plements, 4%;) for a period of 1 week prior to drug or vehicle treatment. Rats were maintained on this diet for the remainder of the experiment (14 days). Groups (n = 6) of rats received various doses of **1a**, **2a**,b, and **3a** [0.2 or 0.4 (mmol/kg)/day in 0.25% methylcellulose] or 0.25% methylcellulose orally twice daily for 14 consecutive days. In all experiments, drugs were given in a total volume of 1.0 mL/100 g of body weight. All rats were fasted 16–18 h before blood collection. Blood was drawn (orbital plexus) from rats under light ether anesthesia one day before (day-1) and 4, 7, and 10 days (day +4, +7, +10, respectively) after drug treatment. After 14 days (day +14) of drug treatment, blood was collected by exsanguination from the abdominal aorta of rats, and livers were excised, rinsed in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 (containing 1.15%, w/v, KCl), blotted, weighed, minced, and homogenized in the same buffer using a glass homogenizer equipped with a Teflon pestle.

Liver Assays. CH and TG's were extracted from the liver homogenates (25%, w/v) by the method of Abell et al.[37] and analyzed by the method of Parekh and Jung[38] and Soloni,[39] respectively.

Serum Assays. All blood samples were placed on ice, and after clotting, serum was separated by centrifugation at 2000g for 10 min. The serum samples were kept at 4 °C and analyzed within 48 h. Serum total CH was measured by the enzymatic method of Allain et al.[40] with A-Gent cholesterol reagent (Abbott Laboratories, Diagnostics Division, Chicago, IL) on an Abbott Bichromatic Analyzer (ABA-100) equipped with a 1:51 syringe-plate. Serum TG concentrations were measured with A-Gent triglyceride reagent (Abbott Laboratories Diagnostics Division, Chicago, IL) on an ABA-100 according to the method of Sampson et al.[41] α-LP CH was estimated by the polyanionic precipitation method[42] using a modification described by Steele et al.[43] Heparin-$MnCl_2$ precipitable LP CH was determined by subtracting α-LP CH values from serum total CH values.

Statistical Evaluation. Data obtained was statistically evaluated by an analysis of variance with repeated measures using GLM of SAS.[44] Differences between groups were determined using the Duncan's multiple range procedure.[44]

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(39) F. G. Soloni, *Clin. Chem.*, 17, 529 (1971).
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(44) SAS Institute Inc. in "SAS Users Guide", 1979 ed., Raleigh, NC, 1979.

L-[4-^{11}C]Aspartic Acid: Enzymatic Synthesis, Myocardial Uptake, and Metabolism

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Sterile, pyrogen-free L-[4-^{11}C]aspartic acid was prepared from $^{11}CO_2$ using phosphoenolpyruvate carboxylase and glutamic/oxaloacetic acid transaminase immobilized on Sepharose supports to determine if it is a useful indicator for in vivo, noninvasive determination of myocardial metabolism. An intracoronary bolus injection of L-[4-^{11}C]aspartic acid into dog myocardium showed a triexponential clearance curve with maximal production of $^{11}CO_2$ 100 s after injection. Inactivation of myocardial transaminase activity modified the tracer clearance and inhibited the production of $^{11}CO_2$. Positron-computed tomography imaging showed that the ^{11}C activities retained in rhesus monkey myocardium are higher than those observed in dog heart after intravenous injection of L-[4-^{11}C]aspartic acid. These findings demonstrated the rapid incorporation of the carbon skeleton of L-aspartic acid into the tricarboxylic acid cycle after enzymatic transamination in myocardium and suggested that L-[4-^{11}C]aspartic acid could be of value for in vivo, noninvasive assessment of local myocardial metabolism.

Amino acids play a central role in myocardial energy metabolism, specifically in the coordination of mitochondrial and cytosolic biochemical processes. This knowledge has been derived from in vitro studies by using, for example, ^{14}C- or ^{15}N-labeled compounds. These nuclides, however, are not adequate in the application of the principles of tracer kinetics to animals and man for the assessment of local biochemical and physiological processes. Positron-emitting radiopharmaceuticals, e.g., labeled with ^{13}N or ^{11}C, are ideally suited for this purpose. ^{11}C and ^{13}N are short-lived radionuclides (20.38 and 9.96 min half-lives, respectively) which decay by positron emission, and their in vivo usage is based on the visualization of the two 511-KeV γ annihilation photons emitted per decay event.[2] In a recent paper we discussed the enzymatic synthesis of ^{13}N-labeled L-amino acids and their utilization for in vivo assessment of local myocardial metabolism.[3] Evidence obtained from a variety of studies[4] suggests that regulation of oxidative metabolism of carbohydrates requires the malate–aspartate cycle, by which carbon skeletons from amino acids can be shuttled into the tricarboxylic acid cycle. Therefore, it seems reasonable to expect that the types of labels in the amino acid, e.g., ^{13}N or ^{11}C, might produce different in vivo tissue distribution and kinetic patterns of positron activity. Prompted by the above considerations, we designed the present study to determine if ^{11}C-labeled L-aspartic acid is a useful indicator for in vivo, noninvasive determination of myocardial metabolism.

Chemistry. We describe herein the covalent immobilization of phosphoenolpyruvate carboxylase[5] and glutamic/oxaloacetic acid transaminase[6] to Sepharose by the cyanogen bromide activation method. This work represents the first example of the use of this technique for the

(1) Address correspondence to School of Medicine, Laboratory of Nuclear Medicine, Division of Biophysics, University of California at Los Angeles, Los Angeles, CA 90024.
(2) See, for example, Hoffman, E. J.; Phelps, M. E. *Med. Instrum.* **1979**, *13*, 147.
(3) Baumgartner, F. J.; Barrio, J. R.; Henze, E.; Schelbert, H. R.; MacDonald, N. S.; Phelps, M. E.; Kuhl, D. E. *J. Med. Chem.* **1981**, *24*, 764.
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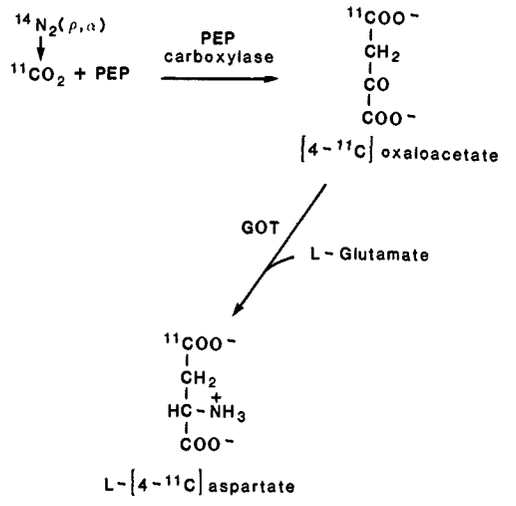
Scheme I



enzymatic synthesis of ^{11}C-labeled amino acids.[7] L-[4-^{14}C]Aspartic acid has been synthesized using a crude extract from chicken liver acetone powder, and the possibility of labeling with ^{11}C has been suggested.[8] Numerous applications of immobilized enzymes have been experimentally realized, and the various advantages of immobilized enzyme reactions compared with the corresponding enzymatic reactions in solution have been reported.[9] In this particular case, the immobilization procedure makes the radiopharmaceutical preparation suitable for animal and patient studies, since potential pyrogenic or antigenic proteins are not present in the final preparation. When $^{11}CO_2$, produced using the $^{14}N(p,\alpha)^{11}C$ reaction on N_2, in combination with the appropriate substrates, was passed through a phosphoenolpyruvate carboxylase column, [4-^{11}C]oxaloacetate was obtained in good yields (Scheme I). In several instances, small amounts of $^{11}CO_2$ were detected in the final product. Because oxaloacetic acid appears to be unstable under a variety of different conditions (e.g., pH,[10] divalent metal ions,[10] ionic resins[11]), producing pyruvic acid and CO_2, partial decomposition of the radiolabeled product could not be discounted. In any event, rapid purification of radiolabeled oxaloacetate produced a radiopharmaceutical suitable for immediate use in biological studies (radiochemical purity >95%). If L-[4-^{11}C]aspartic acid was desired, immediate passage of the solution containing [4-^{11}C]oxaloacetic acid through a second column that contained immobilized glutamic/oxaloacetic acid transaminase converted the ^{11}C-labeled oxaloacetic acid into L-[4-^{11}C]aspartic acid.[12] To indicate further the extent of decarboxylation of oxaloacetate, a solution of [4-^{11}C]oxaloacetate was left at room temperature for 40 min and then passed through the glutamic/oxaloacetic acid transaminase column. The radiochemical transformation into L-[4-^{11}C]aspartic acid was reduced by an average of 5% in comparison to the solution immediately transaminated to L-[4-^{11}C]aspartic acid. In all cases, L-[4-^{11}C]aspartic acid was purified using an AG 50W-X12 exchange column and obtained with >99% radiochemical purity.

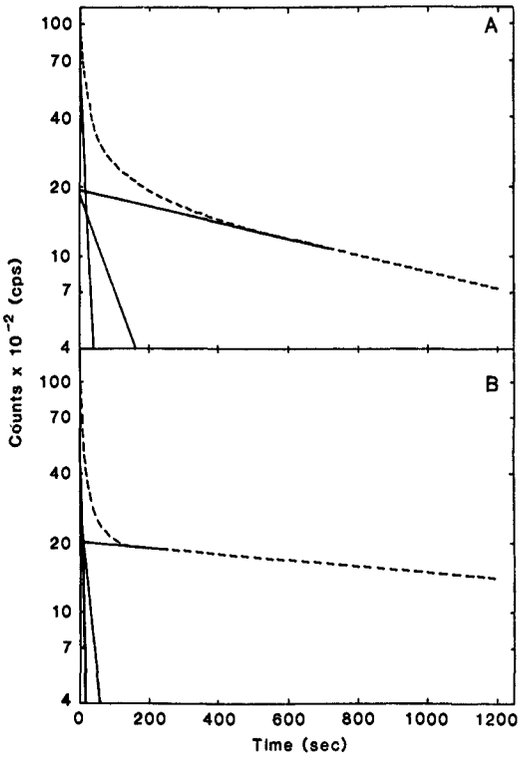


Figure 1. Typical time course of activity in heart subsequent to a left anterior descending coronary artery bolus injection of 0.2 mL of L-[4-^{11}C]aspartic in normal (A) and aminooxyacetic acid treated (B) dogs. The computer-calculated slopes corresponding to the components of the clearance curves are represented by the solid lines.

Myocardial Uptake and Metabolism. Typical clearance curves from the intracoronary injection of a 0.2-mL bolus of L-[4-^{11}C]aspartic acid into dog myocardium are shown in Figure 1. Clearance curves in normal myocardium (Figure 1A) were obtained from four different dogs and consisted of three components with mean half-times of 10.2 ± 4.3 s, 95.6 ± 25.0 s, and 21.80 ± 10.39 min, respectively. The mean residue fraction was 0.13 ± 0.06. The rapid decline in activity subsequent to the bolus injection of L-[4-^{11}C]aspartic acid reflected the clearance of ^{11}C label from the vascular and extravascular space. This

(7) Several ^{11}C-labeled DL-amino acids have been synthesized and mainly used as tumor-localizing agents and for organ imaging; see, for example, Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; Butler, T. A.; Callahan, A. P. In "Radiopharmaceuticals 2", Proceedings of the International Symposium on Radiopharmaceuticals, 2nd, Seattle, WA, Mar 19–22, 1979; Society of Nuclear Medicine: New York, 1979; p 767. D-Amino acid oxidase immobilized on CNBr-activated Sepharose has been used for the resolution of racemic mixtures of ^{11}C-labeled DL-amino acids (Casey, D. L.; Digenis, G. A.; Wesner, D. A.; Washburn, L. C.; Chaney, J. E.; Hayes, R. L.; Callahan, A. P. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 325. Barrio, J. R.; Padgett, H. C.; Ku, H.; Najafi, A. "Abstracts of Papers", 182nd National Meeting of the American Chemical Society, New York, Aug 23–28, 1981; American Chemical Society: Washington, DC, 1981; Abstr NUCL 39). The enzymatic synthesis of a ^{11}C-labeled L-amino acid, L-glutamate, has been recently reported (Cohen, M. B.; Spolter, L.; Chang, C. C.; MacDonald, N. S. *Clin. Nucl. Med.* **1980**, S15, abstract). Unfortunately, the enzymes were not immobilized onto solid supports. Previously, the same authors reported by abstract (*J. Nucl. Med.* **1978**, *19*, 701) that "pyruvate may be transaminated to C-11 L-alanine by glutamic pyruvic transaminase". No experimental details or yields were given.

(8) Hara, T.; Taylor, C.; Lembares, N.; Lathrop, K. A.; Harper, P. V. *J. Nucl. Med.* **1971**, *12*, 361, abstract.

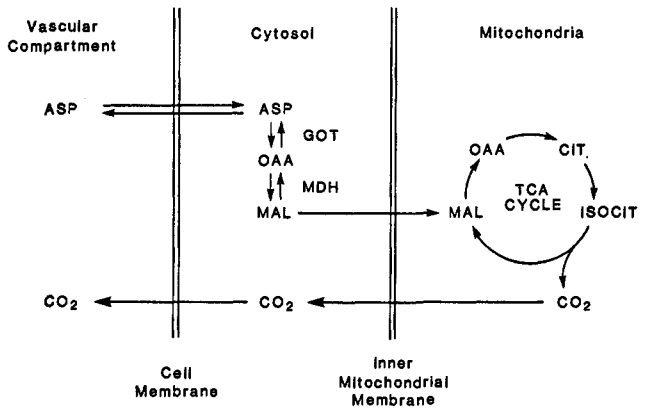
(9) Weetall, H. H.; Suzuki, S. "Immobilized Enzyme Technology"; 1975, Plenum Press, New York and London.

(10) Ito, H.; Kobayashi, H.; Nomiya, K. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 113.

(11) Manley, E. R.; Webster, T. A.; Spivey, H. O. *Arch. Biochem. Biophys.* **1980**, *205*, 380.

(12) ^{11}C-Labeled oxaloacetic acid was also quantitatively converted into [^{11}C]malate in the presence of immobilized malate dehydrogenase.

Scheme II



was confirmed by analyses of the radioactivity arterial–venous difference, which also revealed that $^{11}CO_2$ production reached its peak during the second phase at approximately 100 s after injection and paralleled the release of activity from the heart during the third phase. The elimination of $^{11}CO_2$ production upon myocardial transaminase inhibition with aminooxyacetic acid,[13] as well as the modification of the tracer clearance curve (Figure 1B), confirmed the involvement of transamination reactions. These results indicate that L-aspartate can function anaplerotically in the heart by quickly replenishing tricarboxylic acid (TCA) cycle intermediates. Rapid changes in TCA cycle intermediates in the heart require interaction of the TCA and malate–aspartate cycles,[4,14] which implies that ^{11}C-labeled oxaloacetic acid formed upon transamination of L-[4-^{11}C]aspartate in the cytosol would be converted into ^{11}C-labeled malate for transport into the mitochondria and incorporation into the TCA cycle (see Scheme II). Transport of ^{11}C-labeled oxaloacetic acid through the mitochondrial membrane is unlikely.[14] Intracoronary bolus administration of [4-^{11}C]oxaloacetic acid produced an identical clearance curve with that obtained with L-[4-^{11}C]aspartic acid. Its profile, however, was not modified by transaminase inhibition with aminooxyacetic acid. This is consistent with the idea that cytosolic transamination activity is not the rate-limiting step in the overall kinetic process, at least in normal myocardium.[15] Regulation of carbohydrate and fatty acid oxidation,[14] fatty acid synthesis,[14] and cellular energy metabolism is achieved in the heart by the activity of the TCA cycle. Regulatory mechanisms exerted by amino acids through the action of aspartate (and probably alanine) aminotransferase are important because carbon skeletons from amino acids can be shuttled into the TCA cycle as shown in this and previous work.[3] Alterations in amino acid metabolism characterize patients with chronic ischemic heart disease,[16] and amino acids have been shown to enhance the recovery of ischemic myocardium in vitro.[13] Incorporation of amino acid carbon skeletons into the TCA cycle may contribute to the preservation of ischemic myocardium by providing a source of nonglycolytic energy production in oxygen-deprived myocardium.[17] This study represents an attempt toward the interpretation of these biochemical events from in vivo studies.[18] L-[4-^{11}C]Aspartic acid used with appropriate imaging techniques, such as positron-computed tomography (PCT), can provide noninvasive assessment of myocardial metabolism. PCT images of dog myocardium after intravenous injection of L-[4-^{11}C]aspartic acid revealed low myocardial retention of ^{11}C activity with a heart/lung activity ratio of 1.2:1. Rhesus monkeys, however, exhibited very high myocardial residue fractions with a heart/lung activity ratio of 32.8:1. These data, obtained with a ^{11}C-labeled amino acid, are consistent with the previously observed species-specific myocardial retention of activity of ^{13}N-labeled amino acids.[19]

Experimental Section

Escherichia coli phosphoenolpyruvate carboxylase (EC 4.1.1.31) and porcine heart glutamic/oxaloacetic acid transaminase (EC 2.6.1.1) were obtained from Sigma Chemical Co. Substrates were either from Sigma Chemical Co. or Calbiochem–Behring Corp. Cyanogen bromide activated Sepharose was from Pharmacia; columns and ion-exchange resins were from Bio-Rad Laboratories. Reagent grade chemicals and distilled solvents were used in preparing all solutions.

Enzyme Immobilization. Phosphoenolpyruvate carboxylase (25 units) and glutamic/oxaloacetic acid transaminase (800 units) were immobilized on 500 mg of CNBr-activated Sepharose, using a procedure previously described.[3] The columns were stored at 4 °C in 2 M KCl/30 mM sodium phosphate, pH 7.5. The immobilized enzyme columns were stable and reusable for several weeks.

L-[4-^{11}C]Aspartic Acid. Enzymatic Synthesis. The mixture for the reaction, in a final volume of 5.0 mL, contained 30 mM sodium phosphate, pH 7.5; 25 mM phosphoenolpyruvic acid; 0.3 mM acetyl-CoA;[20] 20 mM $MgCl_2$; 6.0 mM L-glutamic acid; and $^{11}CO_2$ (200–250 mCi).[21] The substrates were passed through the phosphoenolpyruvate carboxylase, immobilized on 1 g of Sepharose support, and, subsequently, a glutamic/oxaloacetic acid transaminase column. These columns were washed with 6 and 3 mL of sodium phosphate, pH 7.5, respectively. The solution was transferred and forced completely through a 0.7 × 4.0 cm AG 50W-X12 cation-exchange resin (hydrogen form) equilibrated with deionized water. The eluate containing unreacted $^{11}CO_2$ and ^{11}C-labeled oxaloacetate was discarded. Since the pH of the cation column was below the pK_a value for the carboxyl group of L-aspartic acid, the positively charged L-[4-^{11}C]aspartic acid was retained on the cation column. The column was washed with 10 mL of deionized water and, finally, L-[4-^{11}C]aspartic acid was eluted from the column by the passage of 10 mL of 0.1 M sodium

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(14) Williamson, J. R.; Cooper, R. H. *FEBS Lett.* **1980**, *117*(Suppl), K73.
(15) The malic enzyme, which catalyzes the decarboxylation of malate and oxaloacetate, is present in the cytosol and to a greater extent in the mitochondria of heart tissue (Lin, R. C.; Davis, E. J. *J. Biol. Chem.* **1974**, *249*, 3867. Nolte, J.; Brdiczka, D.; Pette, D. *Biochim. Biophys. Acta* **1972**, *284*, 497). This enzyme has regulatory functions; namely, it acts as a biochemical shunt for TCA cycle intermediates (Lee, S. H.; Davis, E. J. *J. Biol. Chem.* **1979**, *254*, 420). Nevertheless, it should not be neglected that some of the $^{11}CO_2$ produced upon myocardial metabolism of L-[4-^{11}C]aspartic acid may be the result of decarboxylation reactions catalyzed by malic enzyme.
(16) Mudge, G. H., Jr.; Mills, R. M., Jr.; Taegtmeyer, H.; Gorlin, R.; Lesch, M. *J. Clin. Invest.* **1976**, *58*, 1185.
(17) Taegtmeyer, H. *Circ. Res.* **1978**, *43*, 808.
(18) Except for a preliminary study in which DL-[^{11}C]alanine "showed virtually no localization in the (human) heart or pancreas" (Harper, P. V.; Wu, J.; Lathrop, K. A.; Wickland, T.; Moossa, A. *J. Nucl. Med.* **1980**, *21*, P77, abstract), no attempts have been made to use ^{11}C-labeled amino acids for in vivo determination of their metabolic fate in myocardium.
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(21) Carbon-11 labeled CO_2 was prepared by the $^{14}N(p,\alpha)^{11}C$ reaction by our Biomedical Cyclotron (CS-22 Cyclotron Corp.). (Christman, D. R.; Finn, R. D.; Karlstrom, K. I.; Wolf, A. P. *Int. J. Appl. Radioisot.* **1975**, *26*, 435). The $^{11}CO_2$ was then trapped on a copper coil at −72 °C and subsequently swept with helium into a 3-mL solution of 20 mM NaOH. This solution was then brought to pH 7.5 by the addition of 90 mM sodium phosphate, pH 6.7, and, finally, the other substrates were added.

phosphate, pH 12. Due to the pH of the column after acid washing, concentration, and pH of the sodium phosphate, the fractions containing L-[4-^{11}C]aspartic acid were isotonic and at pH 7.4. The solution was sterilized by passage through a 0.22-μm Millipore filter and transferred into a sterile, pyrogen-free vial. If [4-^{11}C]oxaloacetic acid was desired, L-glutamic acid was omitted from the reaction mixture, and the second enzyme column, glutamic/oxaloacetic acid transaminase, was deleted from the system.[22] [4-^{11}C]Oxaloacetic acid and unreacted substrates were collected in a vial with 1 mL of 0.9 M $NaHCO_3$, and the solution was first acidified with 2 N HCl, stirred for 3 min to expel unreacted $^{11}CO_2$, and then neutralized (pH 7.4) by the addition of 2 N NaOH. The solution was made isotonic and finally passed through a 0.22-μm pore filter into a sterile, pyrogen-free vial. The production of [4-^{11}C]oxaloacetic acid and L-[4-^{11}C]aspartic acid was completed within 15–25 min after cyclotron production of $^{11}CO_2$. The actual product yields were 20–25 mCi of L-[4-^{11}C]-aspartic acid and 30–35 mCi of [4-^{11}C]oxaloacetic acid, with a specific activity of 3.5–5.0 Ci/mmol at the time of injection.

Verification of Radiochemical Properties. The radiochemical purity and specific activity of the L-[4-^{11}C]aspartic acid preparation were verified by using the *o*-phthaldialdehyde (OPT) precolumn fluorescence derivatization procedure as previously described for ^{13}N-labeled L-amino acids.[3] The strongly fluorescent amino acid–OPT product was separated with reversed-phase HPLC[23] (Beckman Model 334, Ultrasphere ODS, 5 μm, 4.6 × 150 mm column; 55% 100 mM potassium phosphate, pH 7.4, and 45% MeOH; flow rate 1.0 mL/min; Varian Fluorichrom fluorescence detector; Ortec Model 406A radioactivity detector; retention time for L-[4-^{11}C]aspartate–OPT complex, 2.5 min). The absence of $^{11}CO_2$ in the final ^{11}C-labeled oxaloacetic acid preparation was verified by direct analysis of radioactive components of the mixture by using reversed-phase HPLC, under similar chromatographic conditions as described above, except that 98% 10 mM potassium phosphate, pH 2.5/2% methanol was used as a solvent: retention time for [4-^{11}C]oxaloacetic acid, 2.5 min; for $^{11}CO_2$, 6.0 min. The specific activity of [4-^{11}C]oxaloacetate was calculated from that obtained for L-[4-^{11}C]aspartic acid.

Myocardial Uptake of ^{11}C-Labeled Substrates. For each experiment the ^{11}C-labeled compounds (20–30 μCi/0.2 mL) were injected into the left anterior descending coronary artery of open-chest instrumented dogs. The venous and arterial blood samples for ^{11}C metabolite determination were taken from the left anterior coronary vein and left atrial appendage, respectively. The myocardial activity was recorded for 20 min; the time–activity curve was corrected for physical decay and plotted on semilogarithmic paper, and the numerical values were printed out at 0.1-s intervals. The residue fraction of ^{11}C activity retained in myocardium was determined with a graphic extrapolation of the third slow clearance phase (*C*) back to the time of the maximal peak (*A*) representing the total amount of activity injected. The residue fraction was computed as the ratio of *C*/*A*. The half-times of the components of the clearance curve were calculated from the slopes (Figure 1). All experimental protocols for the single pass uptake technique have been reported in detail previously,[24] and the technique has been used with ^{13}N-labeled L-amino acids.[3] This method has also been previously employed with other radiolabeled compounds and validated for studies in the brain[25,26] and in the heart.[24,27,28] Inhibition of myocardial transaminases with aminooxyacetic acid was produced as previously described.[13] Tomographic images were obtained with the UCLA positron emission computed axial tomograph, ECAT,[29] as described previously,[30] following intravenous injections of 5 mCi of L-[4-^{11}C]aspartic acid in mongrel dogs or rhesus monkeys. Imaging was begun 5 min after injection.

Acknowledgment. This investigation was supported by the U.S. Department of Energy under Contract DE-AM03-76-SF00012. The authors express their thanks to D. S. Kuhl for his assistance in the initial studies on the synthesis of ^{11}C-labeled L-amino acids and Dr. N. S. MacDonald and his staff for preparing the ^{11}C-labeled CO_2.

(22) The enzymatic synthesis were performed using a remote, semiautomated system as described for the preparation of ^{13}N-labeled L-amino acids.[3]

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1-β-D-Arabinofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (*ara*-3-Deazaadenine)

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The arabino isomer of 3-deazaadenosine was prepared and evaluated for biological activity. It is a mediocre inhibitor of 5-adenosyl-L-homocysteine hydrolase. It was only slightly cytotoxic and slightly inhibitory to the growth of herpes simples type 1 virus in L929 cells.

S-Adenosyl-L-homocysteine (AdoHcy) is the product of the biological methylation reactions in which *S*-adenosylmethionine (AdoMet) serves as the methyl donor, and *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) catalyzes the hydrolysis of AdoHcy in eukaryotes to adenosine and L-homocysteine. AdoHcy is a potent inhibitor of biological methylations and the AdoMet/AdoHcy ratio is thought to be important in cellular control

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