Effect of Chronic Myocardial Ischemia on the Activity of Carnitine Palmitylcoenzyme A Transferase of Isolated Canine Heart Mitochondria

By Jeanie McMillin Wood, Louis A. Sordahl, Robert M. Lewis, and Arnold Schwartz

ABSTRACT
The effect of chronic ischemia on the activity of carnitine palmitylcoenzyme A transferase (PCoAT) was examined. Control mitochondria were isolated from the left ventricle of normal hearts and from the uninvolved areas of ischemic hearts. Mitochondria from ischemic tissue were isolated from the left ventricle of chronically ischemic hearts. Formation of the enzyme product, palmityl-14C-carnitine, was markedly depressed in control mitochondria isolated from ischemic hearts; however, the pH optima of the enzymes from control and ischemic mitochondria were in the same range. After 1 day of chronic ischemia, the kinetics of the enzyme from ischemic mitochondria (compared with that of the enzyme from control mitochondria) showed marked changes in Vmax. Periods of ischemia longer than 1 day significantly decreased the Km of carnitine PCoAT for carnitine from 2.59 ± 0.476 mM to 0.713 ± 0.148 mM, and Vmax was still depressed (15 to 3 nmoles/min mg-1). The Km for palmitylCoA was not significantly affected. Arrhenius plots for the enzyme from ischemic (32 hours) mitochondria revealed a single phasic response to temperature change. Two nonintersecting slopes were characteristic of the normal 1-day ischemic and the normal sonicated activity. As a result of ischemia, changes in the lipid components in the membrane containing carnitine PCoAT were postulated: alterations in the hydrophobic environment of the enzyme produce interference in the binding of palmitylCoA to the second substrate site and may result in a decrease in the Km of the enzyme for carnitine. The single phasic response of the enzyme to temperature change may be additional evidence for membrane lipid alterations. A defect in the transport of long-chain fatty acids may be an early change associated with myocardial ischemia.

KEY WORDS l-carnitine and CO2 production Km changes pH optima palmityl acid transport

Myocardial hypoxia is associated with lipid accumulation, chiefly in the form of triglycerides (1, 2). Since lipid droplets are found only in living ischemic tissue, cellular autolysis is probably not responsible for this phenomenon (3). Decreased rates of fatty acid oxidation enhance the intracellular concentration of fatty acids (4). These increased levels of free fatty acids are thought by some to be deleterious to cardiac function, paralleling periods of myocardial hyperirritability and onset of fatal arrhythmias (5, 6).

The present study deals with changes in the carnitine palmitylcoenzyme A transferase (PCoAT) mechanism that might be partially responsible for the decreased fatty acid oxidation under conditions of chronic left ventricular ischemia in the dog heart. Previously, we demonstrated in isolated mitochondrial preparations that carnitine-stimulated oxidation of palmitic acid was markedly depressed after 1 day of chronic ischemia and abolished after 6–8 days (7). Oxidation of 14C-hexanoic acid, a short-chain fatty acid that is transported independent of carnitine-mediated transfer, was normal at all times subsequent to induction of ischemia (7). The capacity of the electron transport chain for normal rates of fatty acid oxidation was shown to be adequate at these time intervals (7). Concomitant measurements of the carnitine-dependent enzyme responsible for transport of the long-chain fatty acids in ischemia...
acylCoA into the mitochondrial matrix, i.e., carnitine PCoAT, revealed depressed values after the onset of ischemia. Further characteristics of the inhibited enzymatic activity are described in this paper and their possible significance is considered.

**Methods**

Ischemia was produced in the left ventricle of dog hearts by totally occluding the left circumflex artery 1–2 cm from its origin. The epicenter of the infarct was located in the posterior papillary muscle and extended through the posterior wall of the left ventricle. This model of ischemia has been described in detail (8). At the time of termination, left ventricular end-diastolic pressure, aortic pressure, ventricular pressure, and peak isometric pressure developed by the left ventricle after aortic occlusion were measured. Force development (F) over the anterior (control) and the lateral (ischemic) walls was also measured using two separate Walton-Brodie strain gauges, and dF/dt was calculated for both areas. From these data peak dF/F, a sensitive indicator of segmental contractility, and the percent increase in ventricular pressure with aortic clamping, an indicator of total ventricular function and of the ability to sustain peak isometric pressure, were derived. In the studies from which tissue was taken, dF/F in the ischemic area was always markedly impaired, but left ventricular function as a whole was more variably affected. Control experiments used mitochondria isolated from uninvolved areas of the ischemic heart, i.e., the interventricular septum, the right ventricle, or both. The interventricular septum was used in the enzymatic assays as an "uninvolved" control. Other controls were performed on left ventricular mitochondria isolated from control dogs.

No significant differences in metabolic activity were noted in mitochondria isolated from right and left ventricles and interventricular septa. Rates of oxygen consumption in the presence of glutamate and malate during state-3 respiration were 111.3 ± 14.6, 137.9 ± 8.9, and 139.3 ± 11.9 nanoatoms O_2/min mg^-1 for mitochondria from right and left ventricles and interventricular septa, respectively. Carnitine PCoAT activities in mitochondrial preparations from the left ventricle and the interventricular septum in the presence of 160 μM palmitoylCoA and 2.4 mM carnitine were 11.8 ± 1.66 and 13.65 ± 1.9 nmoles palmitoylcarnitine/min mg^-1, respectively. Finally, carnitine-stimulated rates of CO_2 production from labeled palmitate oxidation corresponded to 6.72 and 7.27 nmoles palmitate oxidized/mg protein (average of two separate experiments) for normal right and left ventricular mitochondria.

Mitochondria were prepared in a medium containing 0.18M KCl, 10 mM EDTA, and 0.5% bovine serum albumin (Fraction V, Sigma), as described by Sordahl et al. (9). Polargraphic measurements of respiratory function were performed on all preparations. In general, the rate of oxygen consumption was depressed by 50% (160 to 78 nanoatoms/min mg^-1) after 1 day of ischemia and by 78% (160 to 35 nanoatoms/min mg^-1) after 7 days of chronic ischemia. Oxygen consumption for the uninvolved areas of the ischemic heart ranged from 110 to 182 nanoatoms/min mg^-1.

For sonication, mitochondria were prepared in 0.25M sucrose, 10 mM Tris HCl, pH 7.4, and sonicated at 25 mg/ml, using a Branson ultrasonic probe (Branson Instruments, Inc.). Sonication was carried out for 20-second intervals at maximum power, and the pH was adjusted immediately afterward to 7.0.

Oxidation of 1^14C-palmitate by mitochondria from control and ischemic tissue was measured by trapping metabolic ^14CO_2 after injection of NaOH into sealed incubation flasks. The ^14CO_2 was subsequently released onto filter paper strips soaked with Hyamine hydroxide (p-[diisobutylcresoxyethoxyethyl] dimethylbenzylammonium hydroxide) by addition of HCl in amounts sufficient to acidify the basic solution. The radioactivity of the filter paper strips was determined after elution of the strips with 1 ml of methanol (for counting method, see below).

Carnitine PCoAT activity was measured in a total reaction volume of 0.125 ml as described by Pande and Blanchaer (10) with dl-1^14C-methylcarnitine hydrochloride (ICN Chemical and Radioisotope Division), specific activity 1,580 dpm/nmole l-carnitine. Enzyme activity was determined at 30°C, using 0.1–0.4 mg of mitochondrial protein. The concentration of the radioactive product, palmitoyl-1^14C-carnitine extracted in butanol was estimated by scintillation counting of samples taken from the butanol phase. An external standard method for quench correction was employed in all experiments. The scintillation fluid for radioactive counting was composed of Beckman Biosolve BBS-3 and fluoralyx in toluene. Samples were counted in a Packard TriCarb spectrometer, model 574.

Protein concentration was measured by the Biuret method (11). PalmitoylCoA (85–90% pure), coenzyme A (85–90% pure), ATP, palmitic acid, and l-carnitine were all obtained commercially. Palmitic acid (0.45 mm) was prepared in 0.6% bovine serum albumin to an approximate molar ratio of 5 to 1, acid to albumin.

Statistical analysis of the data was carried out using least-mean-squares regression analysis for best fit. Student's t-test was used to evaluate the significance of the changes produced.

**Results**

Although electron transport chain activity is not thought to be necessarily rate limiting to the oxidation of fatty acids in ischemia, intramitochondrial depletion of substrates and cofactors, reported previously in ischemic tissue (12, 13), could play some role in limiting oxidation of palmitic acid to CO_2. Therefore, the oxidation of 1-^14C-palmitate by mitochondria from uninvolved tissue and from ischemic left ventricle (7 days postligation) was measured before and after sequential addition of selected substrate and cofactors (Table 1). The addition of cytochrome C had little or only a slight effect on either basal or carnitine-stimulated rates of ^14CO_2 production in...
normal and ischemic mitochondria. On the other hand, addition of malate or succinate in low concentrations to stimulate oxidation through the citric acid cycle (14) markedly depressed the incorporation of the 14C-palmitate label into CO2 by the mitochondria from uninvolved tissue. Addition of these substrates had no marked effect on fatty acid oxidation by mitochondria isolated from ischemic tissue (Table 1).

When carnitine PCoAT activity was measured in mitochondria isolated from uninvolved interventricular septa and from tissue which had been ischemic for 7 days, the enzymatic activity in the ischemic mitochondria was significantly depressed (Fig. 1A). Addition of increasing quantities of mitochondrial protein produced a greater total conversion of palmitylCoA to palmitylcarnitine, but the differences in total activity between the enzymes from the control and the ischemic mitochondria became increasingly greater. An examination of pH optimums for the mitochondrial enzymes from ischemic and uninvolved areas revealed a similar pH range for optimal activity (Fig. 1B). However, the effect of increasing the pH toward neutrality on the enzymatic activity was much more marked in the case of the control mitochondria. The rate of increase in control enzyme activity per unit change in pH was approximately double that seen with the ischemic enzyme.

A kinetic analysis of the carnitine PCoAT activity from control or uninvolved mitochondria was compared with the enzyme kinetics of ischemic mitochondria. Figure 2A and B shows data plotted using Lineweaver-Burk and Hofstee analyses. For periods of ischemia longer than 24 hours, an analysis of the data demonstrated a drop in Vmax from 15 nmoles palmitylcarnitine/min mg−1 (Lineweaver-Burk) (or 14.2 nmoles/min mg−1 [Hofstee]) to 3.0 nmoles/min mg−1 (Lineweaver-Burk) (or 3.4 nmoles/min mg−1 [Hofstee]). Therefore, a severe depression of the enzyme activity was seen for this time period of ischemia (32 hours to 7 days). The Km for L-carnitine in the presence of 160 μM palmitylCoA for the enzyme from ischemic mitochondria decreased to 0.713 ± 0.148 mM (Lineweaver-Burk) (or 0.531 mM [Hofstee]). These values for Km decreased by approximately the same order of magnitude from the Km values for control as calculated from Lineweaver-Burk (2.59 ± 0.476 mM) and Hofstee (1.48 mM) plots. The Lineweaver-Burk value for the Km of the enzyme from ischemic mitochondria was significantly less (P << 0.01) than the value obtained for the normal Km. The standard error of the mean for the Km determinations was calculated after plotting the individual data by least-mean-squares regression analysis and extrapolating the lines to an ordinate value of zero. A third set of data included in Figure

### TABLE 1

<table>
<thead>
<tr>
<th>Additions</th>
<th>Control* (nmoles/mg)</th>
<th>Control + carnitine (fold)</th>
<th>Ischemic* (nmoles/mg)</th>
<th>Ischemic + carnitine (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.17</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM cytochrome C</td>
<td>1.27</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mM NAD + 10 μM cytochrome C</td>
<td>1.02</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 μM succinate + 10 μM cytochrome C</td>
<td>0.68</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 μM succinate + 2.5 mM NAD + 10 μM cytochrome C</td>
<td>0.95</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM malate</td>
<td>0.77</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM carnitine</td>
<td>3.74</td>
<td>3.2</td>
<td>0.098</td>
<td>1.4</td>
</tr>
<tr>
<td>5 mM carnitine + 10 μM cytochrome C</td>
<td>3.96</td>
<td>3.11</td>
<td>0.82</td>
<td>1.6</td>
</tr>
<tr>
<td>5 mM carnitine + 2.5 mM NAD + 10 μM cytochrome C</td>
<td>3.51</td>
<td>3.40</td>
<td>0.83</td>
<td>2.0</td>
</tr>
<tr>
<td>5 mM carnitine + 20 μM succinate + 10 μM cytochrome C</td>
<td>1.08</td>
<td>1.60</td>
<td>0.49</td>
<td>1.2</td>
</tr>
<tr>
<td>5 mM carnitine + 20 μM succinate + 2.5 mM NAD + 10 μM cytochrome C</td>
<td>0.97</td>
<td>1.02</td>
<td>0.41</td>
<td>1.08</td>
</tr>
<tr>
<td>5 mM carnitine + 10 μM malate</td>
<td>1.88</td>
<td>2.44</td>
<td>0.30</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The basic incubation medium consisted of 10 mM MgCl2, 45 mM potassium phosphate, pH 7.4, 20 mM KCl, 0.1 mM coenzyme A, 45 μM 1-14C-palmitate (25,500 dpm/mole), 2 mM ATP, and 500 μg of mitochondrial protein. Incubation was at 30°C for 10 minutes. Results are representative of two separate experiments. The control preparation contained 58% interventricular septum and 42% right ventricle from ischemic heart. The ischemic preparation contained left ventricle from ischemic heart.

*Nanomoles palmitate oxidized/mg mitochondrial protein.
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FIGURE 1

A: Effect of increasing mitochondrial protein concentration on total carnitine PCoAT activity. Carnitine PCoAT was assayed as described in Methods. Results are the average of two separate experiments. Circles indicate control activity (mitochondria isolated from interventricular septa). Squares indicate ischemic activity (mitochondria isolated from ischemic left ventricles of dogs killed 7 days postoperatively).

B: Determination of pH optimums for carnitine PCoAT in normal mitochondria and mitochondria isolated from ischemic left ventricle. Carnitine PCoAT was assayed as described in Methods at varying pHs in Tris-HCl buffer. Circles indicate control activity (mitochondria isolated from interventricular septa). Squares indicate ischemic activity (mitochondria isolated from ischemic left ventricle of a dog killed 14 days postoperatively).

2B illustrates the results of two separate experiments with mitochondria isolated at 24 hours of chronic ischemia. The value for Vmax (6.6 nmoles/min mg⁻¹) was approximately 50% of the control value (14.2 nmoles/min mg⁻¹). The Kₘ (1.34 mM) was not greatly different from control (1.48 mM); however, a trend toward decreased Kₘ might have been present at this time period of ischemia.

The Kₘ values for palmitylCoA (measured in the presence of 0.8 mM l-carnitine) at short periods of induced ischemia (20–30 minutes) and at periods of 1 day and longer of chronic ischemia are presented in Figure 3A and B. Short periods of ischemia appeared to have no effect on the kinetics of carnitine PCoAT with respect to its substrate, palmitylCoA. Using the Lineweaver-Burk transformations (Fig. 3A), Vmax was determined to be 6.5 and 8.6 nmoles palmitylcarnitine/min mg⁻¹ for control and 20–30 minutes of ischemia, respectively. These values are comparable to those estimated by the Hofsteet plot, i.e., 6.7 nmoles/min mg⁻¹ for control and 8.1 nmoles/min mg⁻¹ for 20–30 minutes of ischemia (Fig. 3B). Vmax determined under these conditions was less than that observed in Figure 2A and B due to a suboptimal concentration of carnitine (0.8 mM) in the incubation medium. At lowered carnitine concentrations, the inhibitory action of palmitylCoA on enzyme activity is more easily discerned (15). On prolonged ischemia (1–7 days), Vmax decreased to 2.3 nmoles palmitylcarnitine/min mg⁻¹. The values for Kₘ for enzymes from ischemic and control mitochondria were 35 μM and 42 μM, respectively, using the Lineweaver-Burk plot (Fig. 3A). These values were very close to those determined for Kₘ for control, 20–30 minutes of ischemia, and 1–7 days of ischemia, using the Hofsteet plot (Fig. 3B), i.e., 31 μM, 35 μM, and 34 μM, respectively. Therefore, it appears that values obtained for Kₘ for the enzymes from ischemic and control mitochondria are not significantly different.
A: Effect of chronic ischemia on the kinetics of carnitine PCoAT: Lineweaver-Burk analysis. Enzyme activity was determined at varying concentrations of L-carnitine and in the presence of 160 μM palmitoylCoA. Open circles indicate control activity ± SE (mitochondria isolated from normal left ventricles and from interventricular septa from ischemic hearts). Triangles indicate ischemic activity ± SE (mitochondria isolated from ischemic left ventricles of dogs after 1-7 days of chronic ischemia).

B: Effect of chronic ischemia on the kinetics of carnitine PCoAT: Hofslee plot. Enzyme activity was determined at varying concentrations of L-carnitine and in the presence of 160 μM palmitoylCoA. Open circles indicate control activity ± SE (mitochondria isolated from normal left ventricles and from interventricular septa from ischemic hearts). Solid circles indicate ischemic activity (average of two separate experiments) (mitochondria isolated from ischemic left ventricles of dogs at 24 hours of chronic ischemia). Triangles indicate ischemic activity ± SE (mitochondria isolated from ischemic left ventricles of dogs after 1-7 days of chronic ischemia).

A. Effect of chronic ischemia on the kinetics of carnitine PCoAT: Lineweaver-Burk analysis. Enzyme activity was determined at varying concentrations of palmitoylCoA and in the presence of 0.8 mM L-carnitine. Open circles indicate control activity ± SE (mitochondria isolated from normal left ventricles and from interventricular septa from ischemic hearts). Solid circles indicate ischemic activity (short term) (mitochondria isolated from ischemic left ventricles of dogs after 20-30 minutes of ischemia). Values represent the average of two dogs. Triangles indicate ischemic activity ± SE (mitochondria isolated from ischemic left ventricles of dogs after 1-7 days of chronic ischemia).

B: Effect of chronic ischemia on the kinetics of carnitine PCoAT: Hofslee plot. Enzyme activity was determined at varying concentrations of palmitoylCoA and in the presence of 0.8 mM L-carnitine. Open circles indicate control activity ± SE (mitochondria isolated from normal left ventricles and from interventricular septa from ischemic hearts). Solid circles indicate ischemic activity (short term) (mitochondria isolated from ischemic left ventricles of dogs after 20-30 minutes of ischemia). Values represent the average of two dogs. Triangles indicate ischemic activity ± SE (mitochondria isolated from ischemic left ventricles of dogs after 1-7 days of chronic ischemia).
The structural and the functional integrity of the membrane-bound mitochondrial enzymes has been associated with double, nonintersecting slopes for activation energies (16). Therefore, activation energies were calculated for carnitine PCoAT from left ventricular mitochondria of (1) normal dog heart, (2) 24-hour ischemic dog heart, and (3) 32-hour ischemic dog heart and from sonicated mitochondria isolated from normal dog heart (Fig. 4). All of the enzymatic activities studied presented a biphasic response to temperature with the exception of the carnitine PCoAT from the 32-hour ischemic mitochondria. The activity of the carnitine PCoAT from these mitochondria was comparable to that seen at 7 days of chronic ischemia. Vmax was 2 nmoles palmitoylcarnitine/min mg⁻¹ and the value of K_m, determined by the Lineweaver-Burk analysis, was 0.6 mM. The initial slopes of the temperature response for normal and 24-hour ischemic mitochondria represented activation energies of 12.6 and 10.9 kcal, respectively. The second phase of the temperature response corresponded to activation energies of 5.2 and 5.48 kcal for normal and 24-hour ischemic mitochondrial carnitine PCoAT. The break in the slopes occurred between 15° and 20°C. However, the carnitine PCoAT activity of the 32-hour dog heart mitochondria gave a single activation energy of 5.1 kcal, corresponding to the second phase of the Arrhenius plot for the activation energy of the normal enzyme. Although the activation energies for the enzyme in the sonicated preparation were altered, a biphasic response to temperature was still present.

Discussion

The effect of chronic ischemia on certain specific alterations in the mitochondrial enzyme carnitine PCoAT has been described. The activity of this enzyme is thought to be important to the rate of long-chain fatty acid oxidation in normal tissue (14, 17-23). Stimulation of the oxidation of palmitate to CO₂ by carnitine, seen in normal mitochondria, was severely depressed in mitochondria isolated from left ventricles which had been ischemic for 7 days. Addition of cytochrome C, Krebs cycle substrates, or both had little or no effect on increasing the carnitine-dependent rate of palmitic acid oxidation in the ischemic mitochondria. However, in the normal or the control mitochondria, oxidation of palmitate to ^14CO₂ was depressed in the presence of 5 mM carnitine after addition of malate or succinate. These substrates have previously been shown to have a sparing action on the oxidation of palmitoylcarnitine and acetylcarnitine (14, 24). Bremer has suggested a successful competition by the α-ketoacids (with acylcarnitine) (24) for the available mitochondrial CoA. A preference of the mitochondria for the electrons derived from the α-ketoacids and the Krebs cycle intermediates to those derived from the β-oxidation of fatty acids is also possible.

Severe inhibition of carnitine PCoAT activity in mitochondria isolated from ischemic left ventricle was demonstrated. This finding may be consistent with the idea of reduced quantities of active enzyme. However, a decreased velocity of the breakdown of enzyme-substrate complex in some or in all of the enzyme-substrate complexes is also possible. Although brief periods of ischemia (20-30 minutes) had little effect on the carnitine PCoAT in the presence of varying amounts of palmitoylCoA as substrate, periods of chronic ischemia greater than 24 hours noncompetitively inhibited enzyme activity compared with control activities. However, in more recent experiments employing higher (2.4
mm) concentrations of carnitine, the Vmax of carnitine PCoAT was depressed 40% as early as 30 minutes postocclusion (data not shown). During prolonged periods of ischemia (>1 day), an interesting effect on the Km of the enzyme for l-carnitine was observed. Even though Vmax was even more markedly depressed during this time interval, the apparent affinity of the enzyme for substrate, carnitine, was significantly increased. Earlier studies have proposed that palmitoylCoA is not only a substrate for carnitine PCoAT but also is a competitive inhibitor for the second substrate (carnitine) (15). Bremer and Norum (15) observed that high palmitoylCoA concentrations inhibited carnitine PCoAT and elevated the Km toward carnitine. The Ki for palmitoylCoA was reported to be 3 μM (15). This value is similar to that recently found in this laboratory for normal dog heart (Ki < 5 μM). Certain detergents counteract this inhibitory effect of palmitoylCoA, decreasing the Km for carnitine. It was postulated that detergents may bind to hydrophobic sites on the enzyme, thus interfering with the binding of palmitoylCoA (25). Any interference in the binding of palmitoylCoA to an inhibitory site might be expected to raise the Ki for palmitoylCoA. In agreement with this finding, preliminary data on mitochondria from four dog hearts after 7 days of chronic ischemia demonstrate a significant increase in Ki (Wood and Schwartz, unpublished observations). Considering these observations, it seems tempting to suggest that prolonged chronic ischemia might produce similar alterations in the hydrophobic regions of the enzyme. Corrections for the inhibitory action of palmitoylCoA on carnitine PCoAT have given Km values for carnitine varying from 0.25 (15) to 0.45 mM (26), much less than the value of 2.1 mM initially measured by Norum (27). Our values for normal dog heart carnitine PCoAT Km for carnitine (1.5 mM and 2.6 mM) are in agreement with the latter Km observed at high palmitoylCoA concentrations (27).

In line with these observations, further evidence for a change in the hydrophobic regions of the membrane-associated carnitine PCoAT from ischemic tissue is presented after examination of the change in activation energy of the enzyme from normal and 32-hour ischemic dog hearts. Discontinuities observed in the Arrhenius plot are characteristic of several membrane-bound mitochondrial enzyme systems, including the succinate oxidase system from several homeothermic animals (28) and cytochrome oxidase, β-hydroxybutyrate, and α-ketoglutarate oxidase in rat liver mitochondria (16). Since the enzymes and the enzyme systems exhibiting the abrupt discontinuities in activation energy with a change in temperature are localized within or closely associated with the membrane of the mitochondrion (28) and since this change in activation energy does not occur when the lipid component of the membrane is removed or disturbed by detergent, the phase change is most probably associated with this lipid component (28).

A temperature-dependent change in the permeability of the mitochondrion to its substrate has been suggested as a possible explanation for the discontinuity seen in the Arrhenius plot (28, 29). However, when mitochondrial membranes were disrupted by hypotonic swelling, freezing, and thawing or by sonication (16) and any permeability barrier to substrate was most likely removed, the membrane-bound enzyme systems continued to exhibit the discontinuity in the Arrhenius plot (16). Experiments with sonicated mitochondria presented in this paper confirm these results.

These data suggest that specific changes occur in the fatty acid transferase system of mitochondria from ischemic hearts. The changes measured are suggestive of a disturbance in the lipid components of the mitochondrial membrane as a result of prolonged ischemia and may account for a specific metabolic defect in the oxidation of fatty acids observed in ischemic hearts. The irreversibility of this defect may be due to structural changes in the mitochondrial membrane lipid and, perhaps, protein components as well. These studies suggest that a defect in the transport of long-chain fatty acids into mitochondria may be associated with myocardial ischemia.

References
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