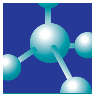

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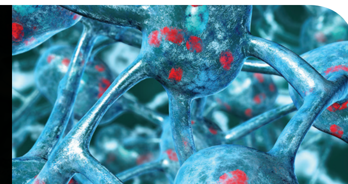

The Impact of Stable Isotope Tracers on Metabolic Research

Tracer methodology has advanced the field of metabolism by enabling the quantification of metabolic reactions *in vivo*. Stable isotope tracers have been particularly important in this regard, as these tracers have made possible a wide range of studies that would not have been possible with radioactive tracers. Early efforts using stable isotope tracers focused on determining the nature of protein "turnover," or the simultaneous processes of protein synthesis and breakdown. As analytical techniques have developed and a wide variety of isotopic tracers have become available, the scope of tracer studies has widened to the point where it would be impossible to even touch on all possible applications. Some specific examples will be discussed.

Methods using stable isotope tracers fall into two general categories: those in which the use of stable isotopes is a preferable option to the use of the corresponding radioactive tracer for reasons of ease of disposal or analysis; and methods for which there are no radioactive tracers available that would enable quantification of the metabolic pathway under investigation.

The ease of disposal of stable isotopes stems from the fact that, unlike radionuclides, they do not undergo spontaneous decay with resulting emissions that have adverse biological effects (hence the name stable isotopes). Stable isotopes are naturally occurring and may be present in significant amounts. For example, slightly more than one percent of all naturally occurring carbon is ^{13}C, and the amount of ^{13}C infused in the context of a tracer study will likely not significantly affect the whole body level of enrichment. Since mice have been raised to have almost entirely ^{13}C in their bodies without apparent adverse effects, we can be quite confident that the experimental use of stable isotopes is safe and that no special procedures are necessary in the disposal of animals given stable isotopes. The potential analytical advantages of stable isotope tracers are twofold. If mass spectrometry is used to measure enrichment, then the ratio of tracer to tracee is measured directly as opposed to the separate measurement of concentration and decays per minute (dpms) and the calculation of specific activity (the expression of tracer/tracee ratio when radioactive tracers are used). Another advantage of stable isotopes stemming from analysis is that the use of selected ion monitoring with mass spectrometry enables definitive proof that the analyte has been isolated in absolutely pure form for the measurement of stable isotope enrichment. Also, the measurement of enrichment in specific positions of a molecule is generally much more feasible with mass spectrometry and stable isotopes than the chemical isolation of radioactive atoms in specific positions in a molecule and subsequent determination of dpms of those isolated atoms. The use of D-glucose (6,6-D_2, 99%) (DLM-349) to measure the rate of hepatic glucose production is the most common example of a stable isotope providing an alternative to the radioactive analogue.

While factors such as safety and convenience are important, the most exciting advances in metabolism stemming from the use of stable isotope tracers generally involve the quantification of metabolic pathways that realistically could not have been measured otherwise. Nitrogen metabolism is the most obvious example, since there is no radioactive isotope of nitrogen. Since nitrogen is the key element that defines amino acids and protein, a wide variety of applications have been derived to quantify various aspects of nitrogen metabolism in the body using ^{15}N as a tracer. Stable isotopes of carbon and hydrogen have also been used to label amino acids in order to perform novel studies of amino acids. Individual amino acids can be labeled with a variable number of heavy stable isotopes in order to produce molecules of different molecular weight that retain the same metabolic functions (isotopomers). Isotopomers can be useful in a variety of approaches. For example, measurement of muscle protein synthesis involves infusion or injection of an amino acid tracer, and measurement of the rate of incorporation over time into the tissue protein. Collection of the muscle protein requires a muscle biopsy. By staggering the times of administration of isotopomers of the same amino acid, one single biopsy can suffice to determine the rate of incorporation over time, thereby enabling the calculation of the rate of muscle protein synthesis.

Stable isotope methodology also enables the concurrent use of the same isotope incorporated in to numerous molecules. Since there are 20 different amino acids in the body, it is often important to study the interaction of the kinetics of multiple amino acids. For example, amino acid transmembrane transport differs for specific amino acids, but there is overlap in carrier functions for particular amino acids. It is, therefore, advantageous to quantify transport rates of different amino acids simultaneously. This can be accomplished using stable isotope tracers of the amino acids of interest because the amino acids are separated by gas or liquid chromatography prior to measurement by mass spectrometry. Therefore, different amino acids with the same stable isotope tracer can be distinguished even though the mass increase caused by the tracer is the same in each case.

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The unique ability to measure by mass spectrometry the enrichment of a variety of molecules enriched with the same stable isotope tracer has been central to the development of many new approaches in the field of metabolic research. The most popular method involves administering deuterium oxide (D, 99.9%) (DLM-4) and measuring the synthetic rates of a wide variety of molecules by determining the rate of incorporation of the D. It is also possible to quantify intracellular reaction rates using both positional and mass isotopomers of the same tracer, most commonly using ^{13}C. Use of positional isotopomers to calculate various intracellular flux rates involves administering a molecule with labeling in a specific position and determining by mass spectrometry the extent of appearance of the stable isotope tracer in other positions of the same molecule, or in specific positions of other molecules. Mass isotopomers have proven useful to determine the enrichment of precursors of the synthesis of polymers such as fatty acids. If multiple labeled precursors (e.g. ^{13}C-acetate) are incorporated into a product that is a polymer of the precursor (e.g. palmitate), then this will be reflected in the mass increase in the product. From the profile of mass increases in the product the precursor for synthesis can be calculated.

Increased sophistication of mass spectrometry analysis has led to the development of the field of metabolomics. The concentrations of a wide variety of compounds, usually in the blood or urine, are measured to develop a profile distinctive of a particular metabolic state. Stable isotope tracers have played an important role in metabolomics, as their use as internal standards enable quantification of the concentration of any tracee for which a stable isotope tracer is available. Although the metabolomics approach has proven useful in some circumstances, there has been some ambiguity in interpreting metabolomics profiles because they reflect only concentrations. For that reason the field of fluxomics is evolving in which a wide variety of tracers are given to the subject before the blood is sampled so that the metabolomics profile can reflect not only concentrations, but also the flux rates of relevant metabolic pathways.

This brief overview is meant only as a introduction to the varied possibilities possible with the use of stable isotope tracers. A key factor in the development and advancement of these applications has been the increasing availability of a wide variety of stable isotope tracers from CIL. The diversity of the CIL products has advanced to the point where the application of new methodologies is limited only by our own insights and creativity.

Further information about stable isotope tracer methodology as applied to metabolic research can be learned at the annual course "Isotope Tracers in Metabolic Research" held in Little Rock, AR, organized by Drs. Bob Wolfe and Henri Brunengraber, and sponsored by the NIH. The course provides an intensive exposure to a variety of techniques and seasoned investigators in the field. For information about the course, please contact Deb Viane at djviane@uams.edu.

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