Localization of Viable, Ischemic Myocardium by Positron-emission Tomography with $^{11}$C-Palmitate

Rene A. Lerch, M.D., Hans D. Ambos, Steven R. Bergmann, Ph.D., Michael J. Welch, Ph.D., Michel M. Ter-Pogossian, Ph.D., and Burton E. Sobel, M.D.

SUMMARY Regional metabolism of exogenous fatty acid extracted by myocardium depends primarily on oxidation. Positron-emission tomography (PET) delineates necrotic myocardium by virtue of its failure to extract labeled fatty acid. In ischemic myocardium, fatty acid is extracted, but metabolized slowly. This study was performed to determine whether viable, but ischemic, tissue could be detected and localized in vivo based on external detection of impaired fatty acid metabolism. Accordingly, regional clearance of $^{11}$C-palmitate was assessed by sequential PET in 15 anesthetized dogs. Clearance was consistently monoexponential from 5-15 minutes after administration of the tracer. In the absence of coronary stenosis (n = 7), clearance was homogeneous throughout the heart, with an average rate constant ($k$) of $-0.060 \pm 0.005$ min$^{-1}$ (± SEM) and a coefficient of variation (CV) of $11.1 \pm 2.1\%$ in each heart. Homogeneity persisted when the heart rate was increased from $84.4 \pm 6.0$ to $202.7 \pm 11.5$ beats/mln with atropine (CV 13.2 ± 3.5%). With left circumflex coronary stenosis (± 70% reduction in vessel diameter), homogeneity of $^{11}$C-clearance under control conditions and with tachycardia did not differ from clearance in hearts without coronary stenosis. However, with stenosis >70% sufficient to induce ischemia without gross infarction, regional clearance of $^{11}$C became markedly heterogeneous under control conditions (CV 28.1 ± 5.5%, p < 0.01 compared with normal hearts) and with tachycardia (CV 34.8 ± 5.4%, p < 0.01). The heterogeneity resulted from reduced clearance of $^{11}$C in regions supplied by the stenotic vessel ($k = -0.044 \pm 0.011$ min$^{-1}$) compared with clearance in well perfused regions ($k = -0.064 \pm 0.011$ min$^{-1}$, p < 0.025), a difference accentuated by tachycardia.

Thus, sequential PET after i.v. injection of $^{11}$C-palmitate delineates zones of viable, ischemic myocardium that characteristically exhibit impaired oxidation of extracted fatty acid.

DEFINITIVE diagnosis of coronary insufficiency, localization of jeopardized zones of myocardium and assessment of therapeutic interventions designed to facilitate myocardial preservation require noninvasive, quantitative methods amenable to sequential applications. However, clinically available procedures are limited. Electrocardiographic changes may be influenced by numerous factors, such as electrolyte shifts, pharmacologic agents and hyperventilation.1, 2 Thallium-201 scintigraphy and technetium-99m radioventriculography with patients at rest and under stress provide sensitive detection of regional abnormalities.3, 4 However, quantitation in conventional single-photon tracer scintigraphy is hampered by the nonphysiologic nature of the tracers, superimposition of three-dimensional myocardium on a two-dimensional display and variable attenuation of tracer radiation as a function of distance from the detector and inhomogeneity of the absorbing tissues.5

We have shown that irreversible injury in dogs with coronary occlusion6 and in patients with spontaneous myocardial infarction7 can be quantified by tomographic detection of diminished accumulation of intravenously injected $^{11}$C-palmitate. We have also shown that isolated perfused rabbit hearts exhibit monoeponential myocardial clearance of $^{11}$C-palmitate extracted from the coronary perfusate.8 Under conditions of constant flow, the slopes of the monoeponential clearance curves from these preparations were directly related to determinants of myocardial oxygen consumption and liberation of radiolabeled $^{14}$CO$_2$. Thus, externally detectable clearance and aerobic fatty acid metabolism were closely related.3 These results suggested that analysis of regional time-activity curves obtained by positron-emission tomography after i.v. administration of $^{11}$C-palmitate might permit detection, localization and quantification of ischemic regions in vivo based on analysis of clearance of activity of radiolabeled fatty acid.

In contrast to inert, diffusible tracers,8 the metabolic fate of an intermediary metabolite is influenced by numerous factors in addition to myocardial perfusion itself, e.g., regional and global cardiac function, local pH, availability of other metabolites, and the hormonal and neurohumoral environment.9, 10 Accordingly, clearance of activity of extracted $^{11}$C-palmitate is not likely to be quantitatively related to flow itself. On the other hand, when flow is so low that metabolic demands cannot be met, clearance of $^{11}$C-palmitate activity in vivo is likely to be depressed, judging from the characteristics of fatty acid metabolism in normal, hypoxic and ischemic heart muscle.11-14 Evaluation of this hypothesis was not possible without instruments that can sequentially acquire data fast enough to permit characterization of clearance from selected regions.

In the present study, we used a new fast-scanning...
positron-emission tomographic device (PETT V). The study was designed to determine (1) whether a phase of monoeponential clearance of activity could be identified in canine hearts in vivo studied serially by positron-emission tomography after i.v. injection of $^{11}C$-palmitate; (2) whether regional clearance of activity is homogeneous throughout the normal canine heart; (3) whether homogeneity persists despite changes in loading conditions; and (4) whether clearance of activity is systematically altered in regions supplied by a coronary artery with stenosis sufficient to induce ischemia without gross infarction.

**Methods**

**Animal Preparations**

Tomographic studies according to the protocol described below were performed in dogs that weighed 18–30 kg. Seven dogs without and eight with coronary stenosis were studied. In dogs subjected to incomplete coronary occlusion, stenosis of the left circumflex coronary artery (LCX) stenosis was produced. The procedure entailed anesthesia with sodium thiopental, 15 mg/kg, prompt intubation, intraoperative support with positive-pressure respiration (Harvard respirator Model 607), and maintenance of anesthesia with 0.5–1.5% halothane in oxygen. The heart was exposed by a left thoracotomy in the fourth intercostal space and suspended in a pericardial cradle. The LCX was dissected free proximal to the first major ventricular branch and constricted with a 2-0 silk ligature tied snugly around both the artery and an inlying 19-gauge needle. The needle was then removed, leaving the artery partially constricted. Two pacing electrodes were attached to the left atrium and exteriorized to the left lateral chest wall. The pericardium was left open and the chest was closed.

**Preparation for Tomographic Procedures**

Tomographic studies in normal dogs and 24–48 hours after the initial operation in those with LCX stenosis were performed after each dog was anesthetized with i.v. sodium thiopental, 15 mg/kg, followed by α-chloralose, 90 mg/kg, and i.v. urethane, 440 mg/kg. The dogs were intubated and ventilated with room air supplemented with oxygen to maintain arterial PO$_2$ > 100 mm Hg. Polyethylene catheters were advanced to the abdominal aorta and the inferior vena cava through a femoral cutdown. A polyethylene catheter was inserted intravenously in the contralateral leg for administration of the $^{11}C$-palmitate. Aortic pressure was measured continuously with a Statham Pb23Db transducer and Grass polygraph (model 7B). To facilitate positioning of the dog in the tomograph, the cranio-caudal location of the heart relative to externally attached radiopaque markers was determined first with a lateral chest x-ray and marked on the chest wall. The dog was placed supine in a Plexiglas half-cylinder and positioned in the tomograph.

**Experimental Protocol**

$^{11}C$-Clearance from Canine Hearts Without Coronary Stenosis

All studies were performed with a recently developed fast-scan positron-emission transaxial tomographic device (PETT V) that permits serial acquisition of data in 60-second intervals from seven transaxial sections simultaneously. After the dog was positioned, a transmission scan was performed to provide data for attenuation correction. Subsequently, $^{11}C$-palmitate (8–15 mCi) bound to 6–10 ml of 4% human albumin in saline was injected intravenously. One-minute emission tomograms were initiated 5, 8, 11 and 14 minutes after injection of the tracer. In selected experiments, additional tomograms (scanning intervals 1–10 minutes) were obtained for as long as 65 minutes after injection. To assess the disappearance of tracer from the blood pool, 1-ml venous blood samples were withdrawn from the caval catheter at 2–5-minute intervals and radioactivity was quantified in a well counter. To permit comparison of blood activity to injected activity, a small diluted sample of the injectate was counted in the same well counter.

To characterize the influence of increased work load on the regional rate of clearance of tracer, a second injection of $^{11}C$-palmitate was given 90–120 minutes after the first injection. The interval between the two injections was sufficient to permit decay of the initial radioactivity accumulated by the heart and a return to background (half-life of $^{11}C$ = 20.4 minutes). Emission tomograms were repeated at the same intervals after injection. However, to augment heart rate, atropine sulfate (0.2 mg/kg) was administered 3.5 minutes after the injection of $^{11}C$-palmitate (1.5 minutes before the first scan). In each case the maximal heart rate was reached within 1 minute after administration of the drug.

Venous blood samples were obtained for assay of plasma free fatty acid immediately before and after each study. Results are averages of duplicate determinations.

$^{11}C$ Clearance from Canine Hearts with Coronary Stenosis

Tomographic studies were performed 24–48 hours after surgery under the same conditions as those used in dogs without coronary stenosis. Immediately after induction of anesthesia, five i.v. injections of lidocaine (1 mg/kg) were administered at 4-minute intervals, followed by an infusion of lidocaine at 40 μg/kg/min to prevent lethal dysrhythmia. Emission scans were performed as in dogs without coronary stenosis. In dogs with left circumflex stenosis, the study in which tachycardia was induced was performed differently. To facilitate implementation of the highest heart rate with stable 1:1 atrioventricular conduction and without severe accompanying arrhythmia, tachycardia was induced by left atrial pacing beginning 1 minute before tomography (4 minutes after injection of $^{11}C$-palmitate), rather than by administration of atropine.
Coronary Arteriography and Cardiac Pathology

After completion of the tomographic studies selective coronary arteriography was performed (Judkins technique) with left anterior oblique and right anterior oblique views by exposure of Cronex 4 (35.6 × 35.6 cm) x-ray films at 1-second intervals during injection of Renograin-76. The percentage reduction of the diameter of the LCX was measured from both projections and a mean value was calculated. After arteriography, each dog was sacrificed by i.v. administration of saturated potassium chloride and the heart was removed. Four to six transmural samples were obtained from the center of the posterior papillary muscle (one sample), from the myocardium perfused by the LCX 1–2 cm distant from the posterior papillary muscle (one to three samples), and from the region perfused by the left anterior descending coronary artery (LAD) (one to two samples). Samples were fixed in 10% formalin, stained with hematoxylin and eosin, and examined by light microscopy.

Synthesis of \(^{11}C\)-Palmitate

The \(^{11}C\)O\(_2\) was produced in the Washington University Medical Center cyclotron (Allis Chalmers) by the \(^{10}\)B(d,n),\(^{11}\)C reaction. Mixing of \(^{11}C\)O\(_2\) with the corresponding Grignard reagent in ether resulted in formation of the magnesium bromide salt of palmitic acid. Palmitic acid, obtained by addition of 1.0 N hydrochloric acid was solubilized in ethanol and the ether was boiled off. Finally, the palmitate-ethanol was added to 1 ml of 25% human serum albumin in 7 ml of 0.9% NaCl and filtered serially through 0.45-\(\mu\) and 0.22-\(\mu\) Millipore filters. The radiochemical purity of the injectate was analyzed by high-pressure liquid chromatography with a Waters M-Bondpak fatty acid column. One hundred percent of the activity was associated with the palmitate peak.

Positron-emission Tomography

The positron-emission transaxial tomograph (PETT V) system was designed by the Washington University Radiation Sciences Division to provide rapid, high-resolution cardiac imaging in animals. It consists of 48 NaI (Tl) scintillation detectors arranged in a circular array. Seven cross-sectional tomograms 10 mm thick in the center of the field, with 15 mm between sections, are obtained simultaneously. At the high-resolution mode used in the present study, the resolution of the instrument is approximately 10 mm (full width at half maximum)\(^{18}\).

To correct attenuation, a transmission scan was performed before the first injection of palmitate. For this purpose, a gallium-68 ring source was placed around the dog. All data were collected on-line with the use of an Interdata computer (Model 70) and stored on tape. After each experiment, images were reconstructed with an Interdata computer (model 7-16). Computer reconstruction included subtraction of random coincidence counts. For the first scan of each study, all seven sections were reconstructed. To determine regional time-activity curves, one to three sections best delineating myocardium of the left ventricle were selected and reconstructed for all consecutive scans. Two to five regions of interest representing volume elements of 1–3 cm\(^3\) were selected in each serially reconstructed section and mean activity per pixel in arbitrary units in each region was computed for all consecutive scans. All counts were corrected for physical decay of the tracer. In the experiments in which regions perfused by the LAD and LCX were compared, the regions were selected based on the general distribution of coronary blood supply (fig. 1). Regions in the dorsal half of a cross section at the midventricular level were selected as representative of myocardium perfused primarily by the LCX, and regions in the anterior half of the cross section at the level of the mitral valve (exhibiting a horseshoe pattern in normal hearts) were selected as representative of regions supplied primarily by the LAD. The prospective categorization of regions of supply by specific vessels in individual hearts used to select regions of interest does not take into account variations in coronary anatomy from dog to dog or overlap in coronary perfusion. Nevertheless, the suitability of the approach used to identify regions supplied primarily by the LAD or the LCX within the limitations of resolution of presently available instrumentation has been demonstrated in studies depicting the tomographic distribution of flow-dependent tracers and its response to selective constriction of the LAD or the LCX.\(^{18}\)

To compare myocardial activity with both blood and injected activity, a cylindrical phantom with three to five selected concentrations of \(^{11}C\)-palmitate was scanned at the completion of each experiment. Aliquots of samples from the phantom were counted in the same well counter as the blood samples and the injectate.

Determination of Regional Clearance Rates of \(^{11}C\)-Palmitate

Figure 2 shows a typical regional time-activity curve obtained over a 65-minute interval. Two components can be identified readily on the semilogarithmic plot. The first portion of the curve appears to be monoexponential from 5–15 minutes after injection of \(^{11}C\)-palmitate, resembling the corresponding portions of curves in isolated hearts. The second component, prominent 25 minutes after injection of tracer, exhibits a half-time exceeding 100 minutes. To assess the dominant early myocardial intermediary metabolism of \(^{11}C\)-palmitate, clearance was determined from the monoexponential portion of the curve. The rate constant (k) of clearance and the correlation coefficient (r) for the first-order function were determined from the regression line obtained by least-squares approximation of the relation between time after injection and the natural logarithm of the regional count rate.
Regions Supplied by Each Coronary Artery

Regions supplied by each coronary artery:

- LAD
- Circumflex

Figure 1. Diagram of the canine coronary artery system as viewed from the left lateral view (left panel) showing the distribution of myocardial regions supplied by the left anterior descending coronary artery (LAD) and the left circumflex coronary artery (Cx) (right panel) in four selected reconstructed transaxial cross sections of the left ventricle (middle panel). The tracer is distributed in a horseshoe pattern at the level of the mitral valve (slice 3) and in a more circular pattern in the sections at midventricular level (slice 4) and near the diaphragm (slice 5). Regions of interest in the anterior half of the cross section at the level of the mitral valve were selected for determination of $^{11}$C-palmitate clearance in myocardium supplied primarily by the LAD and in the posterior half of the cross section at the midventricular level for determination of the corresponding value in myocardium supplied by the Cx.

Statistical Methods

The significance of differences between values measured under control conditions and with tachycardia and between mean clearance rates of $^{11}$C and mean count rates per volume element in regions supplied by the LAD compared with those supplied by the LCX were compared using the t test for paired samples. One-way analysis of variance and a modified t test according to the Bonferroni procedure were used to compare coefficients of variation between groups.

Results

Clearance of $^{11}$C in Canine Hearts Without Coronary Stenosis

Figure 3 shows the time course of radioactivity per volume element in blood and myocardium. Blood activity was monitored by assaying serial venous blood samples in a gamma well counter. Myocardial activity was monitored from a region of interest in the free lateral wall of a reconstructed cross section at the level of the mitral valve. Blood activity cleared rapidly and averaged only 18% of myocardial activity 5 minutes after administration of $^{11}$C-palmitate. Later than 5 minutes after injection, the changes in activity remaining in the blood pool are small compared with changes of activity in myocardium. Therefore, significant distortion of the myocardial time-activity curves by activity in the myocardial and ventricular blood pools potentially included in the region of interest are likely to be modest after this interval.

The Monoexponential Nature of Early Clearance

Myocardial clearance of $^{11}$C was consistently monoexponential from 5–15 minutes after administration of $^{11}$C-palmitate, with an average correlation coefficient of 0.99 ± 0.002 (± SEM) (table 1). The mean individual rate constant of the monoexponential clearance
Activity corresponds times exceeded regions of more variation obtained (cpm)/cm³. The rate constant (k) of this component corresponds to a half-time of 10.6 minutes. A second component, evident later than 25 minutes after administration of tracer, is extremely slow, with a half-time exceeding 100 minutes.

Homogeneity of Clearance Throughout the Normal Heart

Figure 4 illustrates the variation from region to region of $^{11}$C-palmitate clearance detected by positron-emission tomography in a representative dog. Regional clearance rates exhibited considerable homogeneity throughout the heart. The coefficient of variation of regional $^{11}$C-palmitate clearance rate constants under control conditions averaged only 11.1 ± 2.1% among values from all experiments (table 1).

After administration of atropine, the heart rate increased markedly, by an average of 140% ($p < 0.001$) (table 1). However, the monoexponential character of early clearance was well maintained, as evidenced by the unchanged average correlation coefficient of 0.98 for a first-order function compared with that under control conditions (table 1). Further, homogeneity of $^{11}$C-palmitate clearance rate within each heart persisted, as indicated by the low coefficient of variation of the rate constants from different regions during tachycardia. The average clearance rate constant increased by 17% compared with control conditions (NS).

![Figure 2](image1.png)

**Figure 2.** Semilogarithmic plot of regional $^{11}$C activity vs time from the free lateral wall at the level of the mitral valve. Activity is expressed in millions of counts per minute (cpm)/cm³ obtained after calibration of PETT V with the use of a phantom. From 5-15 minutes after tracer administration, clearance of $^{11}$C activity was monoexponential. The rate constant (k) of this component corresponds to a half-time of 10.6 minutes. A second component, evident later than 25 minutes after administration of tracer, is extremely slow, with a half-time exceeding 100 minutes.

![Figure 3](image2.png)

**Figure 3.** Time course of $^{11}$C-activity per volume element in blood and myocardium. Activity in blood was determined by gamma spectrometry of venous blood samples and activity in myocardium by positron-emission tomography. Clearance of blood activity occurs primarily during the first 5 minutes after injection of $^{11}$C-palmitate. Activity remaining in the blood pool is low compared with activity in myocardium during tomographic data collection.

| Hemodynamics, Myocardial $^{11}$C-Palmitate Clearance and Plasma Free Fatty Acid Concentrations in Dogs without Coronary Stenosis During Control Conditions and with Tachycardia |
|--------------------------------------------------|-------------------|-----------------|
| Control                                          | Atropine          | $p$             |
| Heart rate (beats/min)                           | 84.4 ± 6.0             | 202.7 ± 11.5         | < 0.001 |
| Aortic pressure (mm Hg)                         |                   |                  |         |
| Systolic                                         | 183.7 ± 5.8           | 174.3 ± 5.5           | < 0.05 |
| Diastolic                                        | 128.7 ± 5.0           | 139.3 ± 3.5           | < 0.025 |
| Mean                                            | 147.1 ± 4.7           | 151.0 ± 3.9           | NS      |
| Clearance                                       |                   |                  |         |
| $r$                                             | 0.99 ± 0.002          | 0.98 ± 0.003          | NS      |
| $k$ (min⁻¹)                                     | -0.060 ± 0.005        | -0.070 ± 0.006        | NS      |
| CV (%)                                          | 11.1 ± 2.1            | 13.2 ± 3.5            | NS      |
| Plasma FFA (mM)                                 | 0.37 ± 0.08           | 0.48 ± 0.07           | NS      |

Values are mean ± SEM (n = 7).

Abbreviations: $r$ = correlation coefficient of the best fit monoexponential plot (least-squares method from a mean of three to eight regions); $k$ = rate constant of tracer clearance (mean from three to eight regions); CV = coefficient of variation of tracer clearance rate constants within each heart; FFA = free fatty acid concentration.
Hemodynamic and Tomographic Findings

The hemodynamic and tomographic results are summarized in table 2. Tomographic data from four dogs without coronary stenosis, analyzed in the same fashion, are included for comparison. The heart rate during atrial pacing in both groups with stenosis was equal to that after administration of atropine in the group without stenosis. However, the average heart rate under control conditions was markedly higher in dogs with stenosis than in controls.

In the tomographic cross sections from dogs with stenosis in both groups, no defects in uptake of \(^{11}\)C-palmitate were visible, in marked contrast to the diminished uptake evident in zones of frank infarction.\(^7\) The count rate per volume element in the first scan after injection of \(^{11}\)C-palmitate in dogs with stenosis \(\leq 70\)% was \(6.01 \pm 1.03 \times 10^6\) counts per minute (cpm)/cm\(^3\) in the LAD-supplied region and \(6.54 \pm 0.70 \times 10^6\) cpm/cm\(^3\) in the LCX-supplied region. In dogs with stenosis \(> 70\)% the corresponding count rates were \(7.04 \pm 1.12 \times 10^6\) cpm/cm\(^3\) and \(6.45 \pm 1.21 \times 10^6\) cpm/cm\(^3\). Differences between the two regions were not significant in either group. These data indicate the absence of diminished regional tracer accumulation.

Diminished Clearance and Loss of Homogeneity of Clearance Caused by Regional Ischemia

Figure 5 shows time-activity curves from eight regions of interest from one dog with 80% stenosis of the LCX. Compared with the example from a dog without stenosis, considerably less homogeneity of \(^{11}\)C-palmitate clearance is evident. In each of the five dogs with stenosis \(> 70\)% the coefficient of variation of the clearance rate constant was high: average \(28.1 \pm 5.5\)% under control conditions and \(34.8 \pm 5.4\)% during atrial pacing. Both of these values are significantly greater \((p < 0.01)\) than corresponding values under control conditions and during atropine-induced tachycardia in dogs without stenosis (table 1). In contrast, clearance of \(^{13}\)C was homogeneous in dogs with stenosis \(\leq 70\)% similar to dogs without coronary stenosis (fig. 6). Among dogs with stenosis \(\leq 70\)% the coefficients of variation averaged \(14.5 \pm 1.3\)% under control conditions and \(12.4 \pm 3.2\)% with atrial pacing. These values do not differ significantly from corresponding values in dogs without stenosis. Thus, severe coronary stenosis leads to heterogeneity of regional clearance of \(^{11}\)C-palmitate with diminished clearance evident in zones supplied by the partially occluded vessel.

Myocardial \(^{11}\)C-clearance rates, classified according to the source of blood supply, are shown in table 2. The mean clearance rate of \(^{11}\)C in regions perfused by the LAD was calculated for each heart from three regions of interest in the anterior half of the cross section at the level of the mitral valve. The corresponding value for the region perfused by the LCX was calculated from two to three regions of interest in the posterior half of the cross section at the midventricular level.

**Table 2**

<table>
<thead>
<tr>
<th>Region</th>
<th>Clearance Rate (cpm/cm(^3)/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>(6.01 \pm 1.03 \times 10^6)</td>
</tr>
<tr>
<td>LCX</td>
<td>(6.54 \pm 0.70 \times 10^6)</td>
</tr>
<tr>
<td>Atria</td>
<td>(7.04 \pm 1.12 \times 10^6)</td>
</tr>
<tr>
<td>Ventricles</td>
<td>(6.45 \pm 1.21 \times 10^6)</td>
</tr>
</tbody>
</table>

**Figure 4.** Myocardial time-activity curves from six regions of interest in a canine heart without coronary stenosis under control conditions and after atropine. (top) Time-activity curves from a tomographic cross section at the level of the mitral valve. (bottom) Time-activity curves from a cross section at the midventricular level. The clearance of \(^{11}\)C-activity exhibits little variation from region to region. \(k = \) rate constant of monoexponential \(^{11}\)C-clearance.

Clearance of \(^{11}\)C-Palmitate in Hearts with Coronary Stenosis

Morphologic Consequences of Stenosis

Based on the extent of reduction in coronary diameter determined from coronary angiograms, the dogs with LCX stenosis were assigned to one of two groups. Group 1 consisted of three dogs with a reduction in coronary diameter \(\leq 70\)% (range 53–65%) and group 2 consisted of five dogs with narrowing \(> 70\)% (range 73–93%). None of the hearts had regions of myocardial infarction detectable by gross inspection at the time of sacrifice. In hearts from dogs in group 1, no areas of necrosis were detectable histologically. In hearts from dogs in group 2, patchy necrosis was evident, but only in the samples from the region of the posterior papillary muscle. In one dog the injury was limited to the most subendocardial fiber layers, in two dogs it was restricted to the inner 50% of the wall, and in two dogs it extended only irregularly to the epicardium. Thus, most of the region supplied by the stenosed LCX consisted of viable myocardium, based on histologic criteria.
TABLE 2. Hemodynamics and Regional Myocardial $^{11}$C-Palmitate Clearance in Dogs with and without Coronary Stenosis

<table>
<thead>
<tr>
<th></th>
<th>No stenosis (n = 4)</th>
<th>LCX stenosis ≤ 70% (n = 3)</th>
<th>LCX stenosis &gt; 70% (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (beats/min)</td>
<td>Mean AoP (mm Hg)</td>
<td>$k$ (min$^{-1}$)</td>
</tr>
<tr>
<td>Control</td>
<td>88.3 ± 6.9</td>
<td>148.5 ± 4.3</td>
<td>-0.059 ± 0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.061 ± 0.007</td>
</tr>
<tr>
<td>Atropine</td>
<td>201.0 ± 14.9*</td>
<td>150.3 ± 5.6</td>
<td>-0.073 ± 0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.065 ± 0.010</td>
</tr>
<tr>
<td>LCX stenosis ≤ 70%</td>
<td>Control</td>
<td>123.0 ± 15.4</td>
<td>-0.076 ± 0.010</td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td>-0.070 ± 0.013</td>
</tr>
<tr>
<td>Atrial pacing</td>
<td>203.0 ± 6.0*</td>
<td>137.7 ± 3.4</td>
<td>-0.084 ± 0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.079 ± 0.017</td>
</tr>
<tr>
<td>LCX stenosis &gt; 70%</td>
<td>Control</td>
<td>132.0 ± 8.9</td>
<td>-0.064 ± 0.011</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>-0.044 ± 0.011</td>
</tr>
<tr>
<td>Atrial pacing</td>
<td>202.4 ± 5.7*</td>
<td>127.6 ± 5.6</td>
<td>-0.073 ± 0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.044 ± 0.009</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*p < 0.025 vs within group values under control conditions.

†Refers to within group differences between the two regions under the same physiologic conditions.

Abbreviations: HR = heart rate; AoP = aortic pressure; $k$ = rate constant of tracer clearance (from a mean of two to three regions of interest); LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery.

Among dogs with stenosis ≤ 70%, the average rates of myocardial clearance of $^{11}$C in regions supplied by the LAD and by the LCX did not differ significantly under control conditions or during tachycardia. However, in the group with stenosis > 70%, the average clearance rate of $^{11}$C in the region supplied by the left anterior descending coronary artery was significantly higher than in the region supplied by the left circumflex coronary artery.

**Figure 5.** Myocardial time-activity curves from eight regions of interest in a canine heart with a 80% stenosis of the left circumflex coronary artery under control conditions and during atrial pacing. The regions were selected from tomographic cross sections at the level of the mitral valve (top) and at the midventricular level (bottom). Regional $^{11}$C-palmitate clearance exhibits less homogeneity than in hearts without coronary stenosis (fig. 4). The clearance of $^{11}$C markedly decreased in the inferoposterior regions (bottom right), which are supplied primarily by the stenotic left circumflex coronary artery, compared with clearance in the anterosetal regions (upper middle), which are supplied primarily by the left anterior descending coronary artery.

**Figure 6.** Myocardial time-activity curves from seven regions of interest in a canine heart with 60% stenosis of the left circumflex coronary artery under control conditions and during atrial pacing. As is the case in hearts without coronary stenosis (fig. 4), clearance of $^{11}$C is homogeneous throughout the heart and similar among the selected regions of interest.
LCX was reduced by 31% ($p < 0.025$) compared with the average rate in regions supplied by the LAD under control conditions. The difference became even more pronounced, with the reduction averaging 40%, during atrial pacing ($p < 0.005$).

**Discussion**

Results from this study show that myocardial clearance of radioactivity determined by positron-emission tomography after i.v. injection of $^{11}$C-palmitate is homogeneous throughout the normal canine heart and that it remains homogeneous despite altered loading conditions. In contrast, regions supplied by a coronary artery with stenosis > 70% exhibit decreased clearance of tracer compared with the rate in normally perfused regions in the same dog.

Several observations support the hypothesis that analysis of myocardial time-activity curves after injection of $^{11}$C-palmitate provides an index of the rate of oxidative fatty acid metabolism. In isolated perfused rabbit hearts, clearance of myocardial radioactivity after bolus injection of $^{11}$C-palmitate into the perfusate is monoexponential. With perfusion held constant, the rate of $^{11}$C-palmitate clearance and the rate of $^{14}$CO$_2$ production from $^{14}$C-palmitate administered concomitantly change in direct proportion to the change in myocardial oxygen requirements. These findings suggest that the monoexponential phase of $^{11}$C-clearance is closely related to the rate of aerobic metabolism. A corresponding close relationship between the same two variables has been found in vivo in rabbits.

The time-activity curves detected by positron-emission tomography in the present study consistently exhibited a phase of monoexponential tracer clearance analogous to that observed in isolated and in situ rabbit hearts from 5–15 minutes after injection of $^{11}$C-palmitate. The half-time of monoexponential clearance of $^{11}$C averaged 11.6 minutes in normal canine myocardium, a finding in close agreement with values reported by others based on analysis of serial myocardial biopsies. Clearance of activity in the blood occurred primarily during the first 5 minutes after injection of isotope. The low residual activity subsequently remained constant during the interval in which monoexponential myocardial clearance was recorded. Tracer in the extracellular space was washed out rapidly. Accordingly, time-dependent changes in regional myocardial activity detected more than 5 minutes after injection of $^{11}$C-palmitate primarily reflect clearance of activity from $^{11}$C-palmitate initially extracted into myocardium.

Because $^{11}$C-palmitate is metabolized in the same fashion as its physiologic, circulating counterpart, the physiologic fate of this tracer can be interpreted in terms of the extensive information already available from studies of myocardial fatty acid metabolism. The arteriovenous extraction fraction of free fatty acid extracted by the heart is 15–60%. After entering myocardial cells, most of the fatty acid is trapped by thioesterification with coenzyme A. Back diffusion from the small intracellular free fatty acid pool is minimal. One fraction of the thioesterified fatty acid undergoes β oxidation in mitochondria after conversion to acyl carnitine and transport. Another small fraction is incorporated into phospholipids. A relatively large fraction is first incorporated into neutral lipids, subsequently liberated by lipolysis and oxidized.

Based on results of studies with $^{14}$C-labeled fatty acids, the monoexponential phase of the externally detected time-activity curve mainly reflects turnover of the neutral lipid pool. The slow, late component of clearance of $^{14}$C (evident more than 25 minutes after injection of $^{11}$C-palmitate) appears to reflect slow turnover of pools such as the phospholipid pool and a fraction of triglycerides with slow turnover observed by Stein and Stein. In addition, clearance of initially incorporated activity may be somewhat obscured by late myocardial uptake of labeled palmitate released from labeled triglycerides in the liver.

Homogeneity of clearance of tracer throughout the normal canine heart provides a useful baseline for potentially sensitive detection of metabolic changes in ischemic regions. Previous studies have shown that significant differences in oxygen consumption and myocardial blood flow under physiologic conditions exist only between subendocardial and subepicardial layers, but not between different transmural regions of the same ventricle. Although these observations suggest that $^{11}$C-palmitate clearance is likely to be homogeneous throughout the ventricle, potential methodologic limitations of positron-emission tomography could have precluded recognition of the homogeneity. Such limitations include nontransmural sampling, inclusion of activity in nonmyocardial tissue with different tracer kinetics within the region of interest, regional inhomogeneity of background activity and regional differences in counting statistics. In this study, despite these and other potential limitations, clearance of tracer was homogeneous in normal hearts; the average coefficient of variation between different regions of the same heart was only 11.1%. Further, homogeneity persisted even when heart rate was increased markedly.

The mean clearance rate of tracer in normal canine hearts and in regions with unrestricted flow in dogs with coronary stenosis increased only moderately, and was not statistically significant, with tachycardia. The modest extent of the increase is in contrast to findings in isolated rabbit hearts in which interventions imposed to increase oxygen demand increase clearance rate of $^{11}$C considerably. Two factors probably account for the difference. First, compensatory responses in vivo (such as diminution of stroke volume and wall tension due to decreased ventricular dimensions) that blunt the increase in oxygen demand were precluded in the isolated, perfused hearts. Second, elevated plasma fatty acid observed in the present experiments with dogs undoubtedly reduced the increase in myocardial triglyceride use that otherwise would have
occurred with the increased work load. Studies by Crass and co-workers with isolated rat hearts showed that the increase in triglyceride use as a result of stimulation with epinephrine in the presence of low concentrations of fatty acid in the perfusate is abolished when higher levels of fatty acids, comparable to the plasma fatty acid levels measured during tachycardia in the present study, are presented to the heart.29

The effect of ischemia on regional clearance of \(^{11}\)C-palmitate was assessed in dogs with fixed coronary stenosis. The existence of modest, patchy subendocardial necrosis in the region of the posterior papillary muscle in dogs with coronary stenosis > 70% reflects prolonged severe ischemia. Subendocardial hypoperfusion even under resting conditions has been documented with labeled microspheres in dogs with acute LCX stenosis of more than 70%.31 However, most of the myocardium supplied by the partially occluded vessel remained viable, judging from both the histologic findings and the observed capability of the tissue to extract \(^{11}\)C-palmitate. Such regions, however, exhibit markedly depressed coronary flow reserve32 and are therefore prone to metabolic consequences of ischemia during conditions of stress, such as anesthesia and superimposed tachycardia.

In dogs with stenosis > 70%, clearance of \(^{11}\)C-palmitate under control conditions in the region supplied by the compromised LCX was decreased by 31% compared with clearance in the region supplied by the non-occluded LAD. This difference increased to 40% with left atrial pacing. In contrast, no differences in \(^{11}\)C-palmitate clearance were observed between corresponding regions in dogs with less severe or absent coronary stenosis under control conditions or during induced tachycardia.

The inclusion of small necrotic areas in tomographic regions of interest could not account for the observed differences in clearance of \(^{11}\)C. Necrotic myocardium exhibits markedly decreased fatty acid extraction compared with extraction by viable myocardium, which would be reflected by a decreased initial and maximum count rate per sampled volume element.6 Changes in clearance are not a consequence, because the nonviable tissue that fails to extract tracer does not contribute to the observed clearance rate.

Because \(^{11}\)C-palmitate extracted by the heart and incorporated into neutral lipids cannot freely diffuse out of myocardial cells, clearance of tracer is controlled primarily by two factors: oxidative metabolism with resultant formation of low-molecular-weight, diffusible, labeled oxidation products such as \(^{11}\)CO\(_2\), and washout of labeled metabolites, such as short-chain intermediates via the coronary circulation. By themselves, the present results do not distinguish between decreased clearance primarily due to diminished metabolism and decreased washout of products due to low flow. However, in lipid extracts of rabbit hearts perfused at low flow with \(^{14}\)C-palmitate, radioactivity in the aqueous phase containing low-molecular-weight products of oxidative metabolism decreased compared with the activity in the organic phase containing neutral fat.24 These observations suggest that under conditions of low flow, washout of oxidation products by the coronary circulation does not appear to be the rate-limiting step influencing clearance of tracer from myocardium. In addition, clearance of the activity incorporated into triglycerides from rat hearts prelabeled with \(^{14}\)C-palmitate is markedly decreased after induction of hypoxia, even when perfusion pressure is held constant.14 These studies in isolated hearts support the hypothesis that the reduced \(^{11}\)C clearance observed in the present study in regions supplied by the LCX with stenoses greater than 70% primarily reflects decreased oxidation induced by ischemia.

The decreased clearance of \(^{11}\)C under control conditions in zones supplied by a coronary artery narrowed by more than 70% was initially somewhat surprising. Presumably, viable myocardium in such a region would not manifest metabolic consequences of ischemia under resting conditions. However, all dogs subjected to surgery had tachycardia during recovery. Acceleration of heart rate became markedly more pronounced when induction of anesthesia was required again for the subsequent tomographic study. Thus, physiologic stress was present even under control conditions in these dogs. The recovery interval before initiation of the tomographic study was not extended beyond 48 hours so as to complete the study before development of an extensive collateral circulation, which can occur as early as 4–6 days after induction of stenosis in dogs33 and could reduce or preclude ischemia otherwise evident.

We previously showed that severely ischemic regions distal to coronary ligations exhibit markedly decreased palmitate uptake.15 However, in dogs subjected to graded reduction of coronary flow, less severe ischemia (34% of control flow) induced virtually no reduction in fatty acid extraction of 10–15 minutes.84 This appears to reflect two competing phenomena: decreased delivery of activity due to the low flow and an increased extraction fraction due to increased residence time of the tracer.16, 84 Fatty acid metabolism under such conditions is characterized by increased incorporation of fatty acids into triglycerides and by decreased lipolysis and oxidation.18, 14, 84 Accumulation of triglycerides is a hallmark of such regions.84 Accordingly, it is not surprising that the initial count rate observed in the present study in regions with decreased clearance rate was not markedly different from that in control regions supplied by normal coronary arteries. However, small differences in actual myocardial concentrations of tracer may be obscured by regional differences in admixture of non-myocardial tissue in the region of interest and by differences in wall thickness that affect count recovery.87 These factors were not taken into account in the present study because of practical considerations and because they would not be likely to influence measurements of fractional tracer disappearance rates from the same region of interest. Thus, analysis of re-
regional $^{11}$C-palmitate time-activity curves is likely to provide a more sensitive criterion for detection of early consequences of ischemia than static tomographic imaging for recognition of diminished uptake of the tracer.

Detection of ischemia based on analysis of clearance of tracer requires some extraction of $^{11}$C-palmitate. This condition is not met in zones with essentially no blood flow when the tracer is injected$^{18}$ or in zones of infarction.$^{8}$ From a diagnostic point of view in patients, zones of ischemia are generally best detected by provocative testing with stress. The tomographic approach with $^{11}$C-palmitate is amenable to analysis under conditions in which such an intervention could be initiated 3-5 minutes after i.v. administration of the tracer, thereby avoiding inhibition of extraction.

Results of the present study indicate that positron-emission tomography after i.v. injection of $^{11}$C-palmitate may provide a useful approach for improved diagnosis and localization of ischemic zones in patients at rest or undergoing stress. The procedure appears to be valuable not only for estimating the extent of completed infarcts,$^{8,9}$ but also for delineating the extent and distribution of initially metabolically compromised myocardium.

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References


Correlation of Regional Myocardial Blood Flow and Function with Myocardial Infarct Size During Acute Myocardial Ischemia in the Conscious Pig

ROBERT M. SAVAGE, M.D., BRIAN GUTH, B.S., FRANCIS C. WHITE, M.S., ARTHUR D. HAGAN, M.D., AND COLIN M. BLOOR, M.D.

SUMMARY Regional myocardial function and blood flow were determined for 48 hours after permanent occlusion of the left circumflex coronary artery in conscious swine. Systolic wall thickening and end-diastolic wall thickness (EDWTh) were correlated with regional myocardial flow (RMBF) at 15 minutes, 24 and 48 hours after occlusion. Both regional function and blood flow were compared with the extent of myocardial necrosis (determined histologically) after 48 hours in functionally distinct zones. Group 1 (control zones) was characterized by increased systolic wall thickening, EDWTh, RMBF and had no necrosis. Group 2 (marginal zones) had depressed systolic wall thickening (35 ± 3% [mean ± SEM] of preocclusion level at 48 hours) and RMBF (64 ± 6% of preocclusion values), transiently decreased EDWTh and 46 ± 5% necrosis. In Group 3 (ischemic zones), all values were greatly reduced: systolic wall thickening was 3.6 ± 1.2%, EDWTh 76 ± 8% and RMBF 25 ± 9% of preocclusion values; necrosis was 90 ± 5%. Groups 2 and 3 had increased RMBF at 24 and 48 hours from that at 15 minutes after occlusion; however, in neither case was systolic wall thickening greater than that at 15 minutes after occlusion. We conclude that there is close correlation between RMBF, systolic wall thickening and the extent of necrosis present after 48 hours of coronary artery occlusion in the conscious swine; subsequent increases in RMBF to the marginal zone after occlusion are not accompanied by improved regional function.

TENNANT AND WIGGERS first demonstrated the loss of regional contractile function after coronary artery occlusion.1 More recently, the use of chronically implanted ultrasonic crystals has permitted the precise measurement of regional dimensions of the left ventricle in unanesthetized animals.2-4 These studies examined the effect of restricted regional blood flow upon regional myocardial function after coronary occlusion in the conscious dog. Rivas et al.6 studied the relationship between this reduced blood flow and the extent of the subsequent infarct in the canine myocardium.

Theroux and co-workers5-6 identified three functionally distinct zones in the acutely ischemic myocardium: a normal zone that showed increased function after coronary artery occlusion; an ischemic zone that showed immediate dyskinesis and later non-function; and a marginal zone that showed partially impaired function. This marginal zone may represent an area of salvageable myocardium and is therefore of considerable interest.

The dog heart, however, differs from the human heart in several respects that may affect the extrapolation of experimental data to man. The coronary artery
Localization of viable, ischemic myocardium by positron-emission tomography with 11C-palmitate.
R A Lerch, H D Ambos, S R Bergmann, M J Welch, M M Ter-Pogossian and B E Sobel

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