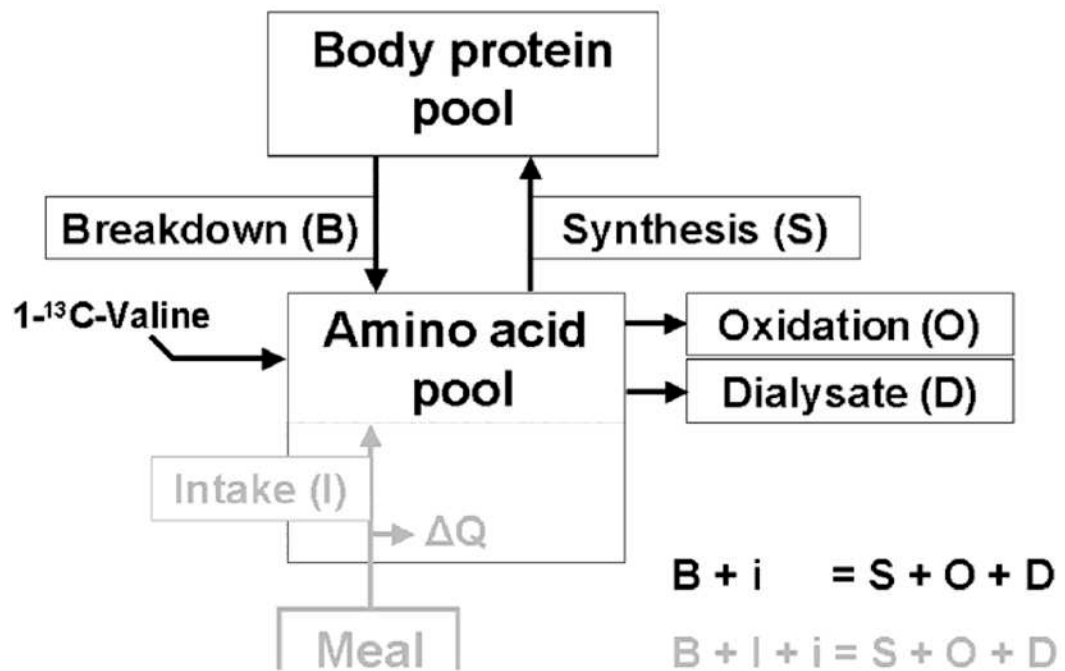




Amino Acid Isotope Tracer Studies



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Preparation of an Isotope Tracer

Background Special precautions must be used to safely administer isotope tracer to patients. The infusates must be prepared under sterile conditions. The isotope tracer must be placed into solution and diluted to an appropriate dose. These solutions are stored in small, sterile vials which are used as needed. One vial is tested for sterility and pyrogens. Many hospitals will require that the pharmacy department prepare the infusate and test for sterility.

Testing the Tracer It makes practical sense to independently test the isotope tracer by mass spectrometry before putting the isotope into solution. It is unwise to assume that you received exactly what was ordered from the isotope manufacturer. Furthermore, a standard curve should be constructed from the isotope tracer(s). It makes good sense to do this in the beginning to insure that all analyses are working properly.

Placing Isotope in Solution The isotope tracer should be solubilized in a 0.9% sterile saline solution at pH 7.4. This should be done under a sterile hood. The final concentration of isotope depends on the amount to be infused. Generally, dilute the isotope so that the total amount of isotope to be infused can be contained in one or two hypovials. Certain tracers, such as tyrosine, have a low solubility in water. Heating the solution slightly will increase the speed of solubility. Other tracers such as palmitate, requiring binding to albumin before infusing to mimic biological processing. It is a good idea to check with the isotope manufacturer or a chemist to determine the solubility of the tracer before placing into solution. **Always save some tracer for mass spectral analysis. Isotope ratio analysis requires standards made from the original tracer.**

Filtering The infusate solution should be filtered through a 0.22 μm filter before placing into small sterile vials.

Storing Infusates The sterile infusate solution can be stored in 10 or 20 ml sterile hypovials. All vials should be dated. Most tracers are stable and can be stored at room temperature. Check with a chemist to be sure of the stability of the tracer. Check sterility and stability if the infusate is not used within 12 months.

Testing Infusates One vial from the batch of prepared infusates should be tested for sterility and lack of pyrogens. Commercial companies are available to provide these services.

Syringe Pumps

Equipment Isotope tracers need to be continuously infused using calibrated syringe pumps. Precise delivery of infusate is crucial to these studies. Harvard Apparatus, Inc., South Natick, MA, (508)-655-7000, sells a complete line of pumps that many researchers have used in tracer studies. It is important that the pump delivers a precise amount of isotope over time, within 1% accuracy, without pulsing. The type of syringe used is not important, but stainless steel or glass syringes will need to be sterilized before the infusion.

Calibration The syringe pump should be routinely calibrated to check for accurate delivery of isotope. To calibrate the syringe pump, pump water through a syringe for 10 minutes. Measure the amount collected by weighing with an analytical balance. Determine the infusion rate (actual) versus the expected infusion rate.

Filtering Always use a 0.22 μm filter at the end of the syringe as a precaution. Use standard tubing to connect the catheter to the patient.

Preparing the Patient

Background The best place to infuse isotope tracers is through a central venous catheter. Surgical trauma patients usually will have these lines in place during their treatment. However, it would be too risky to insert these lines in healthy volunteers. Instead, a small catheter is inserted in the antecubital vein of the arm. Likewise, blood should be sampled from an arterial line. This may not be practical in certain subjects. Alternatively, a heated hand box (70 °C) is used to gather "arterialized" venous blood.

Catheters Short plastic catheters (18 gauge, 2 inch) are inserted into an antecubital vein of the right and left arms. One of the catheters is used for isotope infusion and the other for the withdrawal of blood samples. The catheter used for blood withdrawal is kept patent by infusion of normal saline. This can be accomplished by using a slow-drip IV bag or by syringe, pushing saline through every 15 minutes.

Blood Samples Baseline blood samples should be drawn before the isotope infusion starts. Arterial blood should be collected if a line exists for this purpose. A heated hand box can be used to collect "arterialized" blood. The original paper describing this technique is by Abumrad et al., *Metabolism* 30: 936-940, 1981. These boxes are of simple design and can be easily constructed.

Breath Samples If substrate oxidation is being measured, baseline breath samples and VCO₂ rates need to be determined. Please review the section on collecting breath samples.

Priming Pools Priming the isotope pools will speed the achievement of isotopic steady-state. Please review the section on pool priming.

Infusion Rates Isotope tracers must be infused at certain rates to achieve a measurable isotopic enrichment in the target body fluid. Furthermore, to achieve a steady-state plateau, the isotope must be infused for a certain time period. Please review the section on isotope infusions.

Diets A decision needs to be made about whether the isotope will be infused into the patient in the post-absorptive state (after an overnight fast) or in the fed state. It is possible to conduct a tracer study in the same day during both dietary states. Contact Metabolic Solutions for further details.

Collecting Blood Samples

Blood Tubes Blood should be collected in Vacutainer type tubes, green tops, with heparin added to prevent clotting. The size of the tube will depend on the amount collected.

Collecting Blood from Patients Collect blood from the patient using three-way valves connected to the catheter lines. Withdraw 1-2 ml of blood initially with a syringe and discard. This will remove the saline in the lines. Collect the amount of blood needed with a new syringe. Flush the lines with saline to keep patent.

Filling Blood Tubes Place a 18 gauge needle on the end of the blood collecting syringe. Insert the needle through the stopper and slowly push the blood through. If the blood is pushed too hard through the needle, hemolysis will occur. Hemolysis may affect the results for certain analyses. Gently invert the blood tube to mix the blood with heparin. Store on ice until the plasma can be separated from the blood.

Amount to Collect The amount of blood to collect depends on the size and condition of the subject. For infants, only 1-2 ml of blood may be collected. Generally for adults, 6 ml of blood can be collected which is sufficient for 4 or 5 analyses. Consult Metabolic Solutions for further technical assistance.

Timing of Blood Samples Blood sampling times will vary with the analysis. In general, 2 baseline samples are collected and 4-5 samples are collected at plateau for steady-state kinetic studies. For bolus isotope studies, more time points are required to follow the shape of the decay curve. Metabolic Solutions has specific information about plateau times for many compounds. Please call to have information sent to you.

Storing Blood Samples Whole blood should be immediately spun in a clinical centrifuge for 30 minutes at 2,500 rpm. Carefully, remove the plasma from the tube and aliquot the plasma into small (2 ml) plastic tubes. The cryogenic vials available from many vendors are especially suitable for these purposes. Always aliquot the blood into two or more tubes in case of unexpected loss of the blood sample. In general, blood samples can be stored at -20 °C. Certain exceptions, such as KIC analyses, require the storage of the sample at -70 °C. Blood samples can be stored for a year or longer. Metabolic Solutions can answer all questions regarding blood storage.

Priming Isotope Pools

Background Isotope pools are primed to accelerate the time to achieve plateau. Priming refers to the bolus administration of isotope before the infusion begins. It is similar in concept to flooding the body pools with isotope. The amount of prime necessary to achieve the desired steady-state of isotope can be calculated. Dr. Robert Wolfe gives an excellent description of these calculations in his book entitled, Tracers in Metabolic Research, New York: Liss, 1984.

Prime Doses The ratio of prime dose to infusion rate needs to be calculated and tested experimentally. Many prime doses are known for various compounds. For compounds that turnover fast, the priming doses are low, about 80:1 (prime dose to infusion rate). Compounds with slower turnover rates, such as urea, the prime can be as high as 300:1. Metabolic Solutions can provide all known priming doses.

How to Prime Priming doses are administered a few minutes before starting the infusion. All baseline samples should be collected **before** giving the priming dose. Administer the priming dose through the infusion catheter at a slow rate. The total dose should be given over a 2-5 minute span. The priming dose should not be given as a rapid injection to the patient. Aim to administer the prime as a long and consistent injection.

Advantages The advantages of priming relate to the hastening of isotopic plateau. Many compounds could take longer than 10 hours to achieve isotopic steady-state without priming. Infusions of these lengths could not be tolerated in some patients because of daily treatment regimens. Priming can reduce infusions to under 3 hours in most cases. This significantly cuts isotope costs.

Disadvantages The disadvantage of priming is that the normal physiology of the experimental tracer may be altered due to a rapid intake of tracer. Furthermore, many researchers argue that priming will produce an artificial plateau level that has no physiological meaning. However, many experiments have proved the usefulness of priming doses. All the benefits and disadvantages have to be weighed carefully.

Isotope Infusions

Pump Speed Set the syringe pump to deliver about 0.15 ml solution per minute. This delivery rate should be used as a ballpark number, with a deviation of ± 0.5 ml/min.

Filling Syringes Calculate the total amount of isotope needed for the entire infusion based on the patient's weight and length of infusion. Determine the amount of solution needed to deliver this amount of isotope by multiplying the pump speed by the total infusion time. Allow at least 2 ml extra solution to prevent the syringe from running out of solution at the end. Fill the syringe(s) with this amount of infusate. It is possible to administer many tracers in one syringe. Remember to calculate the dilution if mixing solutions together.

Rates of Infusion Generally, the infusion rate is set to achieve a level of isotope that can accurately be measured by the mass spectrometer and does not substantially alter normal physiology. For example, normally the infusion rate of most isotopes are set to achieve an isotope enrichment of 2-3% in plasma. This rule is for samples analyzed by a GC-Mass Spectrometer. Breath samples will achieve an isotope enrichment of 0.01% and will be analyzed by an Isotope Ratio Mass Spectrometer. Metabolic Solutions can provide many infusion rates for many compounds. In addition, we can calculate approximately the infusion rate for new isotope tracers based on body pool sizes.

Infusate Concentra- The infusate concentration needs to be known precisely. There are two ways to accomplish this. Firstly, the exact dilution of isotope in the hypovials can be **tion** calculated. This requires weighing the isotope precisely to 0.1 mg and diluting using volumetric flasks. In many instances, it may not be possible to do these measurements under sterile conditions. A second method is to save an aliquot of infusate and determine the concentration by an independent method such as an amino acid analyzer or glucose analyzer.

Achieving Isotope Plateau For steady-state kinetic tracer experiments, isotope tracer is infused just long enough to collect 4-5 samples at the plateau enrichment. The time period to infuse isotopes will depend on the turnover rate of that compound. Metabolic Solutions can provide this information upon request.

Collecting Breath Samples

Background Substrate oxidation studies require the collection of breath samples for the measurement of $^{13}\text{CO}_2$ enrichment in expired breath. To determine the oxidation rate requires the plateau enrichment in expired breath and the ventilation rate (VCO_2 , ml/min.).

Equipment for VCO_2 Measurement The ventilation rate can be measured by a commercial metabolic cart (ex. Beckmann) or by a gasometer. The metabolic carts will determine the ventilation rates instantaneously. Obtain an average respiratory rate for at least 6-10 minutes before infusing isotope tracer, at least once during the infusion and at the end of the infusion. If a gasometer is used, (ex. Collins Gasometer, Warren Collins Co., Braintree, MA), the carbon dioxide concentration of expired breath will need to be determined by a medical gas analyzer (ex. Beckman Instruments).

Equipment to Collect Expired Breath Collection of expired breath samples requires the patient to breath into a Rudolph valve (one-way valve that lets oxygen in and carbon dioxide out). The Rudolph valve is connected by plastic tubing to a 3-liter anaesthesia bag. Three-way valves are inserted at the front and end of the anaesthesia bag.

Determining VCO_2 Rate Determine the VCO_2 rate at least two times, before and after the infusion of isotope. The more VCO_2 measurements the more accurate the oxidation rate measurements. To measure VCO_2 rate, follow directions from the manufacturer of the metabolic cart. For those researchers using a gasometer, collect expired breath for two 10 minute periods at the beginning and end of the infusion. All respiratory measurements should be made in a quiet room with subjects awake and recumbent in bed, and the data adjusted to standard temperature and pressure.

Collecting Breath Samples Collect three baseline and 4-6 plateau samples for isotope analysis. The baseline samples are very important because the diet will influence the natural levels of ^{13}C in expired breath. The anaesthesia bag should be connected to a vacuum source to remove all air in the bag before filling. Use the three-way valves to evacuate the bags. Collect about one minute of expired breathing. The bag should be almost filled. Use nose clips on the patient to force all expired breath through the Rudolph valve. For seriously injured patients, face masks are available to collect expired breath. Patients on respirators will require connection of the anaesthesia bag to the ventilatory exit tube.

Collecting Breath Samples (Continued)

Transferring Breath Samples to Tubes Transfer the expired breath in the anaesthesia bag using a 30 ml plastic syringe via the three-way valves. Place a 22 gauge needle on the end of the syringe and fill a 15 ml or 20 ml Vacutainer tube, red top, no additives. Fill each tube only to maximum volume; placing more than the maximum volume will pop stoppers over time. The best Vacutainer tubes to use are those which have not been sterilized. Currently, these tubes require special order from the manufacturer. However, the sterilized tubes have provided good results when contaminants are pumped away during the sample preparation.

Storing Breath Samples Fill a minimum of triplicates of each sample time point. Once the Vacutainer tube is punctured by the instrument the sample is completely removed. Samples can be easily lost during processing. Storing samples in triplicate allows one to always have back-ups to lost samples. The breath samples should be stored at room temperature and are stable for a few months.

Times to Collect Generally, breath samples are collected before the infusion (3 baseline points) and during the isotope plateau (4-6 time points) which also is when blood is sampled.
