting the uptake of FFA from the plasma space would be expected to be its binding to plasma albumin. At physiological pH, almost all of the FFA are in the ionized form and the major part of the anions are bound to albumin. Only very small amounts of the undissociated FFA, the anions and the anionic dimers, are free in solution.30 If FFA molecules pass through the endothelial cells themselves, then, in the absence of a specific carrier transport mechanism, they would be expected to cross their cell membranes in the undissociated form. This permeation may be limited by the low concentration of the non-protein-bound non-ionized species. Alternatively, a carrier mechanism could be mediating the passage of FFA through the endothelial cells. From the data presented here it is impossible to discern whether indeed such a mechanism exists at the level of the capillary. The potential of the capillary exchange process for saturation is virtually impossible to explore in vivo. Increase of FFA concentrations by more than a factor of five or so causes hemolysis.

The sarcolemma, in contrast, has no obvious discontinuities. Here the FFA must penetrate a membrane during their entry. The mechanism of passage—whether by solution in the lipid or by the mediation of a carrier transport mechanism—is again not obvious. The bulk of the evidence from the study of isolated cell systems suggests that uptake of FFA occurs at a rate proportional to the concentration of the unbound species and that no saturation occurs.30 Here, surprisingly, the sarcolemmal permeability of palmitate is found to be slightly larger than that for water, \( 0.19 \times 10^{-4} \) cm/sec vs. \( 0.15 \times 10^{-4} \) cm/sec for water, assuming that the same surface is available for the transfer of both molecules.15 The lack of major difference between capillary and sarcolemmal permeabilities for palmitate makes it probable that the membrane permeation mechanism at each surface is a physical process entailing the dissolution of the undissociated species in the membrane.

In these experiments we have found that when \( k_\text{seq} \) is very high, it is difficult to obtain an unequivocal fit with the model. The computed profiles in Figure 6 for the case when \( k_\text{seq} = \infty \) show that, with normal levels of sarcolemmal permeability, the amount of material returning to the capillary from the interstitial space will be expected to be small compared with the throughput component. After infusion of flavaspidic acid (lower panel, Fig. 4) or of dinitrophenol, the outflow profiles indicate that the material that would ordinarily have returned to the circulation from the cellular space has, instead, been sequestered. The component returning from the interstitial space would become too small to perceive. Since this can only occur if \( k_\text{seq} \) is very large, we have assumed that it has become so, in this instance. With this assumption, we have not then...
been able to obtain deterministic values for $k_m$ by use of the model. Dinitrophenol causes uncoupling of oxidative phosphorylation and, ordinarily, this results in an accelerated utilization of substrate. With this accelerated substrate consumption, dinitrophenol has been found to increase the permeability of the sarcolemmal surface to glucose.\textsuperscript{22, 28} With the difficulties encountered in analyzing the dinitrophenol experiments, we are unable to document whether a similar increase in the permeability of the sarcolemmal surface to FFA also occurs. It is appropriate to note that our data indicate that the increase in the utilization of FFA with uncoupling is not suppressed by high concentrations of lactate, in contrast to the normal control mechanism. Even at high concentrations of lactate, which would ordinarily result in the return of a large amount of FFA from the heart muscle cells, apparently complete removal continues to occur. The mechanism of action of the flavaspidic acid is not clear. It could be acting as an uncoupler, like the dinitrophenol.\textsuperscript{32, 33} Additionally, the flavaspidic acid, which has been shown to have a higher affinity for the intracellular fatty acid-binding Z protein,\textsuperscript{23} may be displacing the FFA to sites with lower affinity, and secondarily leading to their increased utilization; and the displacement of FFA from high affinity intracellular binding sites may itself promote uncoupling.\textsuperscript{34} It is appropriate to note that, in the isolated perfused liver, Mishkin et al.\textsuperscript{35} found that flavaspidic acid increased the uptake of FFA and that, at the same time, an energy-requiring process, esterification, was diminished. While these phenomena are new and their nature is yet to be clarified, they may be of importance in thyrotoxic cardiomyopathy,\textsuperscript{36} where it has been suggested that uncoupling may exist.

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