Biochemical and Morphological Correlates of Acute Experimental Myocardial Ischemia in the Dog

IV. Energy Mechanisms during Very Early Ischemia

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SUMMARY Tissue energy metabolism was examined in posterior (ischemic) and anterior ("control") regions of canine ventricles after 5 and 10 minutes of left circumflex coronary artery occlusion. When compared to identical regions of normal hearts, the following changes were found: (1) decreases in glycolyis and phosphorylase activity in the anterior and posterior regions, (2) depressed state 3 rates of oxygen consumption of isolated mitochondria in both anterior and posterior regions, (3) shifts in optimum substrate concentrations for palmitoyl-CoA (+ carnitine) oxidation by mitochondria in the anterior and posterior regions, and (4) decreases in the apparent zero order and first order rates of mitochondrial palmitoylcarnitine production. These changes correlated with a marked decrease in developed tension in the posterior regions. Depression in tension development in the posterior regions of the heart still was present after 30-60 minutes of reperfusion following a 10-minute period of occlusion. Glycogen content in the reperfused areas was significantly decreased after 60 minutes of reperfusion when compared to normal areas and to control hearts perfused for 70 minutes. After reperfusion, mitochondrial function appeared to return toward "normal." However, the slow restoration of contraction of the ischemic area suggests that cellular mechanisms operative in vivo to restore pump function still might be abnormal. Circ Res 44: 52-61, 1979

THE HEART is an aerobic organ which derives the energy needed for mechanical function from the process of oxidative phosphorylation in mitochondria. When tissue levels of oxygen are reduced by decreased flow through the coronary arteries perfusing the myocardium, mitochondrial metabolism is inhibited. Subsequently, the heart becomes dependent on energy supplied by anaerobic glycolysis for contraction and relaxation. However, after onset of myocardial ischemia, mechanical function rapidly deteriorates although 75-90% of the tissue ATP supplies still are present (Braasch et al., 1968; Gudbjarnason et al., 1970; Neely et al., 1973). Consequently, ventricular failure ensues at a time when steady state levels of tissue high-energy phosphates appear to be adequate. These data suggest that the ATP produced by glycolysis may not be immediately available and/or the rates of production are too low to support contractile events. In any event, they underscore the importance of mitochondrial energy metabolism to the heart. These factors, together with observed structural and functional alterations of mitochondria in ischemic, injured myocardial cells, suggest that mitochondrial damage may be critical in the development of irreversible cell damage (Jennings, 1969). Both impairment of function and irreversible injury may be consequences of some prolonged metabolic abnormality, e.g., accumulation of cellular lactate (and NADH) as major factors responsible for glycolytic inhibition in ischemic tissue (Rovetto et al., 1973; Neely et al., 1972, 1975), accumulation of fatty acyl esters (Shug et al., 1975; Whitmer et al., 1972), or lysosomal activation. (Hoffstein et al., 1975; Ricciuti, 1972a, 1972b).
Myocardial ischemia results in rapid changes in myocardial function that occur almost immediately after coronary occlusion. Since mitochondrial integrity appears to be important to assure cellular viability, we have examined the effects of short-term coronary artery occlusion (5- and 10-minute periods of ischemia) on physiological function as well as mitochondrial oxidative reactions. This period of ischemia was selected because it has been suggested that, at least in experimental animals, the cardiac cellular injury caused by it is reversible (Jennings, 1969; Krug et al., 1966). The effect of coronary reperfusion on restoration of contraction and mitochondrial metabolism was studied to describe the period of reflow required for reestablishment of normal mechanical and biochemical function.

Methods

Thirty mongrel dogs weighing 25-35 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv, with supplemental doses when necessary). Ventilation was controlled by a Harvard respirator (model 600). Electrocardiographic leads were attached to the limbs. After a groin incision, Tygon tubing was advanced retrogradely through the femoral artery to the thoracic aorta and was secured in place and connected to a Statham P23Db transducer (Statham Instruments) to measure systemic arterial pressure.

A left thoracotomy was performed through the 4th intercostal space. The pericardium was opened and the heart exposed. Walton-Brodie strain gauges were sewn to the left ventricular myocardium parallel to the minor axis in areas supplied by the left anterior descending and the left circumflex coronary arteries, respectively. In two dogs, two pairs of ultrasonic crystals were embedded in the myocardium to measure regional myocardial segment length in the areas supplied by the left anterior descending and left circumflex coronary arteries (Franklin et al., 1973; Theroux et al., 1974). To minimize the effect of the isometric strain gauges on segment motion, the crystal pairs were placed in a circumferential orientation approximately 3-4 cm from the respective strain gauges with a spacing between crystals of 1.0-1.5 cm. The distance between the crystals in each pair was measured instantaneously by the ultrasonic transit-time principle, using an instrument constructed in our laboratory (Hartley et al., 1974). All data were recorded on a Brush recorder (Clevite Mark 200, Brush Instrument Co.). Derivatives of the force recordings were calculated using Biotronics Laboratory differentiators (model 620, Biotronics Laboratories, Inc.).

A short area of the proximal left circumflex coronary artery then was dissected free from surrounding tissue and a "reversible" ligature was placed around it. Each dog then was allowed to stabilize for 15-30 minutes, and following this, one of five experimental protocols was used:

1. In six dogs no occlusion was performed. After the stabilization period, measurements were made for 10 minutes, after which the hearts were excised for study. To test the effect of a prolonged perfusion interval on physiological performance, one group of three dogs in which no occlusion was performed was monitored for physiological function for 70 minutes following the stabilization period. Two hearts from this group were excised for biochemical study. In another two dogs, control perfusion was carried out for 4 hours. These hearts were followed for physiological measurements only.

2. In 13 dogs, the ligature was tightened to occlude the left circumflex coronary artery. Measurements were made during a 5-minute period of occlusion. The hearts then were excised for study.

3. In 15 dogs, the ligature was tightened, and measurements were made for 10 minutes. The hearts then were excised for study.

4. In six dogs, the left circumflex coronary artery was occluded for 10 minutes. The ligature then was removed and a 20- to 30-minute reperfusion period was allowed before the heart was removed. Five hearts from this group were processed for biochemical data.

5. In four dogs, the left circumflex coronary artery was occluded for 10 minutes and the artery was reperfused for 60 minutes. The hearts were processed for biochemical data. In another set of six dogs, reperfusion was carried out for 4 hours following occlusion to determine the effect of more extensive reperfusion on physiological performance.

Since physiological function was monitored continuously during the occlusion and reperfusion periods, the following numbers of dogs were grouped for physiological studies: 2-5 minutes of occlusion, 28 dogs (13 dogs in group 2 and 15 dogs in group 3); 10 minutes of occlusion, 21 dogs (15 dogs in group 3 and 6 dogs in group 4); 20 minutes of reperfusion, 10 dogs (6 dogs in group 4 and 4 dogs in group 5); 60 minutes of reperfusion, 4 dogs (group 6); 4 hours of reperfusion, 6 dogs (group 5). All data were grouped for analysis, and a paired Student's t-test was used for statistical analysis.

Biochemical measurements were made on heart muscle samples taken from various regions of the myocardium following the previously described experimental protocols: control perfusion, coronary ligation, or coronary ligation and varying periods of reperfusion. The regions sampled from the ligated hearts represent (1) "control" areas perfused by the left anterior descending coronary artery, i.e., the anterior papillary muscle and the anterior subendocardium at the base of the papillary muscle; (2) "ischemic" areas where flow is decreased following ligation of the left circumflex artery, i.e., the posterior papillary muscle. "Normal" values were obtained from both the anterior and posterior regions of hearts that were perfused for a period of 10 minutes after dissection (but no ligation) of the left
circumflex artery. Statistical analysis of the biochemical data employed Student's t-test (nonpaired variates) for direct comparison to normal, perfused hearts as well as Student's t-test for paired samples to compare control and ischemic regions from the same heart. All data are expressed as the mean ± SE.

Glycogen and phosphorylase activity were measured in homogenates immediately following homogenization of a cardiac muscle sample and in the microsomal fraction isolated from the homogenates as previously described (Entman et al., 1976). Glycogen was measured in two ways to ensure the accuracy of the results. The data presented represent measurements by the phenol sulfuric acid method which essentially quantifies all sugars (Dubois et al., 1956). To ensure that the measurements made were specific for polysaccharides, selected samples also were measured by the following method: serial dilutions of cardiac homogenates or microsomal protein ranging from 10 to 120 μg of protein were incubated in 1 ml reaction mixtures containing 1 mg/ml of Rhizopus glucoamylase (Sigma) at pH 4.5 and 55°C. After 1 hour, the reaction was stopped, and glucose was measured in a manner similar to that previously described (Entman et al., 1976). A rabbit liver glycogen standard curve demonstrated that this method gave almost theoretical yields of glucose. Glycogen values measured by both of these methods were similar.

Total phosphorylase (a + b) was measured in cardiac homogenates and microsomes by a continuous assay method in a Gilford recording spectrophotometer. Reactions were carried out in the presence of 15 mM sodium phosphate, 10 mM 2-(N-morpholino) ethane-sulfonic acid buffer (pH 6.8), 10 mM magnesium chloride, 1 mM dithiothreitol, 0.015 mg/ml glucose-1,6-diphosphate, 1% glycogen, 2 mM AMP, and 0.5 mM NADP. Glucose-1-phosphate production was linked to NADP reduction by 0.015 mg/ml glucose-1,6-diphosphate, 1% glycogen, 2 mM AMP, and 0.5 mM NADP. Glucose-1-phosphate production was linked to NADP reduction by the addition of 15 units of phosphoglucomutase and 15 units of glucose-6-phosphate dehydrogenase. In reactions measuring phosphorylase activation, an ATP-regenerating system containing 2.5 mM phosphoenolpyruvate and 15 units of pyruvate kinase also was added. In some experiments, HEPES, Tris-maleate, α-glycerophosphate, or additional phosphate was used as buffer with no qualitative difference in results. For both the glycogen and phosphorylase assays, protein was measured by the biuret procedure (Layne, 1957).

Mitochondria were isolated from cardiac homogenates in a medium containing 0.18 mM KCl and 10 mM EDTA both in the presence and absence of 0.5% bovine serum albumin (Fraction V, Sigma), pH 7.2, by a method developed in this laboratory (Sordahl et al., 1971). Electron microscopic examination of the mitochondrial pellets revealed little or no contamination with other intact subcellular fractions. Respiratory activity was measured on a Gil-son Medical Electronics Oxygraph by a previously described procedure (Sordahl et al., 1971). and mitochondrial protein was measured by the biuret method (Layne, 1957).

Total mitochondrial carnitine palmityltransferase activity was measured by a modification of the original method of Bremer and Norum (1967) as described by Pande and Blanchaer (1970) using 0.04% Triton X-100, to express latent enzyme activity, 2.4 mM DL-[3H-methyl]carnitine hydrochloride (Amersham/Searle), specific activity 8850 dpm/nmol of l-carnitine and 10–15 nmol palmitoyl-CoA. Suspensions of heart mitochondria were added to the assay tubes at a protein concentration of 0.1–0.15 mg to maintain a constant palmitoyl-CoA to protein ratio of 100 nmol palmitoyl-CoA/mg mitochondria. At saturating concentrations of both substrates and in the presence of Triton X-100, the reaction followed an apparent linear rate up to 1 minute at 30°C. [3H]Palmitylcarnitine formed was measured by scintillation counting in a Beckman liquid scintillation spectrometer (model LS-200B). An external standard method for quench correction was employed in all experiments. The scintillation fluid for radioactive counting was composed of Beckman Bio-Solv Solubilizer and fluorarol in toluene.

A sensitive method for determination of carnitine palmityltransferase activity at physiological palmitoyl-CoA levels was employed using a spectrophotometric assay which measures the quantitative release of CoA-SH upon enzymatic formation of palmitylcarnitine (Bieber et al., 1972). In these experiments, the reaction proceeds to completion, since CoA-SH is removed by a chemical reaction with 5,5'-dithiobis-(2-nitrobenzoic acid).

Results

Physiological Data

After a period of stabilization, the left circumflex artery was ligated and the wall tension developed in the anterior and posterior regions of the left ventricle was monitored with strain gauges placed on the areas perfused by the left anterior descending artery and the circumflex artery. In two dogs, regional muscle shortening was measured with an ultrasonic length gauge. Changes in tension and shortening also were measured after coronary ligation and reperfusion of the myocardium. The tension developed was significantly decreased in the posterior left ventricle by 58 ± 7% and 66 ± 8% after 5 and 10 minutes of occlusion, respectively (Fig. 1), and there was a net increase in tension recorded from the anterior left ventricle (+11 ± 6%, P < 0.05 at 5 minutes after occlusion). Release of the occlusion and reperfusion resulted in a transient increase in developed wall tension as measured by the strain gauge in the circumflex region. However, despite this increase in tension 5 minutes after onset of
reperfusion, developed tension still was significantly lower than control ($P < 0.05$). In fact, continued reperfusion did not alter tension development which remained depressed even after 40 minutes of reperfusion. On release of the ligation, there was a rapid decrease in tension in the anterior wall region ($P < 0.05$ compared to control). In the experiments in which the reperfusion period was 4 hours, there was a depression in tension development in the circumflex region that remained constant at $-40 \pm 5\%$ of control. Two control dogs in which perfusion continued for 4 hours without coronary artery ligation showed no significant changes in tension development during this period, compared to either initial control values before coronary occlusion or to unoccluded hearts perfused for 10 minutes or 70 minutes (see Methods). Tension development in the anterior region of the occluded myocardium decreased gradually during the 4-hour reperfusion period, i.e., at 1 hour by $11 \pm 5\%$, at 2 hours by $16 \pm 4\%$, at 3 hours by $23 \pm 5\%$, and at 4 hours by $27 \pm 3\%$.

The rate of tension or force development ($dF/dt$) in the anterior left ventricle showed no change after occlusion; however, the posterior wall demonstrated decreases of $38 \pm 10\%$ ($P < 0.001$) and $42 \pm 13\%$ ($P < 0.05$) in $dF/dt$ at 5 and 10 minutes after coronary ligation (data not shown). Changes in $dF/dt$ in the circumflex region were similar to recorded developed tension in that area (see above). Aortic systolic pressure decreased from $122 \pm 3$ to $114 \pm 11$ mm after 5 minutes of occlusion ($P < 0.001$), but returned to the control value throughout the period of reperfusion.

Muscle shortening measured by segment length crystals during the 10-minute occlusion followed by reperfusion showed a 30-40% decrease in shortening in both regions of the myocardium. This decrease persisted during the 4 hours of reperfusion. Tension development and muscle shortening both are indices of regional myocardial function, but the development of tension takes place during isovolumic contraction, whereas muscle shortening occurs during ejection. Afterload and preload thus affect these parameters to varying degrees. However, both indices showed approximately the same decrease in the posterior and anterior (supposedly unaffected) regions. In one control dog in which there was no occlusion, muscle shortening as determined by segment length crystals remained at control levels for the entire 4-hour period.

Biochemical Data

Glycogen Levels and Phosphorylase Activity

Both glycogen and phosphorylase activity decreased rapidly in areas of myocardial ischemia. Following a 5-minute period of left circumflex occlusion, the region of the anterior papillary muscle and posterior papillary demonstrated glycogen levels of $62.6 \mu g/mg$ protein (100%) and $51 \mu g/mg$ (81%), respectively. The percentage decrease was similar for both glycogen and phosphorylase and was found in the areas most dependent on left circumflex coronary artery perfusion (Fig. 2). These findings suggested that decreased total phosphorylase activity in cardiac muscle might be related to glycogen depletion. To examine this possibility, phosphorylase activity associated with the cardiac microsomal fraction (presumably sarcoplasmic reticulum fragments) was determined and correlated with the glycogen concentration. There was a linear relationship between glycogen and phosphorylase levels in microsomal fractions in all samples of ischemic and nonischemic (control) myocardium taken from a single heart after 5 minutes of circumflex ligation (Fig. 3). Thus, the total decrease in tissue phosphorylase (Fig. 2) was accompanied by diminution of phosphorylase activity bound to subcellular structures. To test the possibility that lower total phosphorylase might have been due to enzyme inactivation or destruction in ischemic portions of the heart, we removed hearts from normal dogs and placed samples of left ventricle in beakers containing 150 mM KCl kept at $37^\circ$C for periods up to 1 hour in the absence of pacing and tissue perfusion. Glycogen concentration and phosphorylase activity were measured in homogenates taken from these heart samples after different incubation intervals. In contrast to the data recorded for experimentally induced regional ischemia, there was glycogen breakdown but no concomitant phosphorylase depletion (Fig. 4). Thus, tissue perfusion may play an important role in phosphorylase washout.

Because glycogen depletion is a sensitive quantitative marker for early ischemia in the absence of a major sudden increase in cardiac work, glycogen
content was measured in "control" and ischemic areas of the hearts after left circumflex coronary occlusion and after occlusion with subsequent reperfusion. To examine the possibility that the control or anterior regions of the hearts subjected to ligation also might respond to the insult associated with posterior ischemia, glycogen content was measured in the anterior and posterior zones of a normal, nonischemic dog heart. Compared to glycogen levels in the normal papillary muscles and subepicardium, 5 and 10 minutes of ischemia produced a time-dependent depletion of glycogen in both anterior and posterior papillary muscles (Fig. 5A). The anterior subepicardium did not show a significant depletion of glycogen, compared to normal, after occlusion. However, the posterior subepicardium demonstrated a significantly lower glycogen content after 10 minutes of ischemia (Fig. 5B). After 30 minutes of reperfusion, the anterior papillary muscle showed no significant change in glycogen compared either to control or to 10-minute ischemic hearts. Posterior papillary glycogen remained significantly depressed compared to control following 10 minutes of ischemia and 30 minutes of reperfu-
Mitochondrial Function

Mitochondria isolated from normal and ischemic as well as reperfused areas of the left ventricle were examined for their ability to oxidize a number of Krebs cycle and lipid substrates. In the presence of glutamate-malate, 5 minutes of ischemia produced a highly significant decrease in state 3 $QO_2$ (ADP-stimulated) in mitochondria isolated from both anterior and posterior regions of the myocardium (Fig. 6). Compared to 5 minutes of ischemia, the mitochondria from the anterior papillary muscle and subepicardium showed slightly higher state 3 oxidative rates after 10 minutes of ischemia, although these changes were not significant between the two occlusion intervals. The posterior papillary muscle and subepicardial mitochondria remained depressed at the same levels at both 5 and 10 minutes.

State 3 rates of mitochondria from all areas were significantly decreased at 10 minutes compared to control. Only after 10 minutes of occlusion was a significant difference between state 3 respiration of anterior and posterior papillary muscle mitochondria in the same heart demonstrable using Student's $t$-test for paired variates. Thirty minutes of reperfusion resulted in glycogen levels of 74 $\mu$g/mg in both papillary muscles and subepicardial muscle. A: Papillary muscle glycogen. After 10 minutes of occlusion, anterior papillary muscle glycogen has decreased significantly ($0.05 > P > 0.02$), and a significant decrease is demonstrated after 60 minutes of reperfusion ($P < 0.02$). Posterior papillary muscle glycogen is decreased significantly at both 5 and 10 minutes of occlusion ($P = 0.05$ and $P < 0.001$, respectively) and after 10 minutes of occlusion with either 30 or 60 minutes of reperfusion ($P < 0.01$ and $P < 0.005$, respectively). B: Subepicardial muscle glycogen. Compared to normal, no significant differences in anterior subepicardial muscle glycogen were seen. Significant changes in posterior subepicardial glycogen were observed after 10 minutes of occlusion ($P = 0.02$) and 10 minutes of occlusion plus 1 hour of reperfusion ($P = 0.005$).

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** Effect of coronary occlusion and reperfusion on total homogenate glycogen content. Glycogen content is expressed as $\mu$g glucose per mg protein. Bars represent reperfusion time after a 10-minute occlusion interval. Filled bars, ••••, anterior; unfilled bars, ○○○○, posterior. Significance of changes (*) in total glycogen with respect to glycogen content in normal, perfused canine myocardium. A: Papillary muscle glycogen. After 10 minutes of occlusion, anterior papillary muscle glycogen has decreased significantly ($0.05 > P > 0.02$), and a significant decrease is demonstrated after 60 minutes of reperfusion ($P < 0.02$). Posterior papillary muscle glycogen is decreased significantly at both 5 and 10 minutes of occlusion ($P = 0.05$ and $P < 0.001$, respectively) and after 10 minutes of occlusion with either 30 or 60 minutes of reperfusion ($P < 0.01$ and $P < 0.005$, respectively). B: Subepicardial muscle glycogen. Compared to normal, no significant differences in anterior subepicardial muscle glycogen were seen. Significant changes in posterior subepicardial glycogen were observed after 10 minutes of occlusion ($P = 0.02$) and 10 minutes of occlusion plus 1 hour of reperfusion ($P = 0.005$).

![Figure 6](http://circres.ahajournals.org/)

**Figure 6** Effect of coronary occlusion and reperfusion on state 3 oxidative rates of canine heart mitochondria: glutamate as substrate. State 3 (ADP-stimulated) $QO_2$ is expressed as nmoles oxygen consumed per min per mg mitochondrial protein. Bars represent reperfusion time after a 10-minute occlusion interval. Filled bars, ••••, anterior; unfilled bars, ○○○○, posterior. Significance of change (*) in state 3 respiration with respect to respiration of mitochondria from normal canine myocardium. A: Papillary muscle mitochondria. Significant decreases in anterior and posterior papillary muscle mitochondrial respiration were seen after 5 minutes of occlusion ($P < 0.005, P < 0.001$, respectively) and after 10 minutes of occlusion ($0.02 > P > 0.01$ and $P < 0.005$, respectively). Glutamate-malate oxidation was significantly depressed in anterior papillary muscle mitochondria after 10 minutes of occlusion with 20–30 minutes of reperfusion ($P = 0.05$). B: Subepicardial muscle mitochondria. Significant decreases in anterior and posterior subepicardial mitochondria $QO_2$ were seen after 5 minutes of occlusion ($P < 0.005$ for both) and in the posterior subepicardium after 10 minutes of occlusion ($0.02 > P > 0.01$). Both the anterior and posterior subepicardium demonstrated significantly depressed $QO_2$ values after 10 minutes of occlusion and 20 to 30 minutes of reperfusion ($0.05 > P > 0.025$ and $P = 0.05$, respectively).
fusion produced no alteration in glutamate-supported respiration in any of the areas compared to values obtained after 10 minutes of ischemia. In fact, mitochondria isolated from reperfused myocardium using glutamate-malate as substrate were still depressed significantly compared to normal, perfused hearts. However, after 1 hour of reperfusion, the ability of mitochondria to oxidize glutamate appeared restored toward normal levels (Fig. 6). Similar results were obtained when either succinate or pyruvate was employed as the substrate after 5 to 10 minutes of ischemia (Figs. 7 and 8). However, any differences in mitochondrial state 3 oxidative rates did not attain statistical significance at the 5% level when compared to control or ischemic rates after either 30 or 60 minutes of reperfusion with the population size employed (see Methods).

Significant decreases in glutamate-supported state 4 respiration were observed in all areas of the myocardium after 5 minutes of ischemia (0.05 > P > 0.02) when palmitylcarnitine was employed as oxidative substrate. Succinate-supported state 4 rates demonstrated a significant decrease after 5 minutes of ischemia, but only in mitochondria isolated from the posterior papillary muscle (P < 0.025). The respiratory control ratios for anterior and posterior papillary muscle mitochondria for glutamate and succinate, respectively, were not significantly decreased from control (8.7 ± 0.7; 4.7 ± 0.6) at 5 (10.3 ± 1.1; 4.0 ± 0.1) and 10 minutes (7.7 ± 8; 4.0 ± 0.3) of ischemia due to either decreases in both state 3 and state 4 rates after 5 minutes of ischemia or to the partial recovery of subepicardial mitochondrial state 3 respiratory rates after 10-minute occlusion (7.6 ± 0.7, control subepicardium vs. 8.7 ± 0.1, 10-minute occlusion and 4.8 ± 0.4, control subepicardium vs. 4.0 ± 0.3, 10-minute occlusion; glutamate and succinate, respectively).

The ability of ischemic and reperfused myocardial mitochondria to oxidize lipid substrate also was investigated. Because free fatty acid is a major fuel for mitochondrial energy production, oxidation of palmitoyl-CoA and palmitoylcarnitine by mitochondria made ischemic for 5 and 10 minutes and by mitochondria isolated from reperfused myocardium was examined. Oxidation of palmitoylcarnitine was
depressed in all regions of the myocardium after 5 minutes of ischemia, and this depression was highly significant in the mitochondria isolated from the posterior papillary muscle after a 10-minute occlusion period (0.05 > P > 0.02). In general, with this substrate, the depression in state 3 rates after occlusion and restoration of rates after 60 minutes of reperfusion were similar to the patterns observed with Krebs cycle substrates (Figs. 6-8). Since palmitoylcarnitine formation from palmityl-CoA is dependent on the activity of the mitochondrial enzyme, carnitine palmityltransferase, the effect of ischemia and reperfusion on total measurable enzyme rates in the presence of Triton X-100 was determined. At saturating substrate concentrations, the rates of palmitoylcarnitine production follow an apparent zero order process. After 5 and 10 minutes of coronary occlusion, carnitine palmityltransferase activity decreased significantly in mitochondria from all areas of the myocardium (Fig. 9). The rates of palmitoylcarnitine production were slightly lower in the posterior papillary muscle than in the anterior papillary muscle both at 5 and 10 minutes of ischemia. Reperfusion restored the total activity of the mitochondrial carnitine palmityltransferase toward normal levels, and no significant statistical difference between normal and reperfused enzyme activity was observed at any time with the population of animals examined.

The result of polarographic determination of state 3 rates of palmityl-CoA oxidation in the presence of 2.0 mM l-carnitine is shown in Figure 10. Maximal rates of substrate oxidation are dependent on the concentration of palmityl-CoA in the assay medium. In mitochondria isolated from normal pap-
illar muscle, optimal rates of respiration occur in a palmityl-CoA concentration range of 20–25 nmol/mg mitochondrial protein (15–20 μM) Fig. 10A. Analysis of the respiratory response to palmityl-CoA of mitochondria isolated after 5 and 10 minutes of ischemia demonstrates a critical and narrow substrate range for maximal oxidative rates. The results are a summation of data for both anterior and posterior regions since palmityl-CoA oxidation was similarly depressed in both anterior and posterior areas of the heart after coronary occlusion. At levels of palmityl-CoA greater than 10 nmol/mg (or 8 μM), respiration was severely inhibited (Fig. 10A). In agreement with the enzymatic determination of carnitine palmityltransferase activity, reperfusion after a 10-minute occlusion restored the respiratory response to palmityl-CoA both quantitatively and qualitatively (Fig. 10B).

To determine whether short periods of coronary occlusion affect the rates of palmitoylcarnitine production at physiological substrate levels, the mitochondria were incubated with concentrations of palmityl-CoA below and at the critical micelle concentration. The reaction, started by addition of 1.6 mM L-carnitine, followed apparent first order kinetics (Table 1). Under conditions of normal myocardial perfusion, the rate constants decreased as palmityl-CoA increased. This decrease in rate is consistent with the known action of palmityl-CoA as a competitive inhibitor of carnitine palmityltransferase at the carnitine site (Bremer and Norum, 1967).

Since the rates of CoA-SH release were no different after either 10 or 20 minutes of occlusion of the left circumflex artery, the data were compiled using both periods of ischemia. The apparent first order rate constants at all palmityl-CoA concentrations decreased on an average to 79% of normal values in both the anterior and posterior papillary muscle mitochondria. Even though the apparent first order rate constants appeared decreased, palmitoylcarnitine formation by mitochondria from the anterior papillary muscle continued to demonstrate an inhibitory response to increasing palmityl-CoA levels. The inhibitory effects of palmityl-CoA on posterior papillary muscle mitochondrial carnitine palmityltransferase were less apparent.

### Table 1: Effect of Physiological Palmityl-CoA Levels on the Apparent First-Order Rate Constants for Normal and Ischemic Dog Heart Mitochondrial Carnitine Palmityltransferase

<table>
<thead>
<tr>
<th>Palmityl-CoA (μM)</th>
<th>Anterior</th>
<th>Posterior</th>
<th>10-20 min occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal perfusion</td>
<td>Anterior</td>
<td>Posterior</td>
</tr>
<tr>
<td>0.67</td>
<td>7.60</td>
<td>7.58</td>
<td>5.72</td>
</tr>
<tr>
<td>2.00</td>
<td>5.55</td>
<td>5.86</td>
<td>4.82</td>
</tr>
<tr>
<td>3.33</td>
<td>4.39</td>
<td>4.78</td>
<td>3.36</td>
</tr>
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Mitochondria were prepared in the absence of bovine serum albumin as described in Methods. Approximately 0.1 mg mitochondria/ml was incubated at 32°C in the presence of the indicated concentration of palmityl-CoA, 0.9% Triton X-100, 1.1 mM EDTA, 0.12 mM 5,5'-dithiobis-(2-nitrobenzoic acid), and 115 mM Tris-Cl, pH 8.0. After establishment of the base line absorbance, 1.6 mM L-carnitine was added to the sample cuvette. Absorbance changes at 412 nm were measured in an Aminco DW-2 spectrophotometer using the split beam mode. Palmityl-CoA hydrolase activity at these palmityl-CoA levels was negligible and any minimal nonspecific color changes were cancelled out by the reference cuvette. Regression analysis of the kinetic data for determination of the apparent first order rate constants yielded correlation coefficients greater than 0.99 in all cases.

### Discussion

The effect of myocardial infarction on the hemodynamic performance of the surviving heart muscle has been described in a number of studies (Mathes and Gudbjarnason, 1971; Gudbjarnason, 1971-1972; Mathes et al., 1976). Biochemical and physiological parameters have been examined and correlated with changes in contractility in hopes of providing insight into effective clinical management and protection of the jeopardized, remote myocardium.

Changes in contractility, high energy phosphate content and norepinephrine stores have been reported for both the infarced muscle as well as the noninfarcted, or remote zones (Mathes and Gudbjarnason, 1971; Gudbjarnason, 1971-1972; Mathes et al., 1976). Corday et al. (1975), for example, have reported recently the presence of arterial lesions and cardiac edema in both the remote and nonremote (ischemic) areas of the myocardium after occlusion and/or reperfusion. The studies reported in the present paper demonstrate very early decreases in mitochondrial state 3 respiration in both the anterior and posterior myocardium after occlusion of the left circumflex artery. The decrease in mitochondrial function was correlated with depressed glycogen content in the ischemic zone as well as in the "control" areas. Interestingly, glycogen depletion was associated with loss in phosphofructokinase activity. This loss may be dependent on enzyme "solubilization" and release from muscle cells under ischemic low-flow conditions and perhaps during reperfusion as well, when myocardial glycogen levels remain severely depressed. Physiological measurement of cardiac function demonstrated only a transient return toward normal myocardial tension on release of occlusion. In the present studies, 4 hours of reperfusion following coronary ligation did not improve tension development in either the posterior regions, which remained depressed, or in the anterior left ventricle, which demonstrated a progressive decrease in function during this period. Heyndricks and co-workers (1975) also found prolonged depression of function after 5 and 15 minutes of occlusion in the conscious dog.

An uncoupling of energy production and utilization has been proposed in acute ischemia (Gudbjarnason, 1971-1972) and may be related to a hypothesized deficiency in the transfer of energy-rich phosphate from the mitochondria to the site of utiliza-
tion. A possible mechanism by which energy transfer from the mitochondria to the contractile apparatus may be affected during myocardial ischemia is via significant and specific inhibition of the mitochondrial adenine nucleotide translocase by long chain acyl-CoA. Depression of translocase activity in ischemia has been reported previously, as well as increased tissue levels of acyl-CoA (Shug et al., 1975). The removal of the fatty acyl-CoA inhibition of mitochondrial high energy phosphate transfer can be accomplished by the mitochondrial carnitine-dependent palmitoyl-CoA transferase, which provides fatty acid to the enzymes of β-oxidation for rapid energy production. The activity of the isolated carnitine palmitoyltransferase is competitively inhibited by its substrate, palmitoyl-CoA, at the carnitine site (Bremer and Norum, 1967). The results in the present paper substantiate the role of palmitoyl-CoA as an inhibitor of carnitine palmitoyltransferase activity in intact mitochondria at low, physiological substrate levels. The apparent zero and first order rate constants of this enzyme assayed in mitochondria isolated from ischemic tissue are diminished after 5 and 10 minutes of ischemia. These results are of particular interest with regard to the estimated doubling (Whitmer et al., 1972) of acyl-CoA in ischemic heart levels to that would have profound effects on the kinetic recovery of the carnitine palmitoyltransferase upon tissue reperfusion. However, a correlation between a possible inhibition of adenine nucleotide translocation and accumulation of tissue acyl-CoA in no way eliminates the potentially important role of the creatine phosphokinase isozymes in energy transfer to the myofibrils which also may be impaired in myocardial ischemia (Gudbjarnason, 1971-1972).

The data in the present study demonstrate very early defects in mitochondrial oxidative capacity after coronary occlusion. Of great interest in the possible management of the surviving, noninfarcted muscle is the response of the mitochondria in the remote or control regions to the ischemic event. The failure of contractile response in the ischemic area to return to normal following 1 hour of reperfusion suggests that the cellular mechanisms operative in vivo to restore myocardial pump function may still be abnormal after brief periods of reperfusion of the ischemic myocardium.

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