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External Detection and Visualization of
Myocardial Ischemia with 11C-Substrates
in Vitro and in Vivo

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SUMMARY To characterize externally detectable changes in
myocardial metabolism of free fatty acids (FFA) and glucose
associated with ischemia, isovolumically beating rabbit hearts were
perfused under conditions of selected flows with cyclotron-produced,
short-lived (t 1/2 = 20.4 minutes), 11C-labeled isotopes of glucose and
FFA. Tension-time index decreased 83% and lactate production
increased from 0.5 ± 1.9 (SE) to 53 ± 2.1 µmol/min per g of dry
weight reflecting myocardial ischemia after flow was reduced from 20
to 5 ml/min. After 30 minutes of low flow the myocardial accumula-
tion of 11C-octanoate, expressed as the extraction fraction, declined
from 56 ± 15% to 30 ± 3%, reflecting metabolic suppression of FFA
extraction during low flow. Effects attributable exclusively to
prolonged residence time were excluded. Similar results were
obtained with 11C-palmitate. The myocardial avidity for 11C-pal-
mitate was demonstrable by rectilinear whole body scanning in dogs
given 5 mCi of the agent intravenously. Diminished 11C-palmitate
uptake in zones of myocardium rendered ischemic for 20 minutes
prior to reflow in intact dogs was delineated by electrocardiographi-
cally gated positron-emission transaxial computer reconstruction
tomography. Thus, diminished 11C-FFA extraction, externally de-
tectable, accompanied decreased perfusion in isolated perfused
hearts, and decreased 11C-FFA uptake reflecting myocardial is-
chemia in vivo can be evaluated noninvasively by positron-emission
transaxial tomography.

The need to detect and estimate the mass of ischemic
myocardium in vivo has given impetus to the development of
several approaches. The presence and extent of impaired
contractility, altered ventricular diastolic compliance, and
ventricular dyskinesia have been used as indirect indices of
the severity of ischemic insults.1 Electrophysiological altera-
tions have proved useful diagnostically but suffer from
quantitative limitations.2 Although ischemia can be inferred
from analysis of coronary artery anatomy or detected in
studies of regional myocardial perfusion, the local metabolic
consequences cannot be evaluated with available methods.3
Release of constituents such as potassium, lactate, or
enzymes from myocardium, and their detection in coronary
sinus or peripheral blood, provide only gross indices of
altered metabolism or tissue integrity and do not localize or
quantify reversible or irreversible injury.4

During the past two decades the metabolic characteristics
of normal and ischemic myocardium have been clarified
substantially. Data have been gathered primarily in studies
of coronary arteriovenous differences and in investigations
of substrate utilization in isolated perfused hearts subjected
to selected physiological conditions.5,6 In general, aerobic
myocardium preferentially utilizes free fatty acid (FFA) for
energy production. In contrast, FFA oxidation ceases in
anoxic or severely ischemic tissue and glycolytic flux
increases at least transiently. However, the effect of tran-
sient ischemia on uptake as opposed to oxidation of FFA has not been clearly defined.

Altered FFA and glucose utilization has been confirmed in isolated perfused hearts subjected to diminished flow or hypoxia in the presence of \(^{14}C\)-labeled substrates in recirculating systems.\(^{10, 11}\) The present study was undertaken to characterize externally detectable alterations in FFA and glucose uptake with the use of positron-emitting \(^{14}C\)-labeled glucose and FFA in isolated perfused hearts exposed to high or low flow. One goal of the study was to clarify transient changes in substrate uptake without the need for direct biochemical analysis of myocardium under conditions in which repetitive determinations could be made. In addition, the investigation was designed to determine whether externally detectable metabolic alterations characteristic of ischemia could be used to visualize and ultimately quantify ischemic zones of myocardium in vivo.

**Methods**

**REAGENTS AND CHEMICAL PROCEDURES**

Bovine serum albumin (BSA), palmitate, octanoate, and glucose were obtained from Sigma Chemical Company. Albumin was defatted by acidification, adsorption with activated charcoal, and concentration with a Bio-Rad hollow fiber device.\(^{12}\) Palmitate was solubilized in ethanol heated to 40°C and added slowly during a 30-minute interval to 2 liters of albumin (2 g/100 ml) at 40°C, resulting in a final concentration of 0.4 mM. For other experiments, 0.4 mM octanoate was formulated in aqueous solution. The solubilized mixture was allowed to cool to room temperature but used as perfusate at 37°C. Lactate was assayed spectrophotometrically after protein precipitation of effluent with 5% trichloroacetic acid. Results were expressed as \(\mu\)mol produced/min per g of dry weight obtained at the conclusion of the experiment by desiccating the heart to constant weight at 70°C.

**ISOLATED PERFUSED HEART PREPARATIONS**

Hearts were removed from nonfasted, male rabbits (weight, 1.7-2.5 kg) after ligation of the venae cavae, and were perfused retrograde through an aortic cannula with a flow of 20 ml/min with the use of a Gilford perfusion pump. The perfusate consisted of Krebs-Henseleit bicarbonate buffer equilibrated with 95% \(O_2\), 5% \(CO_2\). To obtain isovolumetrically beating preparations, a fluid-filled balloon was inserted into the left ventricular cavity via the atrium and connected by a fluid-filled polyethylene catheter to a Statham 23Db transducer for determination of left ventricular pressure (LVP) and the first derivative of the LVP (dP/dt). Heart rate was maintained constant between 160 and 180 beats/min and slightly above the intrinsic heart rate with the use of left atrial pacing with silver wire epicardial electrodes. Coronary effluent was collected quantitatively via a polyethylene catheter inserted into the pulmonary artery. During a 30-minute equilibration period, 5 mM glucose was the only metabolic substrate included in the perfusate. This interval was selected because it is sufficient to deplete most of the endogenous fatty acid substrate.\(^{14}\)

The relation between coronary flow and extraction of FFA and glucose was determined as follows: Perfusion was initiated with Krebs-Henseleit buffer equilibrated with 95% \(O_2\), 5% \(CO_2\) containing BSA (2 g/100 ml), 0.4 mM octanoate or palmitate, and 5 mM glucose. To approximate high physiological levels of insulin throughout high and low flow periods, insulin (70 \(\mu\)U/ml) was added to the perfusate. Flow was maintained at 20 ml/min with this solution for 20 minutes. To prevent streaming of tracer injectate, 50 \(\mu\)Ci of labeled substrate were injected (at 0.1 ml/sec) into the turbulent perfusate 3 cm proximal to the heart through a sidearm in the aortic cannula. The addition of tracer (100-500 ng) with Krebs-Henseleit solution containing albumin in 0.2-ml samples did not alter the concentration of substrate in the perfusate detectably. The labeled substrate in the myocardium was monitored externally by residue detection for 700 seconds, a period sufficient for recognition of the fast and slow components of the washout curves, but not for delineation of the differences in upstroke slope to peak activity occurring with high and low flow. After an additional 20 minutes, by which time radiation background from the previous injection was negligible, the procedure was repeated.\(^{14}\)

To evaluate extraction of \(^{14}C\)-glucose or \(^{14}C\)-FFA under conditions of low flow, two additional injections were made as follows: Perfusion was continued for 20 minutes so that background radioactivity became negligible. Flow was reduced abruptly to 5 ml/min for 1 minute at which time \(^{14}C\)-glucose or \(^{14}C\)-FFA was injected while low flow was maintained for 700 seconds. The second injection was made under identical conditions 29 minutes after the initial reduction of flow.

To verify the metabolic integrity of each preparation after perfusion at low flow, perfusion then was increased to control values (20 ml/min) for 20 minutes and an additional injection of \(^{14}C\)-labeled substrate was performed.

To confirm the presence of ischemia, lactate in the coronary effluent was assayed at 20-minute intervals throughout each experiment. As noted by others, lactate production was maximal within a few minutes after reduction of flow and declined despite persistence of low flow, presumably because of inhibition of glycolytic flux.\(^{4}\)

**CANINE PREPARATIONS**

To determine whether \(^{14}C\)-substrate uptake delineated ischemia in vivo, studies were performed with closed-chest dogs anesthetized with sodium pentobarbital, 30 mg/kg injected intravenously. The dogs were instrumented 1 week earlier with an exteriorized inflatable occlusive cuff around the left anterior descending coronary artery.\(^{14}\) At the time of each experiment \(^{14}C\)-palmitate was injected intravenously prior to coronary occlusion in a dose of 1-3 mCi in 5-10 ml of a solution of 0.5 ml of polyorbate 20 (Tween 20) diluted with albumin, 2 g/100 ml, in water. After 3 minutes, data were recorded for 10-20 minutes with a positron-emission transaxial tomograph electrocardiographically gated for diastole.\(^{14}\) Images were obtained serially prior to occlusion, immediately after occlusion, and 30 minutes after deflation of the cuff.

**SYNTHESIS OF RADIOACTIVELY LABELED SUBSTRATES**

\(^{14}C\)-glucose was prepared by photosynthesis using Swiss chard leaves, and \(^{14}CO_2\) was prepared by the \(^{18}B(d,n)^{14}C\)
nuclear reaction. \( ^{14} \text{CO}_2 \) in a carrier gas was passed over light-starved leaves for 20 minutes during exposure to a Micro-Lite daylight white lamp. Subsequently, the \( ^{14} \text{C} \)-labeled sugars (glucose, fructose, and sucrose) were extracted rapidly with ethanol and hydrolyzed to glucose and fructose by acidification. Glucose was separated and purified chromatographically as previously described.\(^{16}\)

\( ^{14} \text{C} \)-palmitic acid and \( ^{14} \text{C} \)-octanoate were synthesized by carboxylation of the corresponding Grignard reagent with \( ^{14} \text{CO}_2 \) produced by the \( ^{14} \text{B}(d,n)_{14} \text{C} \) nuclear reaction.\(^{18}\) The resultant \( ^{14} \text{C} \)-palmitate was solubilized in 5 ml of a solution containing 0.5 ml of Tween 20 neutralized to pH 7.4 with dilute hydrochloric acid. Because of the high specific activity, the injectates in the isolated perfused heart studies were diluted with perfusate such that \(<20 \mu l \) of Tween 20 was injected. This quantity of the vehicle had no discernible effect on left ventricular function. In the canine experiments, 1–3 mCi were injected in a 5- to 10-ml volume of a solution containing 0.5 ml of Tween 20 diluted with albumin, 2 g/100 ml in water. The vehicle is a potent histamine-releasing agent not suitable for human use\(^{65}\) and it induced cyanosis, tachypnea, and tachycardia. Recently we have solubilized \( ^{14} \text{C} \)-palmitate in an ethanol-albumin solution at 50°C, obviating the need for Tween (unpublished data).

\( ^{14} \text{C} \)-albumin was synthesized by methylation with \( ^{14} \text{C} \)-formaldehyde and sodium borohydride in a modification of the Rice and Means method\(^{21}\) as previously described.\(^{22}\)

**Quantification of Accumulation of Positron-Emitting Radionuclides by the Heart**

The radioisotope used in the present study was the cyclotron-produced, short-lived (\( t_1/2 = 20.4 \) minutes), positron-emitting \( ^{14} \text{C} \) which gives rise to two 511-keV photons emitted at 180° to each other. The radiation detection system utilized employed two NaI (T1) detectors placed 180° apart, 2 cm from the surface of the heart at the midventricular level. Coincidence detection of the two 511-keV photons restricts the detector response to activity in a well defined region between two opposing detectors. Accordingly, in this system coincidence counting selectively detects events reflecting isotope distribution in the entire isolated perfused heart. It is not capable of delineating regional flow differences in this small target. Background activity is reduced to essentially 0 by coincidence counting.

The detected events were recorded with an on-line LINC computer which corrected for radioactive decay and residual background after sequential injections. Results were expressed as counts per second emanating from the heart as a function of time. Recordings of labeled compounds in the heart were initiated 1 second prior to injection of the radioisotope and continued for 700 seconds. The height of the initial peak (Fig. 1) is proportional to the total activity injected. The initial rapid decline in activity is due to the clearance of labeled substrate from the vascular space and the later part of the curve reflects washout from the extravascular space.\(^{22}\) The activity in the slowly declining plateau phase for FFA is consistent with extravascular washout of labeled substrate, of \( ^{14} \text{CO}_2 \), and of other \( ^{14} \text{C} \)-labeled products of metabolism. An apparent small extraction, less than 7%, which was not subtracted in these studies, could be calculated for \( ^{14} \text{C} \)-albumin curves obtained during periods of low flow. This probably reflected reflux of small amounts of perfusate into the left ventricular chamber and possibly some capillary leakage.

To delineate the maximal net percent of substrate accumulated by the heart from the perfusate in a single transit, an extraction fraction was calculated. This extraction fraction (Fig. 1) is defined as the value obtained by extrapolating a plateau phase of extravascular washout curve back to the time of peak count rate, and then dividing this value of tracer residue by the peak value.

Substrate extraction is dependent on myocardial avidity for the tracer, and the flow-dependent time of the exposure of substrate to the myocardium. To differentiate changes in myocardial uptake which reflected the metabolically mediated change in myocardial avidity for the substrate from alterations of substrate extraction due solely to the kinetics of altered flow, as reflected by the altered vascular transit time (\( t \)), we determined \( f \) with \( ^{14} \text{C} \)-albumin and measured the \( ^{14} \text{C} \)-substrate extraction fraction under conditions of varying flow. The value of \( f \) was calculated by the method of Zierler.\(^{22}\) Increased extraction therefore may occur in predictable fashion, due simply to diminution of flow rate. An experimental design therefore was required to distinguish these flow-mediated changes from changes reflecting altered myocardial metabolism and consequent changes in myocardial avidity for the tracer.

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**Figure 1** A representative time course of count rates observed in the isolated perfused heart after a bolus injection of \( ^{14} \text{C} \)-labeled substrate. The curve consists of: (1) a peak proportional to total activity injected, (2) a rapidly declining portion representing clearance from the vascular compartment, and (3) a slowly declining component representing extraction and subsequent clearance of activity from myocardium. The fraction of total labeled substrate extracted into the tissue is defined as the back-extrapolated intercept value divided by maximum height.
For this reason, for each substrate studied, the effects of altered flow per se were observed by injecting "C-labeled albumin at high and low flow rate. For descriptive purposes, the qualitative term "residence time" is utilized to describe the duration of exposure of tracer to the myocardium, and this period of exposure is directly proportional to vascular transit time (t).

**Visualization of Ischemic Myocardium by Positron-Emission Transaxial Tomography**

A suitable detection system should provide the potential for quantitative localization of altered extraction of tracer and should avoid difficulties of low contrast and resolution associated with conventional two-dimensional display of superimposed regions. Accordingly, we utilized positron-emission transaxial tomography (PETT), a computer reconstruction technique based upon coincidence counting. In the isolated perfused heart experiments, a simplified approach was selected which utilizes the same coincidence mode counting system but no computer reconstruction. In experiments performed with lightly anesthetized closed-chest dogs, the PETT was used to obtain cross-sectional images from 1-cm-thick slices of the ventricular myocardium in vivo. The selection of tracers was based on results of studies in isolated perfused hearts and refined by preliminary canine studies utilizing rectilinear scanning. Of the three "C-metabolities examined, "C-palmitate exhibited several advantages including: (1) high accumulation in myocardium, (2) virtually no accumulation in lung (in contrast to octanoate), (3) noninterfering accumulation in chest skeletal musculature, (4) slow clearance of the activity from the myocardium (clearance half-time of approximately 30 minutes), and (5) rapid removal from the blood pool within 3 minutes.

**Results**

**Documentation of the Physiological Effects of Reduced Flow on the Isolated Perfused Heart**

Under conditions of high flow (20 ml/min) left ventricular pressure (LVP) development, dP/dt, and tension-time index (pressure-time integral, mm Hg sec min^-1) were stable in all preparations studied. An example of LVP and dP/dt under conditions of high flow, low flow (5 ml/min), and subsequent return to high flow after 10 minutes is shown in Figure 2. Similar recovery was seen after flow had been reduced for 30 minutes (n = 4 hearts) and often when flow was reduced for as long as 60 minutes (n = 3). After reduction of flow, the tension-time index decreased from an average (n = 10) of 927 ± 185 to 123 ± 108 (so) with reduction of flow and remained stable during a 30-minute interval of reduced flow. These results demonstrate that tension time had decreased by the time labeled substrate was injected after reduction of flow (see below) and that altered ventricular mechanics persisted and remained stable during the interval of reduced flow required for delineation of myocardial accumulation of "C-labeled substrate.

During initial high flow, lactate appeared in the coronary effluent at an average rate of 0.5 ± 1.9 (so) μmol/min per g of dry weight. After 5 minutes of exposure to low flow (5 ml/min), the myocardium liberated substantially more lactate into the coronary effluent (5.3 ± 2.1 μmol/min per g). After 30 minutes of persistent low flow, lactate in the effluent declined to 2.3 ± 0.6 in keeping with declining glycolytic flux in the face of persistent low flow reported by others.

These results indicate that the preparations used exhibited reversible impairment of ventricular function and increased lactate production typical of changes associated with ischemia when low flow was maintained for 30 minutes.

**Effects of Flow on Clearance of "C-Albumin from the Vascular Compartment in Isolated Perfused Hearts**

After injection of "C-albumin the decline from the initial peak of radioactivity in the heart primarily represents clearance of the vascular space, since albumin is largely confined to this compartment during a single transit through the myocardium. Thus, curves obtained with albumin exhibited a marked lower absolute and often declining level in contrast to that seen after injection of "C-FFA under conditions of identical flow (Figs. 1 and 7), because the decline of radioactivity from "C-albumin is in the main due to clearance of tracer from the vascular compartment, with only a small extravascular residue. As can be seen in Figure 3, the ratio of counts within the field of the detectors at any selected time after the peak at high (20 ml/min) or low (5 ml/min) flow is constant and inversely proportional to the ratio of the flows themselves. Thus, for example, at 500 seconds the values of counts/sec were 200 (at 5 ml/min) and 50 (at 20 ml/min), a ratio of 4:1. Similarly, the values of T are in a ratio of 4:1. Accordingly, the extraction fraction of labeled substrates can increase with increasing transit time under conditions of constant metabolism.

The experiments with albumin were performed for two reasons: (1) to characterize the effects of altered flow on a marker primarily confined to the extracellular space for purposes of comparison to behavior of metabolic substrates.
analyzed in the same system; and (2) because albumin is the vehicle selected for use in transporting FFA in the perfusate in subsequent experiments.

EFFECTS OF ALTERED RESIDENCE TIME DUE TO DECREASED FLOW ON MYOCARDIAL EXTRACTION OF $^{14}$C-GLUCOSE, OCTANOATE, AND PALMITATE

The extraction fraction of any substrate in a nonrecirculating system depends on the duration of contact of substrate with myocardium referred to as residence time and the cellular avidity for the substrate. The effects of increased residence time on the extraction of $^{14}$C-labeled glucose, octanoate, and palmitate were compared after 20 ml/min flow and after only 1 minute of 5 ml/min flow. The results under these two conditions are: $^{14}$C-glucose extraction at 20 ml/min, $3.4 \pm 1.1$ (SE); at 5 ml/min, $16.8 \pm 3.3$ (n = 5); $^{14}$C-octanoate, $56 \pm 15$ and $83 \pm 17$ (n = 10); $^{14}$C-palmitate, $23 \pm 9.7$ and $42 \pm 11$ (n = 5). Representative examples of these experiments are illustrated in Figure 4 and identify the increased extraction initially of all substrates at reduced flow. It should be emphasized that after 30 minutes of low flow, extraction of octanoate and palmitate decreased compared to that after 1 minute because the effect of increased residence time is masked by decreased myocardial avidity due to hypoxia. After 1 minute of flow reduction, as can be seen in Figure 4, the extraction fractions of glucose, octanoate, and palmitate were increased in a fashion that was inversely proportional to the flow reduction and therefore conformed to the hypothetical relationship between decreased flow and increased extraction. In order to delineate the metabolically mediated alterations of myocardial tracer accumulation from those which were secondary to the specific physical phenomena of increased residence time due to flow reduction, we compared sequentially injected tracers after 1 minute and 30 minutes of flow reduction. Since the system utilized did not permit recognition of the metabolically mediated changes of FFA or glucose extraction which occur within 1 minute of flow reduction, we used this point as the baseline for comparison following 30-minute flow reduction. It was noted that octanoate, in contrast with palmitate in the isolated heart, was extracted more completely. A similar disparity was evident even after 30 minutes of flow reduction (Fig. 5).

EFFECTS OF ALTERED METABOLISM DUE TO DECREASED FLOW ON MYOCARDIAL EXTRACTION OF $^{14}$C-GLUCOSE, OCTANOATE, AND PALMITATE

The experiments illustrated in Figures 5 and 6 were designed to determine the effect of prolonged low flow on
myocardial extraction of $^{14}$C-labeled substrates. The increased extraction of FFA resulting from decreasing flow for 1 minute contrasted sharply with the diminished myocardial uptake of both octanoate and palmitate after low flow of prolonged duration (30 minutes). This reduction of uptake, as seen in Figure 5, appears to reflect diminished myocardial avidity for the FFA, resulting in an effect divergent from the residence time effect seen at identical flow after only 1 minute. In panel B of Figure 5, results with palmitate are contrasted to those with albumin, the vehicle for labeled palmitate. These washout curves after 1 minute of low flow indicate the following:

1. Palmitate extraction is high with accumulation of tracer at 700 seconds continuing to be greater than 40% peak concentration.

2. The albumin vehicle accumulation after more than 600 seconds is low and less than 7% of peak concentration. Thus, there is almost complete clearance of the intravascular marker with a remaining slow washout compatible with either small amounts of extravascular albumin or slight reflux through the aortic valve known to occur in these preparations.

3. In contrast to myocardial extraction of FFA after 1 minute of low flow, there is a marked suppression of FFA accumulation after 30 minutes of low flow. The nearly complete suppression of $^{14}$C-palmitate accumulation is evident when the 30-minute washout curve for $^{14}$C-palmitate is contrasted with that of its vehicle, $^{14}$C-albumin. The close resemblance of these two curves indicates that the palmitate accumulation after 30 minutes of low flow is negligible and that the minimal residue parallels that of $^{14}$C-albumin, indicating that the $^{14}$C-palmitate was not extracted into the myocardial tissue to an appreciable extent but rather behaved as a vascular marker. These results indicate that reduction of flow for 1 minute is associated with an altered pattern of palmitate extraction due to changes in residence time but that prolonged reduction of flow is accompanied by marked diminution of extraction (12.5 ± 5.6%, n = 5 hearts). The kinetics of palmitate extraction after 30-minute low flow parallel the kinetics of a vascular marker and its vehicle, $^{14}$C-albumin, since the albumin curves after 30 minutes and 1 minute of low flow are indistinguishable (n = 3 hearts).

Similar results were obtained with octanoate despite the known differences in mitochondrial transport of short chain compared to long chain acyl-CoA derivatives. When flow reduction was maintained for 30 minutes, extraction of octanoate decreased from control values to 30 ± 3 (n = 10), a directionally similar change to that seen with palmitate.

In contrast to the marked reduction of FFA accumulation when the isolated heart was maintained at low flow for 30 minutes, there was no suppression of glucose extraction during prolonged low flow (Fig. 6). Myocardial accumulation of $^{14}$C-labeled glucose essentially remained unchanged.

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

**Figure 5** The effects of decreased flow maintained for 1 minute (open circles) and 30 minutes (filled circles) on the extraction of palmitate by the isolated perfused rabbit heart. As can be seen in panel A, reduction of flow for 1 minute was associated with an extraction fraction for palmitate substantially greater than that seen when flow reduction was maintained for 30 minutes. Thus, during constant flow, the alteration in metabolism resulting from 30 minutes of low flow reduced palmitate extraction. In panel B, the clearance of $^{14}$C-albumin is depicted (filled circles) for comparison. Both experiments in panel B were performed under conditions in which flow was reduced to 5 ml/min for 1 minute prior to the injection of tracer. The calculated extraction fraction for palmitate is substantially higher than the value representing retention of albumin within the field of the detectors. On the other hand, comparison of the curve for palmitate after 30 minutes of low flow (panel A) and the curve for albumin injected after 1 minute of low flow (panel B) indicates that decreased flow persisting for 30 minutes is sufficient to reduce palmitate extraction to the extent that behavior of this tracer begins to resemble behavior of the vascular tracer, $^{14}$C-albumin. As noted in the text, behavior of albumin was the same after 1 minute as it was after 30 minutes of low flow and virtually indistinguishable from the behavior of albumin without reduction of flow.

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

**Figure 6** $^{14}$C-glucose extraction fractions obtained under conditions of high (30 ml/min), "normal" (20 ml/min), and low (5 ml/min) flow. Reduction of flow to 3 ml/min for 1 minute is indicated with circles, and reduction for 30 minutes is indicated with squares. As can be seen, the alteration in extraction fraction of glucose associated with reduced flow was consistent with the hypothetical relationship of extraction fraction and residence time under conditions of constant myocardial glucose accumulation. Accordingly, under these conditions, alterations in extraction fraction of glucose associated with diminished flow are not indicative of a change in myocardial metabolism per se. This may reflect the low absolute values of glucose extraction even under conditions of high flow, hence the proportionately greater error in the calculated value for extraction fraction. Even if differences in the rate of depletion of glycogen were to occur under these conditions, it is unlikely that consistently detectable changes in accumulation of glucose would be observed because of the low extraction fraction of glucose.

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FIGURE 7 Electrocardiographically gated diastolic images obtained with the positron-emission transaxial tomograph after induction of transient myocardial ischemia by constriction of an exteriorized coronary artery occlusive cuff in an intact dog. Each image represents a reconstructed cross-sectional slice through the heart at the ventricular level. Anterior, posterior, left and right are indicated by the letters A, P, L, and R, respectively. In the top panel homogeneous accumulation of $^{11}$C-palmitic acid is evident in the normal left ventricular myocardium. The tomogram was obtained during a 20-minute interval after intravenous injection of tracer. In the center panel, a transmural defect representing failure of accumulation (arrow) of $^{11}$C-palmitate is present anteriorly in an image obtained after 30 minutes of myocardial ischemia. The image shown in the lower panel was obtained during the 20-minute interval immediately following release of the coronary artery occlusive cuff after an interval of ischemia of 30 minutes, hence insufficient to produce extensive infarction. As can be seen, after reperfusion, myocardial metabolic integrity is demonstrable in the area of the previous defect (arrow) and, in fact, the accumulation of tracer in this region exceeds that in adjacent and presumably normal myocardium. As can be seen, the only appreciable uptake of tracer within the thorax is in a region corresponding to the heart.

After 1 minute and 30 minutes of low flow. The extraction of $^{11}$C-glucose was in both cases compatible with augmented uptake secondary to a corresponding increase in residence time, without any discernible metabolically mediated alteration. The reproducibility of determinations of extraction fraction at high flow rates was examined before and after 30 minutes of low flow in two hearts. $^{11}$C-octanoate was sequentially injected in the perfusate at 30-minute intervals. The first two injections were performed prior to reduction of flow; and two subsequent injections were performed 25 minutes after an intercedent interval of low flow (5 ml/min) for 30 minutes when high flow had been restored. In each of the two hearts, extraction fraction varied by less than 5% (range).

Although transient ischemia increases glucose uptake, prolonged ischemia is associated with inhibition of glycolytic flux and the net changes in glucose uptake under these conditions have been variable. From a technical point of view, our results with glucose are less reliable than those with FFA because the extraction fraction of glucose at high flow is less than 5%. Nevertheless, as can be seen in Figure 6, the net effect of prolonged reduction of flow from 20 ml/min to 5 ml/min for both 5 minutes and 30 minutes on accumulation of $^{11}$C-glucose was attributable entirely to increased residence time. The effect of residence time was also seen with a decrease of extraction fraction when flow was high (30 ml/min). Thus, although reduction of flow from 20 ml to 5 ml for 30 minutes increased glucose extraction from 3.4 ± 1.1% to 16.8 ± 3.3%, this change was inversely proportional to the change in flow. This finding, although initially unexpected, is compatible with observations by others using chemical analytic techniques indicating that net glucose uptake changes very little with low flow in the ischemic baboon heart although extraction fraction increases (L. H. Opie, personal communication).

Utilization of $^{11}$C-labeled substrate for delineation of ischemic myocardium in vivo

On the basis of our observations with the isolated perfused heart, we examined the suitability of intravenous administration of $^{11}$C-labeled myocardial substrate for imaging normal, transiently ischemic, and irreversibly injured myocardium in vivo.

As can be seen in Figure 7, intravenous administration of $^{11}$C-palmitate permitted localization of a transiently ischemic zone in the anterior left ventricular wall induced by inflation of an exteriorized coronary occlusive cuff for 20 minutes beginning 3 minutes before the injection. Subsequent injection 3 minutes after release of the cuff led to enhanced accumulation of $^{11}$C-palmitate in the previously ischemic zones in comparison to accumulation in normal myocardium, suggesting reactive hyperemia. We previously have demonstrated that 20-minute occlusions in this model...
do not lead to irreversible injury manifested by release of the MB creatine phosphokinase (CPK) isoenzyme into blood or by evolution of histological changes in myocardium. When ischemia was maintained for 1 hour, however, enzyme release accompanied by subsequent histological changes indicative of necrosis occurred. In the present study, when ischemia was maintained for 1 hour, the region of diminished \(^{14}\)C-palmitate uptake persisted for 24 hours after release of the occlusive cuff. Thus, inferentially, zones of infarction were manifested by persistent impairment of palmitate accumulation in contrast to zones of reversible ischemia which exhibited only transient diminution of \(^{14}\)C-palmitate uptake.

Discussion

Alterations of myocardial metabolism associated with hypoxia or reduced coronary perfusion often have been evaluated in isolated perfused hearts under conditions in which the availability of substrate and the ventricular mechanical function can be controlled conveniently. The present study was designed to determine whether changes in the myocardial accumulation of glucose and fatty acid resulting from ischemia could be quantified externally, and to clarify their time course by sequential monitoring in isovolumic rabbit hearts. Consistent alterations of accumulation of \(^{14}\)C-labeled glucose, octanoate, and palmitate and preferential accumulation of FFA substrates were demonstrable readily in well perfused hearts. Subsequently, the same tracers were used to obtain computer reconstruction tomographic images of ischemic zones in closed-chest dogs.

Oxidation of fatty acid, the primary myocardial energy source, is dependent on fatty acid chain length. Long chain fatty acids are dependent on carnitine acyl-CoA transferase reaction for translocation of the fatty acid from extramitochondrial to intramitochondrial sites, a requirement not involved in oxidation of medium chain length fatty acid. Because of these differences we studied myocardial accumulation of both palmitate (long chain length) and octanoate (medium chain length).

Myocardial accumulation of fatty acid is dependent on both the availability of substrate, a function of the molar ratio of FFA to albumin, and on the metabolic state of the cell reflecting the balance between ventricular myocardial oxygen supply and demand in turn influenced by circulating levels of catecholamines, insulin, and thyroid hormone. The effects of diminution of coronary flow on myocardial metabolism are complex and time-dependent. They differ from effects induced by hypoxia in the absence of impaired perfusion. In the present study, we were concerned with a dynamic evaluation of tracer accumulation. Therefore, alterations in residence time were taken into account.

An abrupt increase of both octanoate and palmitate extraction associated with reduction in coronary flow reflected altered metabolism and altered residence time. It has been shown that exogenous fatty acid metabolism during ischemia may divert FFA from \(\beta\) oxidation with an attendant increase of the triglyceride fraction of myocardial lipid. Thus, in these experiments reported, uptake of FFA during intervals of decreased perfusion probably was due in part to FFA incorporation into triglyceride, despite decreased \(\beta\) oxidation. However, altered extraction of substrate might have occurred simply because of the increased residence time. To differentiate the two mechanisms, we examined the effect of reduction of flow on accumulation of substrate in myocardium. With sequential administration of both labeled octanoate and palmitate, accumulation of FFA decreased markedly during prolonged low flow, without a concomitant change in ventricular mechanics or any limitation in subsequent mechanical recovery after restitution of high flow. Thus, in contrast to glucose, the depression of FFA uptake with 30-minute prolonged reduction of coronary flow was secondary to depressed myocardial extraction of substrate.

Under physiological, aerobic conditions myocardial fatty acid oxidation inhibits carbohydrate utilization and is associated with intracellular accumulation of citrate and isocitrate and decreased activity of phosphofructokinase. With reduction of myocardial oxygen availability, \(\beta\) oxidation of fatty acids decreases profoundly. We anticipated that myocardial accumulation of glucose would increase under these conditions and thereby serve as a directionally opposite index of myocardial ischemia, potentially useful in detecting ischemia in vivo with tomographic imaging techniques. However, the accumulation of glucose by the myocardium was low at all flow rates studied, and substantially less than the accumulation of FFA. The low extraction of glucose under high flow conditions did not permit consistent detection of augmentation of glucose uptake induced by insulin or suppression due to FFA (data not shown). The increased accumulation of \(^{14}\)C-glucose, unlike FFA, persisted when low flow was maintained for as long as 30 minutes. Although the increase in net myocardial accumulation of glucose may serve as a directionally opposite index of reduced flow, it is not indicative of altered metabolic myocardial extraction but reflects decreased flow per se. Furthermore, because of the low extraction of glucose by myocardium, even when flow was low, \(^{14}\)C-glucose did not appear to be well suited for use as an imaging agent. In addition, the dispersion of \(^{14}\)C-glucose throughout many organs, such as lung, as well as in myocardium, precludes precise tomographic delineation of the heart in vivo.

Both palmitate and octanoate accumulated substantially in the isolated perfused heart under aerobic conditions. Palmitate extraction is high under aerobic conditions. Under anaerobic conditions, however, palmitate is confined primarily to the intravascular space when transported by an albumin carrier, as demonstrated by parallel washout curves similar to those of \(^{14}\)C-methylated albumin. Palmitate behaved in a corresponding fashion in vivo and in vitro. When 1–5 mCi of \(^{14}\)C-palmitate were injected intravenously in dogs, tracer was concentrated within 3 minutes in the myocardium, permitting computer reconstruction of transaxial cross-sectional images with no interference contributed by residual palmitate in the blood pool. Despite previous reports of a relative increase in incorporation of \(^{14}\)C-labeled palmitate during myocardial ischemia in dogs, images obtained in the present study by positron-emission tomographic techniques after acute ischemia indicated net decreased accumulation and revealed sharply
defined defects corresponding to the ischemic region of the left ventricle. The release of the occlusive coronary cuff after 20 minutes resulted in an area of increased uptake of palmitate (compared to normal adjacent myocardium) in the previously ischemic zone. The augmented uptake in a zone of previous ischemia apparently is indicative of the reversible nature of injury produced by ischemia maintained for less than 20 minutes. Corresponding changes in S-T segments on surface electrocardiograms in the animal studies (initial elevation and regression with reflow) support this interpretation (data not shown).

11C-labeled octanoate was not useful as an agent for visualization of myocardium. When administered intravenously in dogs, this medium chain length fatty acid exhibited extensive pulmonary distribution possibly resulting from particulate aggregation of the 11C-labeled material. This possibility was excluded, however, by Millipore filtration prior to injection and solubilization in Tween 20. The lack of localization of octanoate in contrast to palmitate in myocardium in vivo may be due in part to the dependence of myocardial avidity for FFA on chain length. In contrast to the case in the studies of isolated hearts in which the perfusate contained only a single FFA, several chain lengths are available in vivo. The presence of physiologically preferred, longer chain FFA, such as palmitate, may have suppressed myocardial accumulation of octanoate. Alternatively, pulmonary or other organ avidity for octanoate may be sufficiently large to preclude effective uptake by the heart.

On the basis of these studies in isolated perfused hearts and preliminary results with canine hearts in vivo, it appears likely that 11C-palmitate will be particularly useful in external detection of metabolic alterations associated with ischemia. With tomographic techniques employing computer reconstruction, differentiation of metabolically jeopardized zones from hypoperfused zones may be possible by comparison of results with labeled physiological substrates to those obtained with tracers with distribution more exclusively dependent upon perfusion. The present findings suggest that metabolism of normal, ischemic, and otherwise diseased myocardium may be amenable to analysis in vivo with the use of 11C-labeled physiological substrates.

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