# Stable isotope usage in developing countries: Safe tracer tools to measure human nutritional status

*"Silent tracers" can help health professionals answer some important questions*

**by Peter D. Klein and E. Roseland Klein**

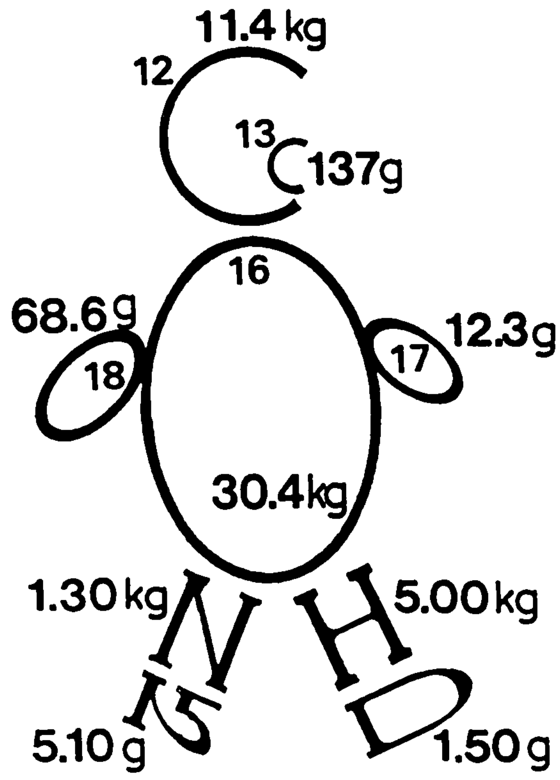
How many calories are used when a nursing mother feeds her infant? How much milk does an infant receive in one week? What is the impact of the environment on the energy needs of children? How adequately does dietary protein sustain the synthesis of body constituents? What types of foods best nourish a child recovering from intestinal diseases such as diarrhoea?

Quantitative answers to questions such as these can be obtained from the use of stable, non-radioactive isotopic tracers. Answers to these questions are important in assessing the nutritional status of infants, children, pregnant women and nursing mothers, as well as that of individuals who subsist on marginal food supplies. Because stable isotopic tracers are completely safe and without hazard, they can be used freely in health, nutrition, and agriculture studies in all populations.

### Which stable isotopes are used?

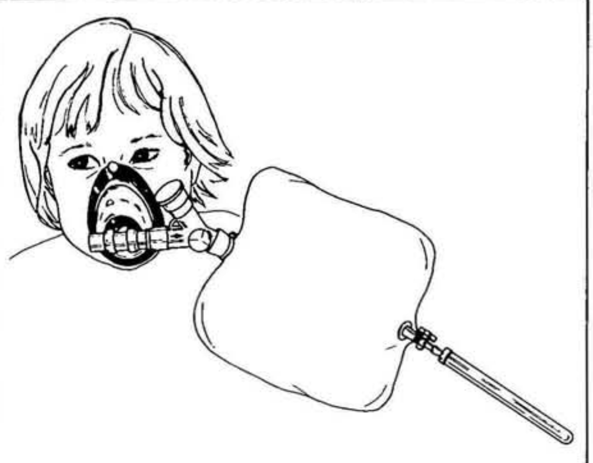
Studies of life processes focus on the behaviour of a limited number of elements: hydrogen (H), carbon (C), nitrogen (N), and oxygen (O). Each of these elements exists in nature in two or more stable forms that differ only in the number of neutrons in their nucleus. In each case, the major light isotope (hydrogen-1, carbon-12, nitrogen-14, or oxygen-16) is accompanied by a constant proportion of one or more minor heavier isotopes whose individual abundances range from 0.02% to 1.11%. An inventory of the human body shows that a 50-kilogram individual has an aggregate of 225 grams of hydrogen-2, carbon-13, nitrogen-15, oxygen-17, and oxygen-18. *(See accompanying diagram.)*

Although there are variations in the proportions of hydrogen-2 to hydrogen-1, carbon-13 to carbon-12, nitrogen-15 to nitrogen-14, and oxygen-18 to oxygen-16, each has a characteristic baseline abundance to which tracer measurements are referred. For purposes of tracer studies, each element can be enriched to 99% or greater in the proportion of the heavier isotope. These procedures make use of slight rate differences in exchange processes (e.g., deuterium) or cryogenic distillations of carbon monoxide or nitrous oxide to remove the lighter isotope and concentrate the heavier species.




The human body contains substantial quantities of stable isotopes, as depicted in this diagram. A person who weighs 50 kilograms, for example, has 225 grams of the heavier isotopes hydrogen-2, carbon-13, nitrogen-15, oxygen-17, and oxygen-18. (Credit: Dr Wada, Mitsubishi-Kasai)

Dr Peter D. Klein is Director of the Stable Isotope Laboratory, and E. Roseland Klein is the Scientific Editor of the US Department of Agriculture/Agricultural Research Service's Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, in Houston, Texas 77030 USA.



Breath samples for isotope ratio measurements of carbon dioxide are readily collected even from young subjects. The samples are stored in evacuated containers until analysis.

The enriched form (e.g., $^{2}H_2O$ or $H_2^{18}O$) may be used directly, $^{13}CO_2$ may be incorporated into plants by biosynthetic procedures, or the isotope may be transformed through organic syntheses into labelled fats, carbohydrates, or amino acids.

## How are stable isotopes used in nutrition studies?

Shortly after deuterium was discovered in 1932, Schoenheimer and Rittenberg conducted the first nutrition study using a stable isotopic tracer. They fed partially hydrogenated (deuterated) linseed oil to two mice and expected the deuterium to be liberated promptly by the oxidation of fat to carbon dioxide and water. They recovered less than half of the anticipated amount of label in the urine, however, and found that the remainder had been incorporated into body fat stores. Thus, Schoenheimer and Rittenberg provided the first demonstration of the dynamic nature of body constituents.

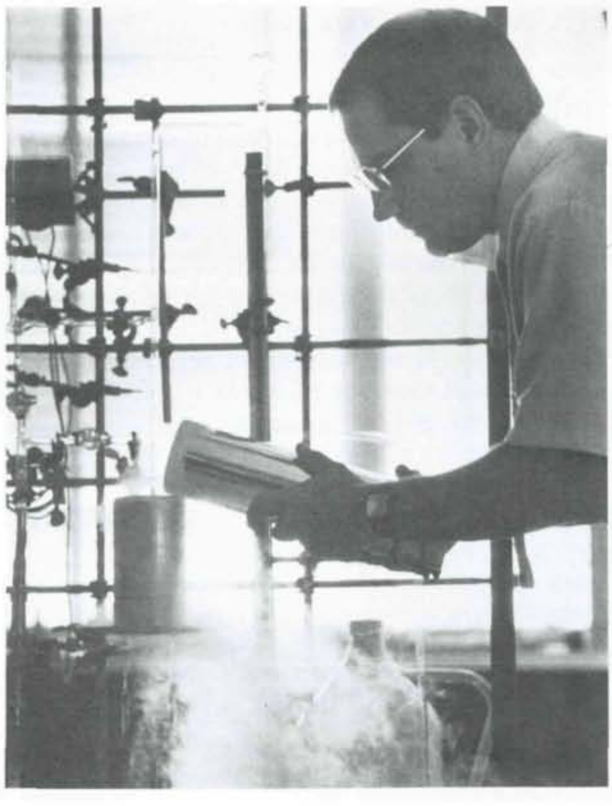
Today, the great appeal of stable isotopic tracers, in addition to their safety, is that they can be administered orally and the metabolic products into which they enter (e.g., body water, respiratory carbon dioxide, urea) can be sampled in breath, saliva, milk, urine, and stool. The use of non-invasive sampling procedures simplifies field studies and enhances subject recruitment and co-operation.

## How are stable isotopic tracers measured?

Stable isotopes are often called "silent tracers" because they emit no externally measurable radiation and their presence in excess of natural levels is detectable only by changes in the ratio of minor isotope to major isotope. For many years, measurement of such ratios has required the use of isotope ratio mass spectrometers in which heavy and light forms of the same molecule undergo separation and quantization. In such instruments, a purified sample of hydrogen gas, carbon dioxide, or nitrogen is admitted through a highly-restricted opening into an ion source under vacuum. The gas molecules are bombarded by a stream of electrons, whereby they acquire a positive charge and are accelerated into a magnetic field. Here the ionized gas molecules become segregated according to mass and strike individual collector plates. In doing so, the ions generate currents that are proportional to their numbers and enable their quantization.

Isotope ratio mass spectrometry provides extremely precise and accurate values of isotopic abundances, but requires a large capital investment and substantial support facilities for sample preparation, purification, and analysis. For this reason, alternative optical spectroscopic techniques are being explored that lend themselves to simpler, less expensive, and more facile analyses. Emission spectrometry has already provided thousands of analyses of nitrogen-15 samples generated in agricultural studies and also is being applied to nutrition studies that utilize nitrogen-15. Infrared absorption measurements are being used to determine the deuterium concentration of body fluids and recently a new infrared heterodyne principle has been described that will provide isotope ratio values (carbon-13/carbon-12) of respiratory carbon dioxide. A low-cost armamentarium of instruments for stable isotope determinations is thus taking shape and should expand and facilitate usage in developing countries.

Solid materials can be combusted to carbon dioxide and water and purified for isotopic analysis on a vacuum line. (Credit: Jack Dykinga, USDA/ARS)



## Generic protocols

Health professionals in every country have recurrent concerns about the nutritional status of specific population segments or age groups. Within this context, a series of generic stable isotope measurements that require little or no adaptation to local conditions should have universal application. A consensus has developed among consultants to the IAEA that these protocols should include tracer studies which:

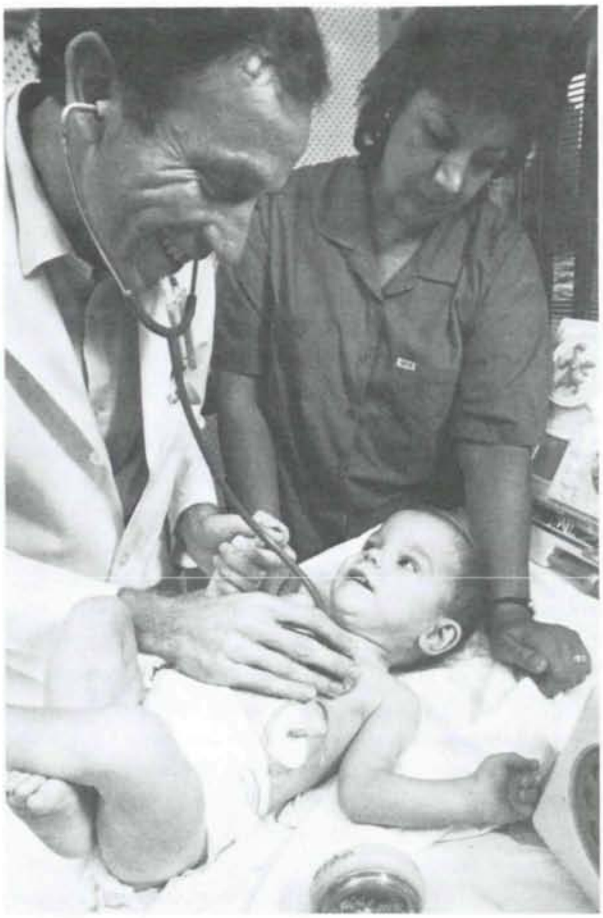
- Estimate total energy expenditure for the individual
- Determine lean body mass, and thereby, per cent body fat
- Provide a simple overall measure of nitrogen flux
- Measure improvement in nutrient absorption and utilization after diarrhoea.

***Estimation of total energy expenditure.*** The caloric expenditure of an individual varies widely during the course of a day; the lowest level occurs during sleep and the peak occurs during periods of exertion, such as work or exercise. Conventional estimates of energy expenditure are based on the rates at which an individual consumes oxygen and produces carbon dioxide and are usually measured in the resting state. Field measurements of energy expenditure by respiratory gas analysis during the full range of an individual's activities are difficult, restrictive, and cumbersome. Moreover, long-term integration of the duration of activities with different activity intensities presents many problems.

A newly validated technique (based on the use of $^2H_2O$ and $H_2^{18}O$) promises to circumvent these difficulties. When doubly-labelled water is administered to a subject, both isotopes mix with body water and are eliminated in body fluids over a period of days. The turnover of body water can be estimated from the daily measurements of hydrogen-2 concentration in urine or saliva samples. When the samples are analysed for oxygen-18, the values will reflect a more rapid excretion rate than that for deuterium because the oxygen-18 is also incorporated into exhaled carbon dioxide. The difference in excretion rates between oxygen-18 and hydrogen-2 tracers thus reflects the volume of carbon dioxide produced over the period of observation. This parameter can be used to calculate the total energy expenditure of the subject.

Depending upon climatic conditions and metabolic activity levels, the doubly-labelled water technique will quantitate energy expenditures accurately over 5 to 18 days. Such measurements will indicate, for example, whether significant differences exist between populations in the energy required to perform similar physical tasks.

***Determination of lean body mass.*** Anthropometric measurements of height, weight, and skinfold thicknesses are used frequently to estimate the proportions of lean body mass and total body fat in individuals. These estimations are based on specific population values, regarded as appropriate for the individual, which have been validated against direct measurements of total body



**An assessment of body composition using isotopes of water reveals the partition of dietary energy deposited as lean body mass or fat stores. (Credit: Jack Dykinga, USDA/ARS)**

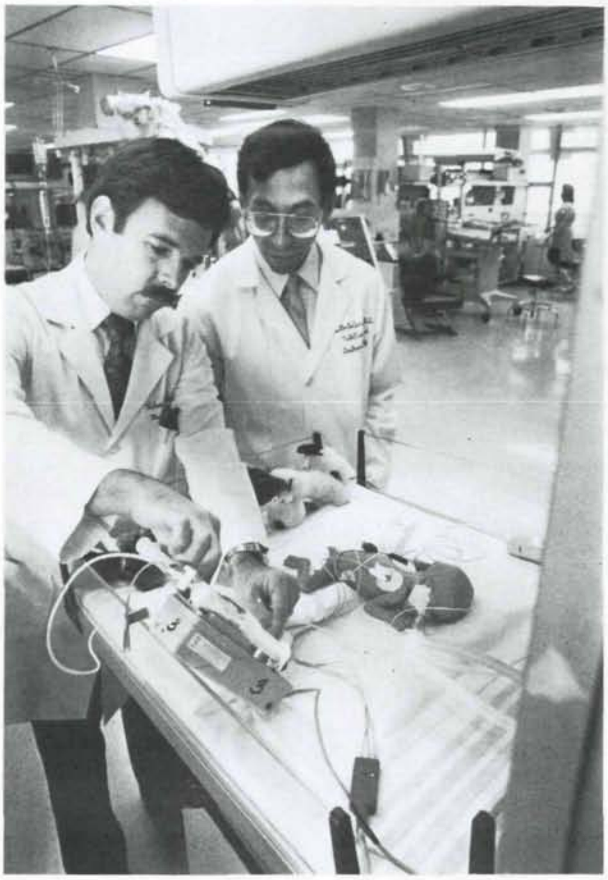
water by isotope dilution. A tracer dose of water labelled with hydrogen-2 or oxygen-18 is administered and allowed to equilibrate for 4 to 6 hours. The isotope concentration in saliva or urine will reflect the dilution undergone by the isotope. In normal healthy individuals, this space — which represents total body water — comprises approximately 73% of lean body mass but may be higher in malnourished individuals. When lean body mass is calculated, the difference in body weight is the amount of adipose (fatty) tissue. Evaluation of changes in body composition are essential after starvation and during catch-up growth and maturation, as well as during pregnancy and lactation.

***A simple measure of overall nitrogen flux.*** Dietary nitrogen (protein) is broken down into individual amino acids which participate in the synthesis of whole-body protein and which undergo catabolism to urea and ammonia. During periods of stress, the catabolic processes that act on body protein predominate over synthetic processes and a negative balance is established between dietary intake and catabolic losses. Although careful analyses and records of dietary nitrogen intake and excretion can reveal long-term trends in nitrogen balance, an operational measure is needed that reflects whole-body protein turnover. Such a measure is provided by the administration of a single oral dose of an amino acid, or preferably a protein, labelled with nitrogen-15. An example is yeast grown in a medium containing $(^{15}NH_4)_2SO_4$. Urine is collected for 9 to 12 hours and the amounts of tracer nitrogen in urinary $NH_3$ and in urea are determined. Either an arithmetic or a harmonic average of these two values provides a reliable estimation of whole-body protein turnover that is insensitive to changes in nonprotein nitrogen metabolism. The test is easily administered, sample collection is simple, and the analytical requirements are modest. Thus the method is ideal for surveys of dietary protein adequacy in refeeding after kwashiorkor or other forms of malnutrition.

***Nutrient absorption and utilization after diarrhoea.*** Breast-fed infants born in developing countries often have repeated episodes of diarrhoea after they are weaned. These disease episodes cause dehydration and interrupt nutrient absorption as a result of intestinal mucosal damage by the infecting organism. During the period of infection, nutrient intake is insufficient to maintain infant growth and development. Regeneration of small intestinal capacity is essential before solid foods can be tolerated, nutrients can be absorbed, catch-up growth can occur, and normal growth can be resumed. Rice water or gruel has been proposed as an alternative to other rehydrating fluids because the necessary carbohydrates or starches would be fed in a form that is easy to assimilate. Demonstration of the efficacy of such a feeding regimen is aided by the production of rice labelled with carbon-13 (by exposure to $^{13}CO_2$ during periods of photosynthesis). The rice plant efficiently incorporates the labelled carbon dioxide into the rice grain in the forms of starch, protein, and fat. When the rice is cooked and fed, digestion and absorption of the starch can be detected (and measured) from the appearance of labelled carbon dioxide in breath samples. The degree of malabsorption can be estimated from the recovery of tracer carbon in total stool carbon. Together, these measurements can guide the development of refeeding practices based on indigenous foods.

### Importance in nutrition programme planning

Health professionals in every country seek to reduce infant mortality, overcome malnourishment and growth stunting of children. They also seek to provide adequate nutrient intakes of pregnant and lactating women, and to determine the required nutrient intakes for daily activities of their general populations. The magnitude of agricultural resources required to meet these objectives and the manner in which they are allocated can only be established when accurate assessments of the existing needs have been achieved. Given the variety of field conditions, levels of technical training, and instrument availabilities, future progress requires safe, simple, and reliable techniques for these measurements. As experience with stable isotopic tracers grows, the use of these tools has the potential to provide answers to major nutritional questions of international scope.



**Non radioactive tracers are used to establish nutrient requirements of premature infants. (Credit: Jack Dykinga, USDA/ARS)**

### IAEA plans co-ordinated research programme

Concern about radiation doses sometimes limits the use of radioisotopes as tracers in human nutritional and medical studies, even though only a very small dose is involved. In many advanced countries, attention has been shifting to the possible application of *stable* isotopes, which are inherently so safe that they can be used even in studies of infants, children, and pregnant or lactating women.

As part of work in this field, the IAEA is planning a new co-ordinated research programme on applications of stable isotopes in studies of human nutrition and nutritionally related diseases. This is expected to focus on measurements of protein turnover and energy expenditure in selected population groups, mainly in developing countries. It is also anticipated that the Agency may be able to provide support for work of this kind through technical co-operation projects and fellowship training.

In general, these and other related IAEA activities in the field of nutrition and health are designed to exploit the potential of nuclear analytical techniques and isotope-aided tracer studies in monitoring and assessing the nutritional status of human populations and their exposure to toxic pollutants.

Further information may be obtained from the IAEA's Division of Life Sciences.