INVESTIGATIVE NUCLEAR MEDICINE

Evaluation of Myocardial Metabolism, with N-13- and C-11-Labeled Amino Acids and Positron Computed Tomography

Eberhard Henze, Heinrich R. Schelbert, Jorge R. Barrio, James E. Egbert, Herbert W. Hansen, Norman S. MacDonald, and Michael E. Phelps

UCLA School of Medicine, University of California at Los Angeles, Los Angeles, California

To evaluate the utility of labeled L-amino acids (AA) for imaging regional myocardial AA metabolism by positron computed tomography (PCT), the myocardial uptake and clearance of Ala,* Glu, Gln, Asp, Leu tagged with N-13, and of C-11-tagged Asp, and oxaloacetate (Oxal), were examined in 44 experiments at control, during ischemia, and after transaminase inhibition. The myocardial time-activity curves recorded after intracoronary tracer injection had two clearance phases (an early and a late) for all N-13 AA, and three (early, intermediate, late) for the two C-11 compounds, with significantly different clearance half-times of 18.7 ± 8.0 (s.d.) sec for the early phase, 141.7 ± 56.5 sec for the intermediate, and 61.2 ± 43.5 min for the late phase. The residual fractions ranged from 0.07 to 0.23 in normal myocardium, and consistently increased with ischemia by 0.02–0.07 for N-13-labeled Ala, Glu, Asp, and Leu, but not for N-13 Gln and the C-11 compounds. Transaminase inhibition shortened the half-times of the late phases of N-13-labeled Ala, Glu, Asp, and Leu; had no effect on $t_{1/2}$ of N-13 Gln and C-11 Oxal; and resulted in a loss of C-11 CO_2 production and of the intermediate phase for C-11 Asp. On the PCT images, N-13 activity from labeled Ala and Glu was not decreased in an ischemic segment despite a significant flow reduction, as demonstrated by N-13 NH_3 imaging and labeled microspheres. From the results, a three-compartment tracer kinetic model is proposed for the noninvasive quantification of Krebs-cycle activity, protein synthesis, and metabolic derangements related to ischemia.

J Nucl Med 23: 671–681, 1982

While generally considered as the basic elements for protein synthesis, several amino acids are linked through their intermediary metabolism to carbohydrate metabolism; they also participate in maintaining the cytosolic and mitochondrial redox balances and can play an important role in removing ammonia from myocardium (*1–4*). The carbon skeletons of Ala,* Glu, and Asp are important intermediates of aerobic and anaerobic glycolysis and can replenish intermediates in the citric acid cycle (*4*), while the corresponding α-ketoacids of branch-chain amino acids such as Leu enter specific metabolic pathways. Asp and Glu further participate in the malate-aspartate shuttle for indirect transportation of cytosolic NADH into the mitochondrial matrix, and for coordination of the cytosolic and mitochondrial redox potential (*2*). Gln and Ala are considered the vehicles for the removal of ammonia from normal and ischemic myocardium (*3,4*). The existence of an anaerobic pathway in ischemic and/or anoxic myocardium has been postulated; it uses Glu and Asp and yields GTP and ATP by substrate phosphorylation in animal models (*5,6*). Consistent with such a hypothesis are (a) the ob-

Received Jan. 26, 1982; revision accepted April 5, 1982.

For reprints contact: Eberhard Henze, MD, UCLA School of Medicine, Div. of Nuclear Medicine, Los Angeles, CA 90024.

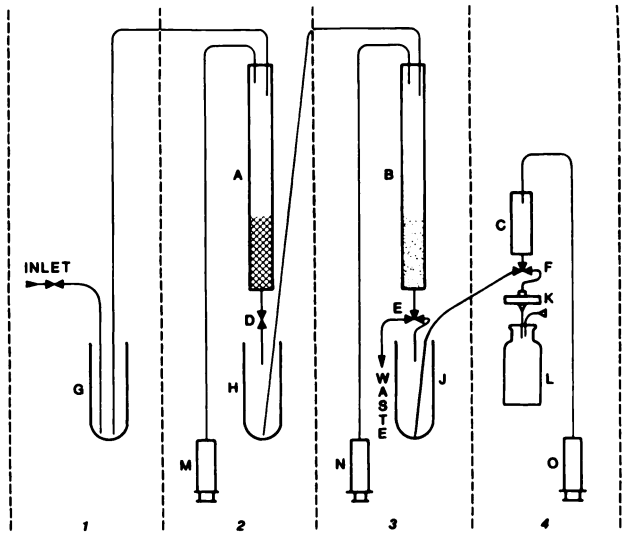

FIG. 1. Remote semiautomated system used for enzymatic production of N-13- and C-11-labeled L-amino acids. System has been conceptually divided into four operational units: (1) preparation of substrates and cofactors, (2) enzymatic synthesis, (3) purification, and (4) sterilization. Specific components are: A = enzyme column(s); B = ion-exchange column; C = reservoir vessel; D to F: solenoid valves; G to J: vessels; L = injection vial; K = 0.22-μm pore membrane filter; M, N, O = syringes.

servation of an increased myocardial extraction of Glu in patients with coronary artery disease (*7*); and (b) the beneficial effect of amino acids, especially Glu, on the mechanical recovery of ischemic rabbit septa (*8*) or cardioplegic human myocardium (*9*). Finally, alterations in myocardial protein synthesis will be reflected by derangements in myocardial amino acid turnover. Protein synthesis has been reported to be altered in a variety of myocardial disorders such as ischemia, cardiomyopathy, hypertrophy, and heart failure (*10–13*).

Amino acids can now be labeled with positron-emitting nuclides (such as N-13 and C-11) with a high yield, a high degree of purity, and no loss of their biochemical properties, and they can be used in man (*14–17*). They could be of value, together with positron computed tomography (PCT), for the in vivo study of normal and abnormal myocardial intermediary and protein metabolism and/or synthesis. Nitrogen-13-labeled amino acids have already been used for tomographic PCT imaging of the heart (*18,19*). However, no systematic studies have yet been performed to delineate the kinetics of these labeled amino acids and their relationship to metabolism and protein synthesis. Such studies are important for interpretation of the PCT images as well as for developing a basis for tracer kinetic modeling.

It was therefore the objective of this study to examine uptake, clearance, and retention of N-13- and C-11-labeled amino acids and related intermediates of the citric acid cycle, in normal and ischemic myocardium as well as after transaminase inhibition. The techniques using single-pass residue fraction and PCT imaging were used to define their utility for the noninvasive study of regional myocardial metabolism by PCT.

MATERIALS AND METHODS

Radiopharmaceutical preparation. The following compounds were synthesized and their kinetics studied: N-13 substituted in the α position in the L-amino acids Ala, Glu, Gln (α-Gln), Asp, and Leu, and in the ω position in Gln (ω-Gln); and C-11 in C_4 position in oxaloacetate (Oxal) and Asp.

N-13 Glu, N-13 Ala, N-13 ω-Gln, N-13 Asp, C-11 Oxal, and C-11 Asp were prepared as reported earlier from our laboratory (*15,16*). N-13 Leu was synthesized as described by Cooper et al. (*17*). N-13 α-Gln was prepared from N-13 Glu and 30 m*M* sodium phosphate (pH 7.5), 20 m*M* $MgCl_2$, 6.8 m*M* ATP, and 20 m*M* NH_4Cl in a total volume of 5 ml, using the procedure described earlier for N-13 ω-Gln (*16*). The enzymatic syntheses were performed by remote, semi-automated control of the chemical unit operations as described in a preliminary report by Barrio et al. (*20*), as shown schematically in Fig. 1.

Animal instrumentation. Twenty mongrel dogs weighing from 19.5 to 35.5 kg (mean 25.5 kg) were studied after an overnight fast. Each dog was anesthetized with sodium pentobarbital (25 mg/kg) and ventilated with room air. After a left thoracotomy, the pericardium was incised widely and sutured to the chest wall to form a cradle in which the heart was suspended. Myocardial blood flow was measured with radioactive microspheres injected into the left atrium using the arterial reference sample technique (*21*). Systemic blood pressure was monitored continuously through a catheter positioned in the aorta and connected to a pressure transducer,† and was recorded together with the ECG on a direct-writing physiologic recorder.‡ Arterial blood samples were withdrawn through a second catheter advanced through the femoral artery into the aorta. Additional instrumentation depending upon the experimental protocol is described below.

Intracoronary injection studies. A. Control and ischemia. In eight dogs, in addition to the basic instrumentation an electromagnetic flow probe and a mechanical screw-type occluder were placed around the left anterior descending coronary artery (LAD). The labeled amino acids were injected into the LAD through a 30-gauge needle§ inserted distally to the flow probe. After a control experiment, LAD flow was reduced by 60–70% until hypokinesis and/or akinesis of the anterior myocardium occurred, the onset of which was determined

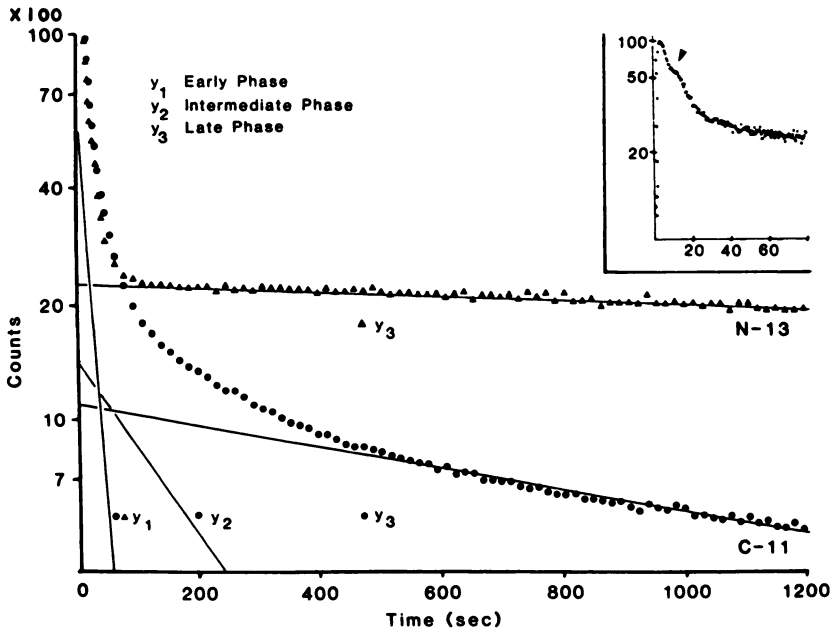

FIG. 2. Two typical time-activity curves for amino acids labeled with N-13 (triangles) and C-11 (dots). Initial portion of these curves is illustrated with expanded time scale in right upper corner. Arrow indicates recirculation of blood activity through left ventricle. Computer curve-fitting revealed three phases (y_1, y_2, y_3) for C-11-labeled compounds but two phases (y_1, y_3) for N-13-labeled compounds.

visually. Fifteen minutes later, the tracer bolus was injected and, while ischemia was maintained, myocardial radioactivity was recorded for 20 min. The occlusion was then released and the myocardium allowed to recover for 40–60 min. Ischemia was then induced again by reducing LAD flow for 15 min. The screw-type occluder was slowly released until LAD flow reached control levels (thus preventing reactive hyperemia), followed immediately by tracer injection and recording of myocardial activity while flow was maintained at control levels. The objectives of these experiments were to eliminate the effects of flow on extraction, washout, and initial metabolic trapping of labeled amino acids in ischemic myocardium, and to establish the tracer kinetics during slow recovery. At the time of tracer injection (only 3–5 sec after normal flow was re-established) the metabolic state of the myocardium supplied by the LAD was still ischemic (*22*) whereas blood flow was normal. This experimental approach, rather than an anoxia model with controlled flow, was used because it resembles more the pathologic situation in coronary artery disease—namely, perfusion after demand-induced ischemia or reperfusion after coronary spasm or thrombolytic interventions. Eight compounds were tested in these experiments: N-13-labeled Ala, Glu, α-Gln, ω-Gln, Asp, Leu, and C-11-labeled Oxal and Asp.

B. Effect of transaminase inhibition: Myocardial uptake and clearance of N-13-labeled Ala, Glu, ω-Gln, Asp, and Leu, and of C-11-labeled Asp and Oxal, were studied in seven dogs at control and after transaminase inhibition with amino-oxyacetate. After the control experiments, myocardium supplied by the LAD was pretreated with intracoronary infusion of amino-oxyacetate solution (0.2 mmol/ml-min) for 15 min. Blood flow in these experiments was approximately 100 ml/min-100g tissue. The above infusion rate therefore resulted in a concentration 2.0 m*M* of amino-oxyacetate in the perfusing blood. This concentration has been reported sufficient to inhibit transaminase activity (*8*). The infusion was maintained throughout the entire 20-min study period. Myocardial C-11 CO_2 production across the myocardium supplied by the LAD was measured after injection of C-11 Asp at control and after amino-oxyacetate infusion. Arterial blood was withdrawn from the left atrium, and venous blood through a cannula inserted into the anterior interventricular vein. For analysis of the C-11 CO_2 activity in arterial and myocardial venous blood, two 2-ml aliquots of the 5-ml blood samples were dispensed into separate test tubes and 0.9 *M* bicarbonate (1 ml) and water (4 ml) added to each aliquot. One aliquot was acidified with 1 ml of 6 *N* HCl, the other was made basic by adding 1 ml of 0.1 *N* NaOH. The acidified sample was placed in a hot-water ultrasonic bath for 5 min to dispel C-11 CO_2, and the C-11 activity in the duplicate tubes was determined. The difference in C-11 activity between HCl- and NaOH-treated duplicate samples represents the amount of C-11 CO_2 in blood. This approach has been validated in our laboratory. The retrieval of C-11 CO_2 by acidification was consistently in excess of 95%, whereas 100% of C-11 CO_2 was permanently bound in alkaline medium.

C. Data collection and analysis: The approach used in our laboratory has been described in detail elsewhere (*23,24*). Briefly, after injection of a 0.2-ml bolus containing 10–30 μCi of the labeled compound (with a mass of approximately 2–5 nmol) into the LAD, the retention of N-13- or C-11-labeled tracer in myocardium, and its subsequent clearance, were recorded with a shielded and

collimated 7.5- by 5.0-cm NaI(Tl) scintillation detector positioned over the heart. The field of detection encompassed the myocardium supplied by the LAD but excluded the site of tracer administration. Data were collected in 0.1-sec increments, corrected for decay, and stored in a digital computer.

Two time-activity curves typical for the N-13- and C-11-labeled compounds tested are shown in Fig. 2. Time zero represents the time of maximum count rate, this being proportional to the total amount of activity injected. These counts were normalized to 10,000. The early portion of the curve (shown expanded horizontally in the right upper corner of Fig. 2) demonstrates a vascular component and activity recirculating through the left ventricle. Because this initial part of the time-activity curve (30 sec) is not related to metabolism (see Results) it was excluded from further data processing. Least-squares curve-fitting by a computer revealed two monoexponential phases (early and late) for all N-13-labeled amino acids, but three monoexponential phases (early, intermediate, and late) for the C-11-labeled compounds. The clearance rates or slopes of these phases are described by their half-times ($t_{1/2}$). The fraction of radioactivity retained in myocardium was defined as the residual fraction, and is equal to the intercept of the late phase divided by the maximum count (10,000). This method of residue detection and analysis has been used previously and found valid for tracer kinetic studies in the heart (*23–25*) and in brain (*26,27*).

Tomographic Studies. A. Tomographic imaging. Tomographic imaging was performed in five dogs with the UCLA positron computer tomograph (*28*). In addition to the basic instrumentation, an electromagnetic flow probe, a snare, and a screw-type occluder were placed around the proximal LAD. Two pacing electrodes were attached to the left atrial appendix. The screw-type occluder was then tightened until reactive hyperemia to a 10-sec total occlusion (with the snare) was abolished. This resulted in a reduction of control flow by 55% ± 12 (s.d.) (n = 5) measured with Co-57- and Sn-113-labeled microspheres.

Each dog was positioned in the tomograph, and transmission images were recorded for subsequent correction for photon attenuation (*25*). One cross section through the mid level of the ventricle was selected for imaging. Demand-induced ischemia was then produced by atrial pacing, raising the heart rate from 117 ± 14 bpm to 196 ± 23 bpm, and was maintained throughout the image acquisition. Before injection of N-13 ammonia and N-13-labeled Glu or Ala, radioactive microspheres were injected into the left atrium. Also, myocardial perfusion was imaged after intravenous injection of the flow tracer N-13 ammonia (*23,24*). Sixty minutes later, after physical decay of N-13 ($T_{1/2}$ = 9.9 min), ischemia was induced again by rapid atrial pacing 15 min before intravenous injection of 10 mCi of N-13 Glu (four experiments) or N-13 Ala (two experiments). PCT imaging of the same myocardial cross section was again begun 4 min after injection for a period of 5 min. The dogs were then killed.

B. Data collection and analysis: Blood flow of the imaged cross section was calculated from the tissue microsphere concentrations and was correlated to the relative tracer concentrations of N-13 ammonia, N-13 Glu, and N-13 Ala, determined from regions of interest assigned to the PCT images as described previously (*23*). The ratio of activity in ischemic to normal segments was used to determine the relative flow reduction from the N-13 ammonia study (F) and relative tracer residue of labeled amino acids (R) in the ischemic segment. The ratio R/F relates the activity of N-13-labeled amino acids to blood flow in the ischemic segment.

All mean values are given with their standard deviations (s.d.). For statistical analysis, Student's t-test for unpaired and paired data was used.

RESULTS

Figure 2 shows two representative myocardial time-activity curves, recorded after intracoronary bolus injection of N-13 Ala and C-11 Asp and corrected for radioactive decay. The initial part of one of the curves is shown in expanded time (0.1-sec intervals) in the inset of the same figure. It illustrates the temporal changes immediately after tracer administration, i.e., the upslope, peak, and initial clearance of activity. The peak of the curve is proportional to the total activity injected. The subsequent short phase of rapidly declining activity represents the vascular component and depends on coronary blood flow. The half-time of this phase averaged 4.6 ± 1.2 sec (n = 22) at normal flow and increased ($p < 0.5$) to 5.5 ± 2.2 sec (n = 9) with low-flow ischemia. This vascular phase was apparent with all compounds tested and indicates that the initial extraction of the N-13- and C-11-labeled tracers in canine myocardium is less than 100%. Because of the incomplete initial extraction, considerable activity recirculates, as demonstrated by a recirculation peak at approximately 15 sec after injection. This initial recirculation is pronounced in our dog model after intracoronary injection because (a) the activity recirculates through the left-ventricular blood pool after only one lung passage, and (b) the scintillation probe used does not separate myocardial from blood-pool activity. The onset of initial recirculation (i.e., the time from peak activity to the onset of the recirculation peak) was noted in all experiments and averaged 7.4 ± 2.0 sec (n = 38), which is comparable to the minimum recirculation time of 0–15-labeled red blood cells shown by the same model (*23*). Because of the relatively large vascular phase and considerable recirculation, the initial extraction fraction cannot be as-

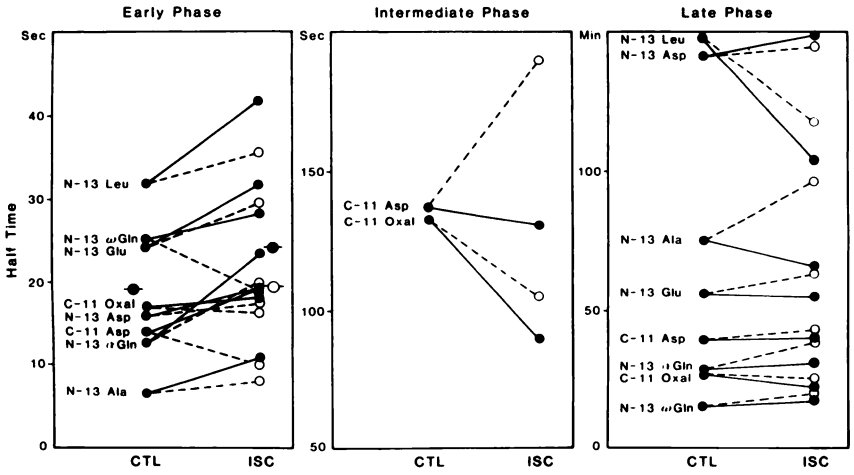

FIG. 3. Clearance half-times at control (CTL) after low-flow ischemia (ISC, solid lines), and during slow recovery after ischemia (ISC, dashed lines) during early, intermediate, and late phases. (Glu = glutamate, Gln = glutamine, α- or ω-labeled, Ala = alanine, Asp = aspartate, Leu = leucine, Oxal = oxaloacetate).

sessed accurately, as was possible for N-13 NH_3 (*23,24*). This initial portion of the time-activity curve (30 sec) is, however, not directly related to metabolic turnover of the labeled amino acids in myocardium and was therefore deleted from further analysis.

Tracer kinetics under control conditions. The time-activity curves for all N-13-labeled amino acids under study were similar but differed distinctly from those recorded with the C-11-labeled compounds, which as a group cleared from myocardium in a rather consistent pattern. Curve-fitting of the time-activity curves demonstrated two clearance phases (early and late) for the N-13 amino acids, but three (early, intermediate, and late) for all C-11-labeled tracers. Each of the three phases had significantly different half-times. As shown in Figs. 3 and 6 (in the control panel), the half-time of the early phase averaged 18.7 ± 8.0 sec (n = 15); for the intermediate phase—present only on the time-activity curves of C-11 labeled tracers—it was 141.7 ± 56.5 sec (n = 4), and for the late phase 61.2 ± 43.5 min (n = 15). The clearance rate of the early phase was virtually the same for N-13- or C-11-labeled tracers. The late phase identified two subsets with significantly ($p < 0.05$) different $t_{1/2}$: one subset with a $T_{1/2}$ of 32.5 ± 6.2 min (n = 7), which included all C-11 tracers and N-13-tagged α- and ω-Gln; and a second subset with a $t_{1/2}$ of 94.3 ± 37.5 min (n = 8) that included the remaining N-13 amino acids.

The residue fraction was relatively low, due to the presence of a relatively large nonextracted portion of the labeled amino acids and the rapid clearance rate of the early phase; it ranged from 0.07 to 0.23, with a mean of 0.17 ± 0.05 (n = 15).

Effect of ischemia. The two interventions (i.e., low-flow ischemia and slow recovery) had different effects on the half-times of the early clearance phase. The rate was significantly slower ($p < 0.01$) with low-flow ischemia ($t_{1/2}$ = 24.5 ± 19.5 sec) than in the experimental runs during recovery with normal flow ($t_{1/2}$ = 19.6 ± 9.1). The intermediate phase's $t_{1/2}$ decreased for both C-11 Asp and Oxal during low-flow ischemia but increased during the recovery period for C-11 Asp.

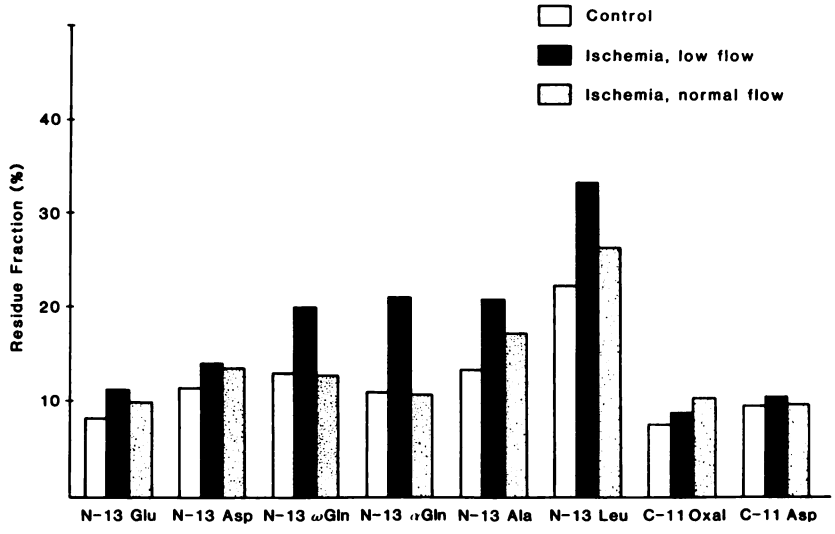

FIG. 4. Residue fractions of N-13- and C-11-labeled amino acids at control, ischemia, and during recovery after ischemia with normal blood flow. (Abbreviations as in Fig. 3).

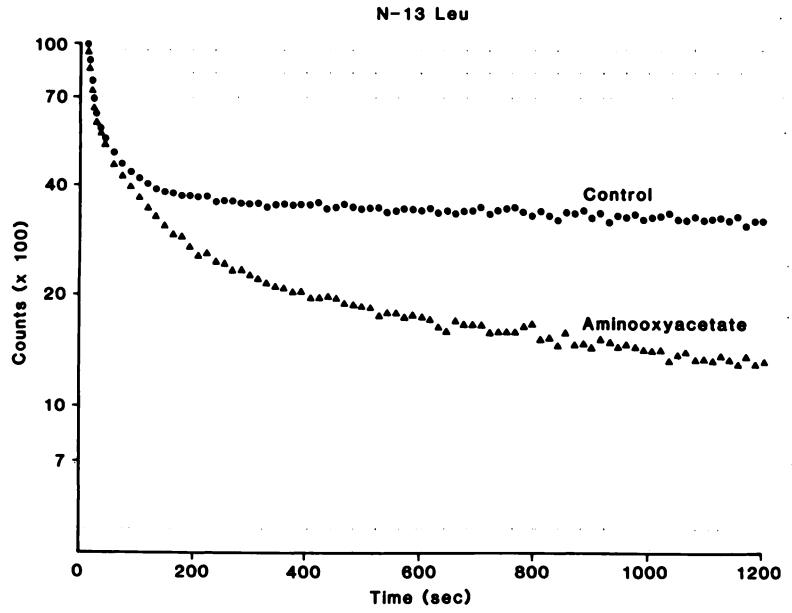

FIG. 5. Effect of transaminase inhibition with amino-oxyacetate on time-activity curves for N-13 activity, recorded after intracoronary injection of N-13 leucine.

During the late phase, and regardless of the type of flow intervention, only very minor changes in $t_{1/2}$ were recorded for all compounds tested with the exception of N-13 Leu. The N-13 Leu had the slowest clearance during the late phase at control and demonstrated a faster clearance in response to both types of ischemia.

As shown in Fig. 4, the residue fraction consistently increased with low-flow ischemia for all compounds tested. This effect, however, was less pronounced during the recovery period and was negligible with N-13 α- and ω-Gln and the C-11-labeled compounds.

Effect of transaminase inhibition. Amino-oxyacetate reduced the metabolic trapping of the N-13-labeled amino group, as indicated by a more rapid clearance rate of the late phase. This is shown in Fig. 5 for a typical experiment with N-13 Leu. The results for all compounds tested are summarized in Fig. 6. Nitrogen-13 ω-Gln and C-11 Oxal were unaffected by transaminase inhibition, while the $t_{1/2}$ of the late phase decreased from 86.7 ± 19.5 min to 40.6 ± 16.9 min ($p < 0.1$) for N-13-labeled Ala, Glu, Asp, and Leu. The effect of transaminase inhibition, however, was most pronounced on C-11 Asp, as shown in Figs. 6 and 7. The typical triphasic appearance of the time-activity curve changed to biphasic, with a loss of the intermediate phase and a lack of C-11 CO_2 production.

PCT imaging. Figure 8 shows a set of typical PCT images obtained after pacing induced ischemia and injection of N-13-labeled ammonia, Glu, and Ala. Table 1 lists the ratios for flow in ischemic and normal myocardium, determined by the microsphere technique and N-13 ammonia (I/N), and the ratios for N-13 tissue activity after N-13 Glu and N-13 Ala administration. The ratios for flow determined by microspheres are in good agreement with those measured in vivo by N-13

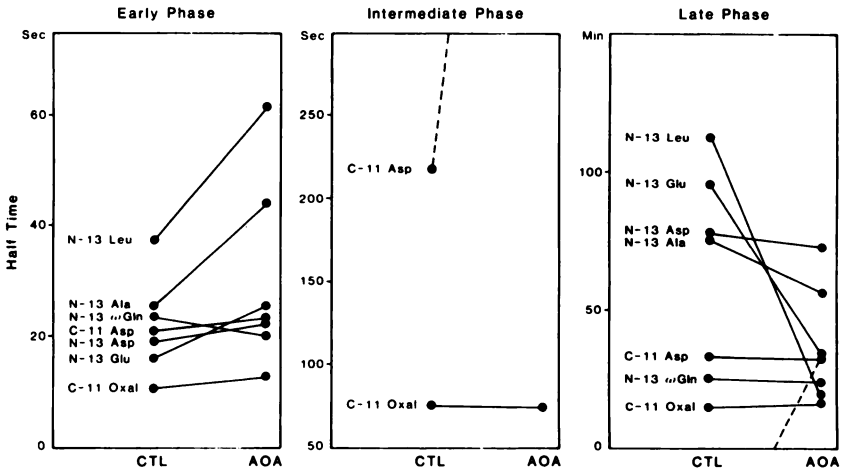

FIG. 6. Clearance half-times of N-13- and C-11-labeled amino acids at control (CTL), and after transaminase inhibition with amino-oxyacetate (AOA) during early, intermediate, and late phases. Dotted line of C-11 Asp during intermediate and late phases after transaminase inhibition demonstrates that this phase has completely disappeared or its half-time is now comparable to the late phase's half-time. This indicates that transamination is necessary for channeling the carbon skeleton (C-11 Oxal) into the Krebs cycle. Thus, C-11 Asp behaves like N-13 Asp after transaminase inhibition. (Abbreviations as in Fig. 3).

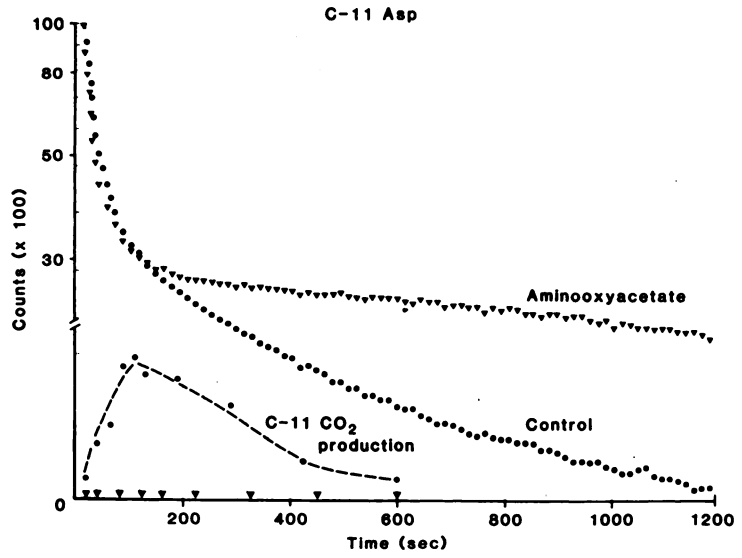

FIG. 7. Effect of transaminase inhibition with amino-oxyacetate on myocardial time-activity curve of C-11 aspartate. Typical triphasic appearance of time-activity curve (solid circles; control) has shifted towards a biphasic time-activity curve (triangles). This was paralleled by complete block (lower triangles) of C-11 CO_2 production that is usually present at 100–200 sec after intracoronary injection.

ammonia PCT imaging. However, the ischemic-to-normal ratios for myocardial N-13 activity in the N-13 Glu and N-13 Ala PCT images were consistently higher than the corresponding flow ratios. Hence, as shown in Table 1, the N-13 tissue activity in the ischemic segment on the N-13 Glu and the N-13 Ala PCT images was two to three times the N-13 activity on the perfusion images, indicating that the extraction of both labeled amino acids into the ischemic segment, as represented by the portion in the late phase, was considerably in excess of flow—in other words, disproportionately greater than the flow reduction.

DISCUSSION

This study was designed to examine the utility of labeled amino acids for the evaluation of myocardial metabolism by PCT. This new imaging technique not only permits display of tomographic, cross-sectional images but also allows noninvasive quantitation of tissue tracer concentrations and, thus, of metabolic turnover rates (*29,30*). Design of tracer kinetic models for metabolic processes requires an understanding of the relationships of the tracer kinetics in specific metabolic pathways that can be examined under controlled experimental conditions, such as with single-pass uptake techniques and selected metabolic interventions.

Nitrogen-13- and C-11-labeled amino acids could be of potential value for imaging myocardial metabolism by PCT for two reasons: First, L-amino acids can be labeled relatively easily with N-13 or C-11 without altering their physiologic properties (*14–19*). The possibility of labeling either the amino group or the carbon skeleton would have great impact on modeling the tracer kinetics and the specific pathway evaluated. Second, amino acids are important intermediates for myocardial protein synthesis and energy metabolism in normal and diseased myocardium (*1–13*).

Tracer kinetic modeling. The results of this study can be best interpreted by means of the compartmental model shown in Fig. 9. Each of the labeled compounds tested exhibited a prominent vascular component representing the unextracted fraction of activity that remains in the vascular space. This fraction can be estimated from the sum(s) of the sizes of all other components of the time-activity curve (intercepts of y_1, y_2, and y_3 for C-11-labeled compounds, and of y_1 and y_3 for N-13 amino acids, respectively) and total activity injected (10,000 cps). This fraction ranged from 40–60% for all compounds tested. Thus, in canine myocardium, only about 50% of the labeled compound leaves the vascular space and enters the interstitial space.

Because of the relative uncertainty of curve-fitting—due to a substantial vascular phase and contamination of the primary curve by recirculating activity—we did not determine quantitatively the differences in initial extraction between control state and that after metabolic interventions. Simple visual interpretation of the time-activity curves, however, indicated that this vascular component and the tracer recirculation—and,

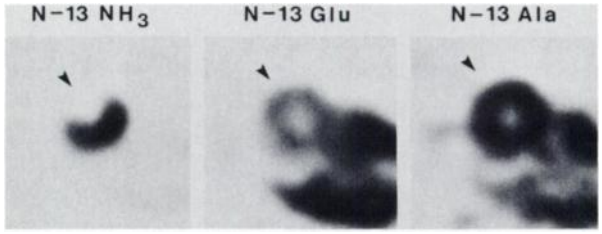

FIG. 8. Positron computed tomographic images obtained after i.v. injection of N-13 ammonia, N-13 Glu, and N-13 Ala, injected during pacing-induced ischemia of anteroseptal myocardium (arrow). Ammonia image demonstrates decreased perfusion here, whereas retained N-13 activity in same segment is disproportionately higher after N-13 Glu and N-13 Ala administration. (Abbreviations as in Fig. 3).

TABLE 1. PERFUSION, N-13 NH_3, N-13 Glu, AND N-13 Ala UPTAKE IN ISCHEMIC AND NORMAL MYOCARDIUM DURING PACING-INDUCED ISCHEMIA

Dog no.	Myocardial Perfusion Miccrospheres (ml/min-100g)				N-13 ammonia	Uptake of N-13 Amino Acid
	I	N	I/N	I/N*	I/N†	R/F
						N-13 Glu
1	51 ± 11	128 ± 33	0.4	0.33	0.72	2.18
2	67 ± 17	130 ± 40	0.52	0.52	1.06	2.04
3	47 ± 16	125 ± 28	0.38	0.33	0.73	2.23
4	30 ± 8	112 ± 40	0.27	0.17	0.48	2.83
5	47 ± 12	132 ± 28	0.36	0.34	0.97	2.87
						N-13 Ala
4				0.41	1.11	2.71
5				0.35	1.15	3.29
Mean ± 1s.d.	48 ± 13	125 ± 34	0.38 ± 34	0.35 ± 0.1	0.89 ± 0.25	2.59 ± 0.45

* I = ischemic; N = normal; I/N = ratio of tracer tissue concentrations in ischemic to normal myocardium; R/F represents the ratio of I/N^+ over I/N,* and indicates retention of N-13 activity after intravenous injection of N-13 Glu or Ala compared with flow in ischemic segment.

thus, the initial extraction—were very similar in all experimental runs, regardless of the type of label or the study protocol.

Once the N-13- or C-11-labeled amino acid has entered the interstitial and/or cytosolic compartment (Compartment 1 in Fig. 9) it may diffuse back into the plasma, or the labeled amino group may be subject to rapid transamination. The newly formed amino acid may also diffuse back into the plasma. That the latter indeed occurs could be demonstrated by qualitative HPLC in a limited number of experiments. Venous blood draining from an ischemic segment 20 sec after intracoronary bolus injection of N-13 Glu contained mainly N-13 Glu but also small amounts of N-13 Ala (E. Henze, et al., unpublished data). The half-time of the early phase was similar for all compounds tested; it averaged 18.7 ± 8.0 sec, which is comparable to that for freely diffusible tracers such as O-15 H_2O or Xe-133 (*25*). The prolonged half-time during low-flow ischemia shown in Fig. 3 provided further evidence for the assumption that the compounds tested exhibit a component of clearance that resembles diffusible tracers during the early phase, because flow and washout are inversely related for diffusible tracers according to Kety's formula (*32*).

From Compartment 1 the labeled amino acids can enter a second pool (Compartment 3 in Fig. 9) consistent with metabolic trapping, such as incorporation into proteins, storage in an amino acid pool, or involvement in intermediate amino acid cycles such as the malate-aspartate shuttle (*2*). This compartment is represented by the late slow phase ($t_{1/2}$ = 94.3 ± 37.5 min) on the time-activity curves for N-13 labeled Ala, Glu, Asp, and Leu. However, certain metabolic steps—such as transfer of N-13-labeled amino groups by transamination—cannot affect the tracer kinetics for N-13 Gln or for C-11 Asp and C-11 Oxal during the late phase, because the removal of the ω-amino group of Gln is not subject to transamination but is due to glutamine synthetase activity. The activity of this enzyme is very low in respect to deamination. This limits the incorporation of these tracers into the metabolic pool represented by Compartment 3, and can thus account for the faster clearance of these latter compounds during the late phase. The $t_{1/2}$ of the late phase for N-13 Glu, C-11 Asp, and C-11 Oxal (32.5 ± 6.2 min) is therefore comparable to that of the late phase for N-13 amino acids after transaminase inhibition ($t_{1/2}$ 40.6 ± 16.9 min). Thus, a substantial fraction of Compartment 3 (unmasked by transaminase inhibition) seems to represent a slow metabolic pool related to N-13-labeled amino acids associated with protein synthesis and protein degradation.

Carbon-11 Asp in general exhibited a kinetic pattern like that of N-13-labeled amino acids during the early phase. The nearly identical tracer kinetics of C-11 Asp and C-11 Oxal, and the appearance of C-11 CO_2 occurring 100–200 sec after administration of C-11 Asp, suggest that initial transamination occurs rather rapidly. The carbon skeleton subsequently enters the citric acid cycle, after which the C-11 label is released into the tissue CO_2 pool as an additional compartment (Compartment 2 in Fig. 9). This compartment is represented by the intermediate phase of the time-activity curves of C-11 Asp and C-11 Oxal. This phase therefore reflects the metabolic breakdown of Oxal in the citric acid cycle, and its half-time of 2–3 min is comparable with the 3–8 min reported for the CO_2 clearance from β-oxidation of C-11 palmitic acid or C-11 acetic acid (*33,34*), and may indicate citric acid cycle activity. Therefore, the tracer

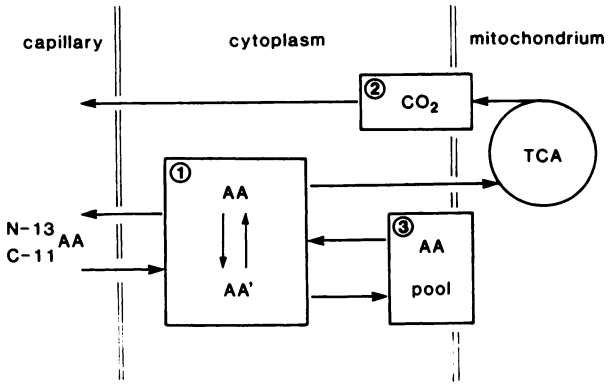

FIG. 9. Proposed three-compartment model for uptake, retention, and clearance of N-13- and C-11-labeled amino acids (AA). Discussion in text. (TCA = tricarboxylic acid cycle, AA′ = amino acid newly formed after transamination).

kinetics of C-11 Asp and C-11 Oxal appear to adequately be described by a three-compartment model.

The validity of this model for the noninvasive measurement of amino acid metabolism in man needs further investigation. The tracer will be administered intravenously rather than as an intracoronary bolus. This will affect in particular the initial uptake and washout kinetics. The knowledge of the arterial-blood time-activity curve (input function) and the myocardial tissue kinetics will be essential for a deconvolution approach. Both the input function and myocardial tracer concentrations, however, can be obtained, quantitatively and nontraumatically, by serial PCT imaging of the heart itself, as recently shown in our laboratory (*31*).

Effect of transaminase inhibition. When transamination was inhibited, the intracellular distribution of C-11 Asp appeared to be limited to two compartments, resulting in a biphasic time-activity curve nearly identical to that of N-13 Asp after transaminase inhibition. Moreover, C-11 CO_2 production was abolished, whereas the kinetics of C-11 Oxal were not effected by amino-oxyacetate (see Fig. 6). For N-13-labeled amino acids, transamination is apparently necessary for long-term retention of the amino nitrogen, as discussed above. The clearance of the late phase for all N-13 L-amino acids (Fig. 6) was therefore accelerated after amino-oxyacetate infusion—with the exception of N-13 Gln, as mentioned earlier.

Effect of ischemia. During ischemia, more N-13 nitrogen became trapped in the myocytes, as shown by the higher residue fraction. In low-flow ischemia this could be explained by the diminished washout of tracer substrate (from Compartment 1) during the early phase. More labeled nitrogen will then be available for metabolic incorporation, due to the reduced competition of back diffusion to plasma. However, the residue fraction was also consistently elevated for N-13-labeled Ala, Glu, Asp, and Leu during the period of slow recovery after ischemia, indicating that an additional mechanism exists in metabolically ischemic myocardium to account for increased retention of the amino group. Further work is needed to clarify this observation. An increased need for amino acid carbon skeletons for intermediary anaerobic pathways—the citric acid cycle or the aspartate-succinate pathway (*5*)—and a decreased availability of pyruvate and α-ketoglutarate as carriers could cause an accumulation of the labeled amino nitrogen. One possibility is that the enzyme glutamate dehydrogenase deaminates N-13 Glu, and the increased N-13 residue activity observed during ischemia reflects labeled ammonia originating from the labeled amino acids.

No conclusive changes were observed with the C-11-labeled compounds during ischemia. Either the activity of the citric acid cycle was still normal in our ischemia experiments, the metabolic derangements did not affect the C-11 tracer kinetics, or Oxal was channeled into the postulated aspartate-succinate shuttle, with release of activity in the form of C-11 succinate (*5,6*).

The increased residue of N-13 activity on the PCT images subsequent to injection of N-13-labeled Ala and Glu, despite markedly reduced flow during pacing-induced ischemia, confirmed our observations from the intracoronary injection studies. Nitrogen-13 activity from these labeled amino acids was disproportionately high relative to flow in the ischemic segment on the images obtained 5–10 min after i.v. injection, or consistent with the increased residue fraction in our first-pass studies. The image quality for N-13 Glu and Ala was rather poor compared with that of N-13 NH_3 (heart-to-lung ratio of only 1.63 for N-13 Glu and 2.83 for Ala, but 74.9 for N-13 NH_3). This is not unexpected because the residue fraction for N-13 Glu was 7.3% and for N-13 Ala 14.4% (Fig. 4) as compared with 80% for N-13 NH_3 (*23,24*). The results of the PCT studies suggest the possibility of imaging ischemic myocardium by a double-tracer technique using N-13 NH_3 or Rb-82 (*35*) as flow tracers and N-13-labeled amino acid for PCT imaging of ischemic myocardium.

Our results do not appear do be entirely consistent with the reported or postulated interactions of amino acids with metabolism in myocardial ischemia (*4,6,7*). Although quantification of the initial tracer extraction was limited, as discussed earlier, we could exclude a markedly higher extraction of N-13 Glu and a decreased uptake or accelerated release of N-13 Ala during ischemia, as one would expect from the observations of Taegtmeyer et al. (*4*), Sanborn et al. (*6*), and Mudge et al. (*7*). Two reasons could explain this discrepancy. First, we cannot follow the pathway of the specific labeled amino acids, but can trace the fate only of the labeled amino group. As seen by the early onset of C-11 CO_2 release at 100–200 sec after C-11 Asp administration, initial transamination of exogenous amino acids seems to occur almost instantaneously in dog myocardium. This

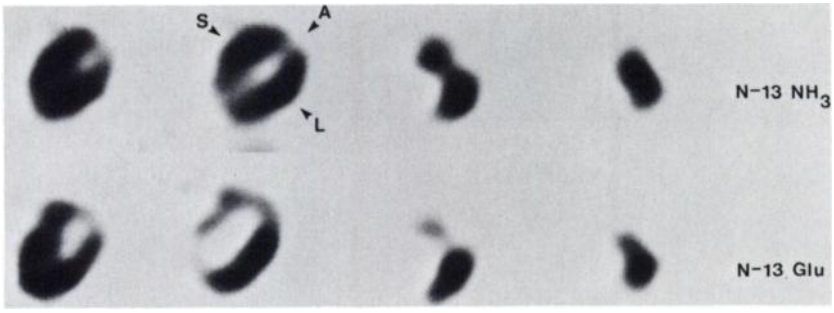

FIG. 10. Positron computed tomograms obtained from patient under treatment of fibrosarcoma supplied by LAD, after injection of N-13 ammonia and N-13 glutamate (N-13 Glu) subsequent to selective infusion of adriamycine into left anterior descending coronary artery. N-13 NH_3 images (upper panel) demonstrate normal blood flow to interventricular septum and lateral wall, whereas there is markedly decreased uptake and/or retention of activity in interventricular septum after i.v. injection of N-13 Glu.

may explain the relative uniformity of our time-activity curves regardless of which amino acid was labeled. Second, there is obviously a marked species-related difference in amino acid metabolism between dog, monkey, and man with respect to uptake and retention of N-13- and/or C-11-labeled amino acids in myocardium. Gelbard et al. (*36*) found high residue activity in myocardium in man after administration of N-13 Glu, compared with canine myocardium. In our laboratory, the residue of N-13 Ala, N-13 Glu, and C-11 Asp on PCT images was found comparable with that of N-13 NH_3 in monkey myocardium but was far less in dogs (*15,19*).

In addition, preliminary patient studies in our laboratory, as shown by an example in Fig. 10, revealed high myocardial uptake of N-13 Glu, with high-contrast tomographic images comparable to those obtained with N-13 ammonia. The heart-to-lung ratio in this study averaged 8.5 ± 1.8 for N-13 ammonia and 5.4 ± 1.1 for N-13 Glu in the normal posterolateral wall. This implies that the residue fraction of N-13 Glu is about 60–70% in man, and thus is much higher than in dogs. Tracer kinetic studies using a first-pass uptake technique are needed to evaluate whether this increased residue of activity is due to a higher initial extraction or less washout during the early phase. The study shown in Fig. 10 further suggests that N-13 Glu may be of potential value for PCT imaging in derangements of human myocardial protein synthesis. This patient received a selective infusion of adriamycine into the LAD for treatment of a fibrosarcoma attached to the left-ventricular anterior wall and supplied through the LAD. While flow in the septum was normal, as shown by the N-13 ammonia PCT images, N-13 Glu uptake and/or incorporation was depressed, presumably because of a regional decrease in protein synthesis after adriamycine administration (*11*).

We conclude from our studies that labeled amino acids are amenable to tracer kinetic modeling. C-11 Asp or C-11 Oxal may be of value for measuring Krebs-cycle activity, while N-13 labeled amino acids may be useful for imaging ischemic but viable myocardium because of the increased residue fraction during ischemia. Further direct histo-radiochemical assays are needed to validate the model, in particular under ischemic conditions, i.e., to evaluate the significance of Glu and Asp as energy substrates via the aspartate-succinate pathway. Which specific N-13-labeled amino acid is to be used seems not to be an important issue because of the very fast initial transamination. Our data also suggest that measurements of protein turnover seem possible because the clearance of the late phase of the C-11-labeled compounds (or of N-13-labeled Ala, Glu, Asp, and Leu after transaminase inhibition) could be related to amino acids that have been incorporated into protein. Recent results obtained in our laboratory with C-11 Leu, N-13 Val, and N-13 Ile (*37*) were suggestive of the kinetics of the N-13- and C-11-labeled tracers reported in this paper. Thus, whether the C-11 or N-13 label, or some other amino acid, is preferable for the latter purpose needs further evaluation.

FOOTNOTES

* The common three-letter abbreviations for the L-amino acids are used throughout this article: Ala = alanine; Glu = glutamate; Gln = glutamine; Asp = aspartate; Leu = leucine; Val = valine; Ile = isoleucine.

† Statham P23Db.

‡ Gould-Brush 200.

§ Infusion set No. 5310, Deseret.

ACKNOWLEDGMENT

This work was supported by Contract DE-AM06-76-SF000012 between the U.S. Dept. of Energy, Washington, DC and the Univ. of California at Los Angeles.

REFERENCES

1. TAEGTMEYER H: Metabolic responses to cardiac hypoxia. Increased production of succinate by rabbit papillary muscles. *Circ Res* 43:808-815, 1978
2. SAFER B: The metabolic significance of the malate aspartate cycle in heart. *Circ Res* 37:527-533, 1975
3. WATANABE T: Significance of ammonia in myocardial metabolism. *Jpn Circ J* (Suppl) 32:1811, 1968 (abst)
4. TAEGTMEYER H, PETERSON MB, RAGAVAN VV, et al: De

novo alanine synthesis in isolated oxygen-deprived rabbit myocardium. *J Biol Chem* 252:5010-5018, 1977

5. HOCHACHKA P, OWEN TG, ALLEN JF, et al: Multiple end products of anaerobiosis in diving vertebrates. *Comp Biochem Physiol* 50B:17-22, 1975
6. SANBORN T, GAVIN W, BERKOWITZ S, et al: Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. *Am J Physiol* 237(5):H535-541, 1979
7. MUDGE GH, JR, MILLS RM, JR, TAEGTMEYER H, et al: Alterations in myocardial amino acid metabolism in chronic ischemic heart disease. *J Clin Invest* 58:1186-1192, 1976
8. RAU EE, SHINE KI, GERVAIS A, et al: Enhanced mechanical recovery of anoxic and ischemic myocardium by amino acid perfusion. *Am J Physiol* 236:H873-879, 1979
9. ROBERTSON JM, VINTEN-JOHANSEN J, BUCKBERG GD, et al: Prolonged safe aortic clamping (4 hours) with cold glutamate enriched blood cardioplegia. *Circulation* 64: Suppl IV-147 1981 (abst)
10. SCHREIBER SS, ORATZ M, ROTHSCHILD MA: Post ischemic reperfusion and anoxic perfusion in the isolated heart: alteration in distribution of radionuclides and in protein synthesis. *J Physiol* (Paris) 76:777-784, 1980
11. GOODWYN JF: Daunorubicin cardiomyopathy. In *The Heart*. J. W. Hurst Ed. New York, 1978, McGraw-Hill, pp 1573-1574
12. MEERSON FZ, ALEKLUA GM, ALEXANDROV PN, et al: Dynamics of nucleic acid and protein synthesis of the myocardium in compensatory hyperfunction and hypertrophy of the heart. *Am J Cardiol* 22:337-347, 1968
13. GUDBJARHANSON S, TELERMAN M, BING RJ, et al: Protein metabolism in cardiac hypertrophy and heart failure. *Am J Physiol* 206:294-298, 1964
14. GELBARD AS, CLARKE LP, LAUGHLIN JS: Enzymatic synthesis and use of ^{13}N-labeled L-Asparagine for myocardial imaging. *J Nucl Med* 15:1223-1225, 1974
15. BARRIO JR, EGBERT JE, HENZE E, et al: L-[4-^{11}C] Aspartic acid: Enzymatic synthesis, myocardial uptake and metabolism. *J Med Chem* 25:93-96, 1982
16. BAUMGARTNER F, BARRIO JR, HENZE E, et al: ^{13}N-labeled L-amino acids for in vivo assessment of local myocardial metabolism. *J Med Chem* 24:764-766, 1981
17. COOPER AJL, GELBARD AS: The use of immobilized glutamate dehydrogenase to synthetize ^{13}N-labeled L-amino acids. *Anal Biochem* 111:42-48, 1981
18. BUDINGER TF: Amino acid uptake by the myocardium. In *Nuclear Cardiology*, J. T. Willerson, Ed. Philadelphia, F.A. Davis Company, 1979, pp 59-60
19. HENZE E, BAUMGARTNER F, SCHELBERT HR, et al: Myocardial uptake and utility of N-13 labeled glutamate, glutamine and alanine for positron emission tomography of normal and ischemic myocardium. *Clin Nucl Med* 5:S17-18, 1980 (abst)
20. BARRIO JR, ROBINSON GD, NAJAFI A: Remote systems for chemistry using large amounts of cyclotron produced radionuclides. *J Nucl Med* 21:P91, 1980 (abst)
21. HEYMANN MA, PAYNE BD, HOFFMAN JIE, et al: Blood flow measurements with radionuclide-labeled particles. *Prog Cardiovasc Dis* 20:55-79, 1977
22. MOCHIZUKI S, NEELY JR: Energy metabolism during reperfusion following ischemia. *J Physiol* (Paris) 76:805-812, 1980
23. SCHELBERT HR, PHELPS ME, HOFFMAN EJ, et al: Regional myocardial perfusion assessed with N-13 labeled ammonia and positron emission computerized axial tomography. *Am J Cardiol* 43:209-218, 1979
24. SCHELBERT HR, PHELPS ME, HUANG SC, et al: N-13 ammonia as an indicator of myocardial blood flow. *Circulation* 63:1259-1272, 1981
25. METZGER JM: Myocardial blood flow and oxygen consumption using $H_2{}^{15}O$ and ^{15}O-hemoglobin. PhD Thesis, Washington University, St. Louis, MO, 1972
26. PHELPS ME, HOFFMAN EJ, RAYBAUD C, et al: Factors which affect cerebral uptake and retention of $^{13}NH_3$. *Stroke* 8:694-702, 1977
27. RAICHLE ME, EICHLING JO, STRAATMANN MG, et al: Blood brain barrier permeability of ^{11}C labeled alcohols and ^{15}O labeled water. *Am J Physiol* 230:543-552, 1976
28. PHELPS ME, HOFFMAN EJ, HUANG SC, et al: ECAT: a new computerized tomographic imaging system for positron-emitting radiopharmaceuticals. *J Nucl Med* 19:635-647, 1978
29. SCHELBERT HR, HENZE E, PHELPS ME: Positron emission computed tomography of the heart. *Semin Nucl Med* 10: 355-373, 1980
30. PHELPS ME: Positron computed tomography studies of cerebral glucose metabolism in man. Theory and application in nuclear medicine. *Semin Nucl Ned* 11:32-49, 1981
31. HENZE E, HUANG SC, PLUMMER D, et al: Retrieval of quantitative information from positron emission computed tomographic images for cardiac studies with C-11 palmitate. *J Nucl Med* 22:P21, 1981 (abst)
32. KETY SS, SCHMIDT CF: The determination of cerebral blood flow in man by use of nitrous oxide in low concentrations. *Am J Physiol* 143:53-71, 1945
33. SELWYN AP, ALLAN RM, PIKE V, et al: Positive labeling of ischemic myocardium: a new approach in patients with coronary disease. *Am J Cardiol* 47:481, 1981 (abst)
34. SCHON HR, ROBINSON JR, SCHELBERT HR, et al: Kinetics of C-11 labeled palmitate in ischemic myocardium. *Am J Cardiol* 47:414, 1981 (abst)
35. BUDINGER TK, YANO Y, DERENZO SE, et al: Rb-82 myocardial positron emission tomography. *J Nucl Med* 20: 603, 1979 (abst)
36. GELBARD AS, BENUA RS, REIMAN RE, et al: Imaging of the human heart after administration of L-[N-13] glutamate. *J Nucl Med* 21:988-991, 1980
37. HENZE E, EGBERT JE, BARRIO JE, et al: Kinetics of N-13 labeled branched-chain L-amino acids in normal and ischemic myocardium. *J Nucl Med* 23:P 1791, 1981 (abst)