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The Rudolf Schoenheimer Centenary Lecture

Isotopes in nutrition research

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The present lecture begins with a brief overview of the professional and scientific journey taken by Rudolf Schoenheimer, before turning to a discussion of the power of isotopic tracers in nutrition research. Schoenheimer's remarkable contributions to the study of intermediary metabolism and the turnover of body constituents, based initially on compounds tagged with ^{2}H and later with ^{15}N, spanned a mere decade. It is difficult, however, to overestimate the enormous impact of Schoenheimer's research on the evolution of biological science. After a relative hiatus, following Schoenheimer's death in 1941, in the use of stable nuclides as tracers in metabolism and nutrition, especially in human subjects, there is now an expanded and exciting range of techniques, experimental protocols and stable-isotope tracer compounds that are helping to probe the dynamic aspects of the metabolism of the major energy-yielding substrates, amino acids and other N-containing compounds, vitamins and mineral elements in human subjects. Various aspects of the contemporary applications of these tracers in nutrition research are covered in the present lecture.

Rudolf Schoenheimer: Stable-isotope tracers: Isotope labelling techniques: Isotope measurement

In an editorial, entitled Breakthroughs 1997 appearing in *Science* (Bloom, 1997), advances such as (1) somatic cell nuclear transfer and (2) exploration of Mars by the Sojourner robot were duly recognized. Further, in terms of predicting what might be 'hot' this year (1998), the short feature ended with a reference to Mark Twain who had noted, 'you can't depend on your eyes when your imagination is out of focus' and offered that the exercise of imagining may be worth practising. A truly imaginative mind was that of Rudolf Schoenheimer (Fig. 1), whom this lecture honours on the approximate occasion of his birth in Berlin, Germany, 100 years ago. It is reasonable to speculate with some confidence that if there had been a Breakthroughs 1935 published then in *Science*, his first series of experiments on the metabolism of partially-deuterated linseed oil (Schoenheimer & Rittenberg, 1935) would have made the list. This experiment showed that 'the fats of the depots are not inert storage materials but are constantly involved in metabolic reactions' (Schoenheimer, 1942). This was the first isotopically-based recognition of the turnover of body constituents. A few years later, in 1939, after Schoenheimer had turned to the use of DL-[^{15}N]tyrosine, it might well have been anointed the 'Molecule of the Year'. In 1992, this prize went to NO (Koshland, 1992) and today, from the recent extensive media coverage and attention, at least in the USA, the awardees might well be the angiogenesis inhibitors, angiostatin and endostatin. The point is that Schoenheimer's experiments and discoveries surely must have been just as exciting then as the major breakthroughs are in today's biology.

In the present lecture, which has been developed in collaboration with my colleague, Alfred Ajami, on isotopes in biochemical, metabolic and nutritional research, it seems fitting to start at the beginning! Hence, we will outline first, in brief, the professional and scientific journey taken by this remarkable physician and scientist, before we turn to a more contemporary setting that might provide some further useful examples, based largely on our own interests, of the power of isotopic tracers in that area of biology with which most of us here closely identify, i.e. nutrition.

Abbreviation: PET, positron-emission tomography.

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Fig. 1. A photograph of Rudolf Schoenheimer taken in about 1937. Provided by Professor Karl Bernhard, Basel, through the courtesy of Professor Peter Klein, Houston.

Schoenheimer and his legacy

A detailed historical account of Schoenheimer's work has been written by Kohler (1977), and this is supplemented by additional shorter essays about his life and the evolution of his remarkable scientific career (Ratner, 1979; Berthold & Klein, 1991; Cohn, 1995; Olson, 1997). These literature sources provide the basis for the first part of this presentation.

The scientific journey taken by Schoenheimer more or less culminated in his famous publication entitled *The Dynamic State of Body Constituents* (Schoenheimer, 1942). This book was put together by David Rittenberg, Sarah Ratner and Hans Clarke, from drafts of the three Edward K. Dunham lectures given at the Harvard Medical School in 1941 by Schoenheimer before his tragic death on 11 September of that year. As Ratner (1979) has reminded us, the components of body tissues, until shortly before that time, had been regarded as being structurally and metabolically inert. Indeed, Schoenheimer (1931) stated at the beginning of a review of his work on sterol metabolism (to which I will refer again, and which had been carried out at the University of Freiburg), 'It is only a relatively short time since we assumed that plants only could synthesize complex compounds whereas animals were forced to obtain these complex compounds indirectly from plants and that in modifying these complex compounds for their specific needs only slight chemical changes are necessary'. The novel concepts and new findings presented in this small but substantial book (Schoenheimer, 1942) were based on investigations carried out over a period of approximately 7 years, and they included the first *in vivo* stable-isotope tracer experiments of mammalian biochemistry. Although commonplace in research today, the application of isotopes as tracers was then entirely original to the study of intermediary metabolism, and it followed upon the work of Hevesey (1923) who, some years earlier, used a radioactive isotope of Pb to measure its translocation in plant tissues. Parenthetically, however, the history behind the tracer concept is much older, as was pointed out by Robertson (1983) who stated: 'The origins of the tracer concept are lost in antiquity, if this is taken to include such examples as the use of a cowbell to locate a herd and similar applications . . . The word 'tracer' [therefore] has many meanings. The post office uses it to designate a method for determining the fate of lost mail. The military uses it to mean a type of ammunition that marks the trajectory of projectiles by leaving a trail of smoke or fire. In the present context the term tracer will be restricted to meaning a marked form of a substance that is used to determine certain properties of the substance in biological systems. These properties include the exchangeable mass or volume of the substance, its localization, its pathway through chemical reactions, and its transfer rates into, out of, and through components of the system.'

Together with Hans Krebs and others, Schoenheimer was laid off from his position at the University of Freiburg due to the economic and political events of the time, which included expulsion by the Nazi regime of Jews from university service. He emigrated to New York City, and in 1933 joined the Department of Biochemistry at Columbia University, College of Physicians and Surgeons. Importantly, 1 year earlier Harold Urey had discovered ^2H (Urey *et al.* 1932), for which he was to receive a Nobel Prize 6 years later. Given Schoenheimer's imagination, along with the encouragement of Urey and Clarke, and with the collaboration of Rittenberg, he seized the opportunity to exploit this nuclide as a tracer in a series of far-reaching investigations. The findings were published in fourteen papers, over a 3-year time period, under the general title of Deuterium as an indicator of intermediary metabolism.

Fig. 2 depicts the differential rates of change in the ^2H enrichment of saturated fatty acids in liver *v.* the depots following feeding of mice with a 'dilute solution of heavy water' (Schoenheimer 1936–7). It was abundantly clear to Schoenheimer at this time that body fats were in a state of rapid flux. Further, as summarized in tables taken for illustrative purposes from his Harvey lecture (Schoenheimer, 1936–7; Table 1), he had shown that (1) 'the larger part of the dietary fat, even when given in small amounts, is first deposited in the fat tissues and is not oxidized' and (2) feeding mice with [^2H]stearic acid gave rise to labelled unsaturated fatty acids. These findings anticipated, by more

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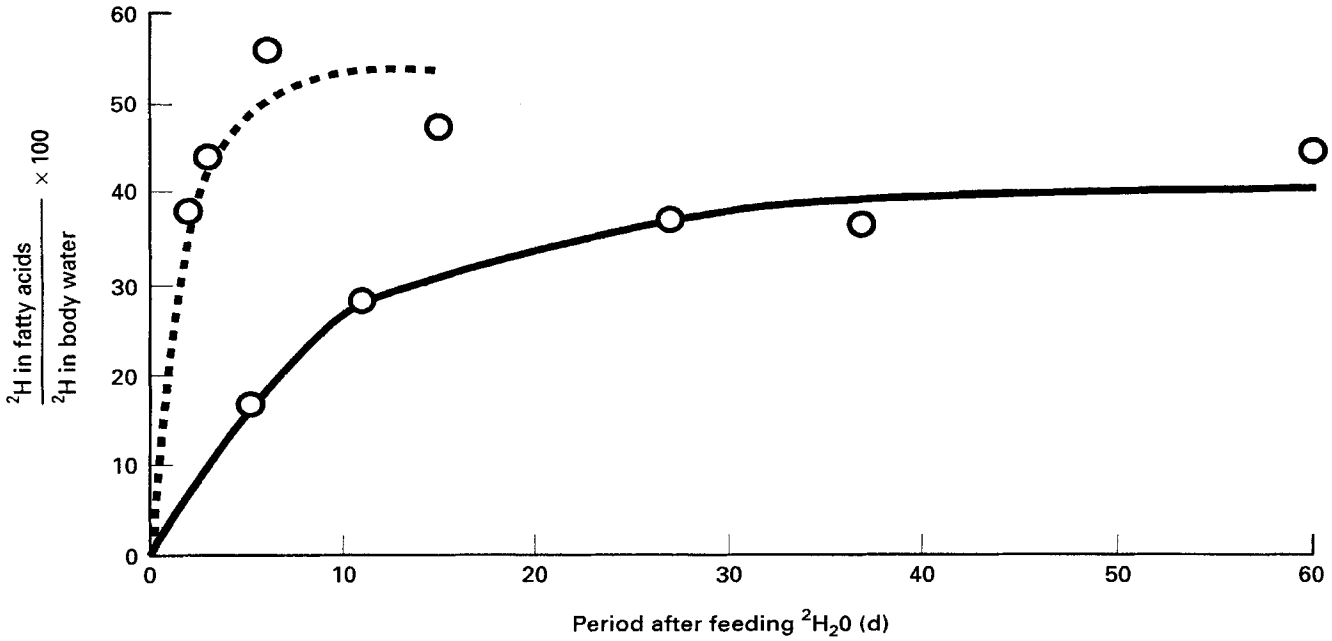

Fig. 2. The rate of saturated fatty acid synthesis in liver (O---O) and depots (O—O) as determined by incorporation of ^2H following feeding mice with a dilute solution of ^2H_2O. (From Fig. 4 of Schoenheimer, 1942.)

than 50 years, a partial metabolic basis for the macronutrient balance and body weight–composition regulation hypothesis that has been put forth by JP Flatt (1995), although questioned, with reference to obesity causation, by others (Seidell, 1998; Willett, 1998).

In 1937, Urey and his collaborators succeeded in concentrating ^{15}N, and again within 1 year, and under Schoenheimer's leadership (Schoenheimer & Rittenberg, 1938), the stage was set for yet another ground-breaking series of research papers. These appeared under the general title Studies in protein metabolism, sixteen of which were published between 1939 and 1942; quite a publication record! These studies required the development of efficient methods for the synthesis of ^{15}N-labelled amino acids, which was accomplished in 2 years (Schoenheimer & Ratner, 1939), and their sensitive analysis, for which David Rittenberg's essential contribution should be recognized (Rittenberg *et al.* 1939).

The first *in vivo* ^{15}N-labelled amino acid tracer study was conducted with the purpose of determining whether glycine of dietary origin or that supplied by body tissues was used for the formation of hippuric acid (Rittenberg & Schoenheimer, 1939). Following this, a critical test of the Otto Folin (1905) hypothesis was conducted in an adult rat with the aid of DL-[^{15}N]tyrosine (the seventh paper in the Studies in protein metabolism series; Schoenheimer *et al.* 1939*b*). According to this hypothesis, ingested N was largely excreted (exogenous metabolism), with only a small amount of dietary N being retained to replace wear and tear of endogenous metabolism. But, only about 50 % of the [^{15}N]tyrosine given in the diet to an adult rat for 10 d was excreted (see Ratner, 1979). This study forcibly refuted Folin's (1905) hypothesis, and it further revealed the reality of body protein turnover. This quantitatively important component of N metabolism in mammalian organisms remains a scientific challenge today, in respect to elucidating the mechanisms involved, identifying the metabolic and cellular mechanisms that causally link synthesis and breakdown under normal conditions and the nutritional corollaries of this turnover.

To describe in greater detail the continuing and exciting series of tracer studies by Schoenheimer seems unnecessary, but one is worthy for special mention. Specifically, we have been struck by a ^{15}N study concerned with the investigation of creatine–creatinine metabolism (Schoenheimer, 1942). Here isotopically-labelled creatine was fed to rats and the ^{15}N was found to be rapidly eliminated via the

Table 1. The investigation of intermediary metabolism with the aid of ^2H by feeding three mice on a diet containing 10 g ^2H-labelled fat (^2H 5·74 atom %)/kg for 4 d and by isolating the unsaturated fatty acids from mice fed on labelled saturated fatty acids ([^2H]stearic acid; ^2H 8·66 atom %) (From Schoenheimer, 1937)

^2H-labelled fat consumed (mg)	^2H-labelled fat recovered in fat tissue (mg)	Percentage of dietary fat stored in fat tissue	Burned dietary fat recovered as ^2H (%)	^2H content (atom %) of:		
				Total fatty acids	Saturated fatty acids	Unsaturated fatty acids
251	119	47	20	1·70	2·58	1·16

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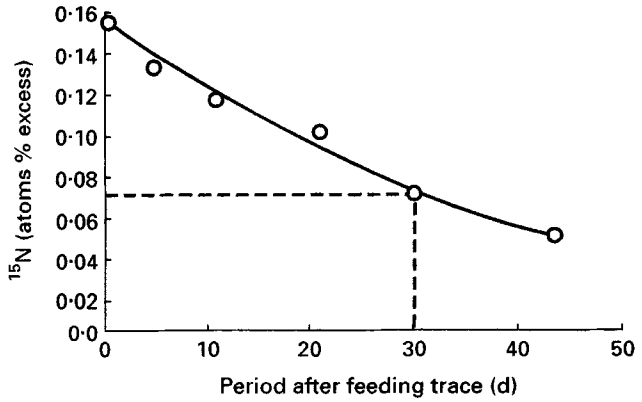

Fig. 3. Pattern of elimination of [^{15}N]creatinine after feeding rats labelled creatine (half-life of elimination of ^{15}N 29 d). (From Fig. 6 of Schoenheimer, 1942.)

urine. From these findings an estimate was made of the half-life of elimination of the ^{15}N, determined to be 29 d (Fig. 3). Taking into account the total creatine content of the animal tissues, the quantity of creatinine excreted via the urine daily amounted to about 2 % of body creatine, and it was shown that the isotopic concentration of muscle creatine and urinary creatinine was the same. This is, in general terms, the global *in vivo* picture of creatine synthesis and turnover that exists today, and relatively little further study of organ and whole-body creatine metabolism has been undertaken since then, except that which has been reviewed by Waterlow *et al.* (1978). We think it is timely to now extend and expand these tracer studies of creatine metabolism, especially in view of the current interest in creatine supplementation, muscular performance and body composition (Grindstaff *et al.* 1997; Volek *et al.* 1997; Kreider *et al.* 1998).

These various ^{15}N tracer studies of amino acid (N) and protein metabolism changed the fundamental understanding of mammalian protein and amino acid metabolism and, again, the general picture that emerged from these investigations still applies today. We wonder whether even the brilliant Schoenheimer could have ever imagined the intricate role played by protein turnover in the development, maturation and functioning of the organism, as well as the variety of cellular mechanisms responsible for this turnover. The growing details about the ubiquitin-proteasome pathway (Ciechanover & Schwartz, 1998) is an example of our contemporary understanding of one of these mechanisms, and in this context it is a process that is fundamental to many diverse cellular functions, such as cell cycle progression, antigen presentation, fertility, activation of transcription factors and cell death (Ghislain *et al.* 1993; Palombella *et al.* 1994; Harding *et al.* 1995; Drexler, 1997; Mbikay *et al.* 1997).

In spite of the power of ^{2}H and ^{15}N as investigative tools, as revealed by Schoenheimer's studies, their application in metabolic and nutritional research declined following Schoenheimer's death. This appears due, at least in part, to (a) Urey's loss of interest in and reduced commitment to a promotion of the application of stable isotopes in biological research (Kohler, 1977) and (b) the discovery of the long-lived radioisotope of C (^{14}C) by Ruben & Kamen in February 1940 (Kamen, 1963).

Nevertheless, several factors have since then combined to promote an extensive, elaborate and exciting use of stable isotopes in biological research over about the past three decades. In our view, a major return to an appreciation for the value of stable isotopes in human metabolic and nutrition research was signalled by the work of Waterlow and his colleagues in Jamaica (Picou *et al.* 1969) on the use of [^{15}N]glycine as a means of studying whole-body protein turnover in infants.

Isotopes in today's nutrition research

This seems to be a reasonable point at which we might now turn to the more contemporary scene of isotopes in metabolic and nutrition research, a topic that has been the subject of extensive reviews (Halliday & Rennie, 1982; Janghorbani & Young, 1982; Matthews & Bier, 1983; Janghorbani, 1984; Bier, 1987; Wolfe, 1992; Abrams, 1994; Crews *et al.* 1994; Aggett, 1997; Demmelmair *et al.* 1997; Patterson, 1997), including presentations at past Nutrition Society meetings (Garlick & Millward, 1972; Halliday & Read, 1981; Buckley, 1988; Beylot, 1994). The focus, for various reasons, will largely be concerned with stable isotopes as tracers, using, where appropriate, some examples taken from the research carried out in our laboratories at the Massachusetts Institute of Technology. Here, it should be noted that we use the term stable isotope rather loosely, to mean a non-radioactive isotope that is less abundant than the most abundant naturally-occurring isotope (Patterson, 1997).

First, it is worth noting that there have been impressive advances in the analytical and technical aspects of tracer use in nutrition research since Schoenheimer described his use of a Zeiss interferometer for measurement of ^{2}H, a method based on the difference in refractive index between water and $^{2}H_2O$. Where greater sensitivity was necessary, measurement of the density of the water was made by a submerged float method (Rittenberg & Schoenheimer, 1935). At that time, the falling drop method was also used to measure the concentration of ^{2}H in water (Cohn, 1995). In Table 2 we list the full palate of the analytical techniques that are now implemented routinely or undergoing intensive development within the life sciences. Some of them are more widely used than others in the application of isotopically-enriched tracers in nutritional investigations. In addition, we give here a historical as well as futuristic emphasis to this summary of analytical trends and techniques. For example, spectroscopy has progressed steadily from static single-line absorptiometry (one-dimensional), through the current period of two- and three-dimensional imaging, moving inevitably towards four-dimensional holography and virtual reality as the form of data display. One can also envision the concomitant trend away from traditional forms of analogue spectroscopy towards miniaturized arrays of digital sensors on the surface of Si and C.

With respect to the use of stable isotopes, a relatively more contemporary development is in relation to NMR

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Table 2. Summary of trends and techniques in use of nuclides in biochemistry and metabolism

	Nuclide type		Approximate time line		
	Stable	Radioactive	1930–1950	1980–2000	2030–2050
Analytical trends					
Physical separation and single-line spectrometry	X		X		
Physical separation and particle-emission counting		X	X		
Chromatographic separation and 2D or 3D spectrometry	X			X	
In situ 3D real time imaging (tomography)		X		X	
In situ 2D real time imaging (tomography)	X				X
Separation and analysis on Si 'chips'	X	X			X
Holographic (virtual-reality) imaging (4D)		X			X
Analytical techniques					
Accelerator MS		X		X	
Atomic-emission spectroscopy	X	X		X	
Autoradiography: Analogue		X	X		
Digital		X		X	
Capillary electrokinetic chromatography	X	X			X
^{13}C NMR spectroscopy	X			X	
^{13}C NMR tomography	X				X
Charge-coupled-diode imaging radiography		X			X
Charge-transfer microelectrode detection	X	X			X
Charge-coupled fibreoptic spectroscopy	X	X		X	
Chemical-field-effect-transistor spectroscopy	X	X			X
Chemical-ionization MS	X	X		X	
Chemical-reaction-interface (GC or LC) MS	X			X	
Continuous-flow-gas isotope-ratio MS	X			X	
Densitometry	X	X	X		
^{2}H NMR spectroscopy	X			X	
^{2}H NMR tomography	X				X
Digital autoradiography		X		X	X
Diode i.r. laser spectroscopy	X			X	
Diode microspectroscopy	X	X			X
Dispersive i.r. spectroscopy	X		X		
Electron-capture spectroscopy	X	X		X	
Electron-impact-ionization MS	X	X	X		
Electrospray-ionization MS	X			X	
Fast-ion-bombardment MS	X			X	
Flame-ionization MS	X	X			X
Fluoroscopy: Analogue		X	X		
Digital		X		X	
Gamma scintigraphy		X		X	
Gamma spectroscopy		X	X		
Gamma tomography		X		X	
Gamma holography		X			X
GC–MS	X	X		X	
Gas isotope-ratio MS	X	X	X		
Gas-phase Geiger counting		X	X		
Gravimetry	X	X	X		
Plasma-atomic-emission spectroscopy	X	X		X	
Plasma MS		X		X	
Ion-cyclotron-resonance MS	X				X
Ionization electrometry		X	X		
Ionization electroscopy		X		X	
Laser desorption ionization MS	X	X		X	
LC densitometry	X			X	
LC–MS	X	X		X	
Liquid scintillation counting		X	X		
Microchannel chromatographic densitometry	X	X			X
Microelectrofluoresence spectroscopy	X	X			X
Molecular-emission spectroscopy	X			X	
Molecular-recognition-biochip detection	X	X			X
Molecular-recognition-transistor detection	X	X			X
Neutron-activation spectrometry	X	X		X	

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Table 2. *continued*

	Nuclide type		Approximate time line		
	Stable	Radioactive	1930–1950	1980–2000	2030–2050
Neutron-capture spectrometry		X		X	
NMR microscopy	X				X
Optical-emission spectroscopy	X		X		
Optical time-of-flight pulse nanospectroscopy	X				X
Photoacoustic spectroscopy	X			X	
Photochromic transistor detection	X	X			X
Photon-emission nanospectrometry	X	X			X
Photon-emission tomography		X		X	
Photon-emission holography		X			X
Positron-emission tomography		X		X	
Positron-emission holography					X
Pyrolysis (GC or LC) MS					
Solid scintillation		X	X		
Surface-enhanced-affinity chromatography	X	X			X
Thermospray-ionization MS	X			X	
^{3}H NMR spectroscopy		X		X	

2D, 3D, 4D, two-, three- and four-dimensional respectively; LC, liquid chromatography; MS, mass spectrometry.

studies, where the magnetic property of the nucleus is the variable of interest rather than the mass of the nucleus. In this context, stable isotopes have opened up opportunities to study macromolecular structure and structure–function relationships. As yet these applications of NMR, in comparison with mass spectrometry, have found more limited use in nutritional studies. However, NMR has been applied in elegant experiments to study, for example, C flux in the Kreb's cycle (Wiechert & de Graaf, 1996), the metabolism and control of glucose at the level of skeletal muscle, and the regulation of liver and muscle glucose and glycogen synthesis (Shulman, 1997; Cline *et al.* 1998; Rousell *et al.* 1998), as well as glutamate–glutamine cycling in the brain (Sibson *et al.* 1997). A possible limitation of this technique in metabolic studies is the amount of tracer that often has to be given; for example, a protocol for measurement of the rate of movement of glucose-C to glutamate in the brain required a dose of [1-^{13}C]glucose that raised blood glucose concentration by about three-fold (Fitzpatrick *et al.* 1990). On the other hand, NMR gives information about metabolic conversions or the fate of a given isotope and its positional information, which positron-emission tomography (PET) does not. NMR offers the possibility of studying monoisotopic elements, which is not feasible by mass spectrometry.

We do not intend to dwell much further here on the technical or analytical aspects of isotopes. However, with particular reference to the extraordinary changes that have occurred since the time of Schoenheimer's experiments, it might be worth speculating about the sort of advances that are likely in the future; in turn, these will probably revolutionize use of isotope tracers in nutrition research.

The more recent technical advances (Roth, 1997) that we are able to exploit today in stable-isotope tracer studies include those that might be regarded as involving single techniques (isotope-ratio mass spectrometry, NMR), multiple or hyphenated techniques such as GC–mass spectrometry, GC–combustion–isotope-ratio mass spectrometry, or hybrid techniques (mass spectrometry–mass spectrometry). However, it is likely that future major technical advances and improvements will involve the miniaturization, with automation, of isolation or separation and detection techniques (Effenhauser & Manz, 1994). In contrast to the earlier GC–mass spectrometry and isotope-ratio mass spectrometers and the associated vacuum line preparation system which required significant space and sometimes more than one large room, we can now work with a GC–mass spectrometer that fits on a small desk or buy a portable GC–mass spectrometer (which will fit under the seat of an aeroplane), and for which the mass resolution and sensitivity is equal to that of the instruments used, for example, in the early 1980s. Further, the pilot production of a hand-held DNA sensor for measurement of DNA sequences, made possible because of improvements in DNA chip technology, has been announced recently (Wilson, 1998). The rapid development of Si technology has been at the heart of the advances in microtechnology and fabrication of miniaturized electrochemical sensors, for example (Arquint *et al.* 1994), but C electronics are now likely to contribute to this continuing revolution (McEuen, 1998).

In this context, in areas or topics such as molecular nanotechnology (Kaehler, 1994), the microchip and Si micromachining become increasingly important. Microchips and C nanofibres give the ability to miniaturize current 'bench-top' experiments (Hadd *et al.* 1997), and it is entirely conceivable that an isotope-ratio mass spectrometer with arrays of Faraday cup detectors, could be micromachined into a single chip; a 'lab-on-a-chip' concept (Woolley *et al.* 1998). Further, the analyses of stable-isotope content or enrichment may not require a mass spectrometer. For example, electrochemical sensors based on ion-sensitive-field-effect transistors and chemical-field-effect transistors, in principle, can respond to minute differences in molecular mass by virtue of 'host–guest' cavity effects, for example, between ^{12}C and ^{13}C. Additionally, microcolumn liquid chromatography (column

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size 25 μm i.d.) has been used to separate and detect, for example ^{2}H-labelled and protiated noradrenaline and adrenaline (Hsieh & Jorgensen, 1997).

In short, the future will undoubtedly involve both the simplification and miniaturization of multiple steps involved in the analysis of the isotopic content of compounds in biological matrices. The major difference from the analytical procedures followed by Schoenheimer and those that most of us use today is that they will, in the future, be conducted in 'molecular' space, rather than in 'human' space, and they will be many orders of magnitude faster. In Schoenheimer's time the isotopic enrichment of his various analytes was teased out, literally, with hammer and tongs: extraction, physical fractionation, micro-degradation, differential isolation by derivatization, re-isolation and then gravimetric, densitometric or spectroscopic elemental composition determinations. Today, much of this work is left to chromatographs and mass spectrometers, with the aid of an occasional robot. But, perhaps ironically, the future may well again take us back to the past, except that all of what Schoenheimer had laboured over will become the province of computerized intelligent agents carrying out laser and plasma thermochemolyses on intact biological matrices, to be followed by ultra-microcapillary electrokinetic separations and quantification of isotopologues (molecules of the same structural type and nominal class that differ incrementally in molecular mass by virtue of the number of contained isotopic labels, e.g. [1-^{13}C]pentane, [1,2-^{13}C]pentane, [1,2,3-^{13}C]pentane and [1,1,2,2-^{2}H]pentane) or isotopomers (molecules of the same molecular mass and nominal elemental composition that differ from each other by virtue of the position and type of contained isotopic label, e.g. [2-^{13}C]pentane and [3-^{13}C]pentane) by chemical-field-effect transistors. Imagine, therefore, about 50 years from now and think of the physician, 'Bones', in Star Trek; he has a whole-body analyser on his index finger that measures all metabolic functions by sensing chemical, electrochemical, thermochemical and radiative signals over the surface of the body. Why not envisage the same futuristic situation for detection of pathophysiologically relevant metabolites from specific stable tracer probes which have been dispensed as diagnostic investigative and imaging agents?

While such microanalytical techniques permit the study and analysis of single cells, many of us who use tracers in nutrition research are interested in understanding how the more than ten trillion (1×10^{13}) cells of multiple lineages that interact to form the tissues and organs of the body are metabolically co-ordinated, and how the regulatory mechanisms responsible for normal homeostasis operate and are affected by nutritional factors. In this context, promoted by the technical advances previously noted briefly and a second area of evolution in the use of isotopes in nutrition research, there has been the birth of a multiplicity of tracer paradigms that are now used in metabolic and nutritional research. In Schoenheimer's experiments, single stable-isotope-labelled compounds were administered to mice or rats via the diet or drinking water. Today, we see a rich array of different stable-isotope tracer protocols, and a partial indication of this variety is presented in Table 3. The earlier approaches in both experimental animals (Schoenheimer, 1942) and in human studies (Picou & Taylor-Roberts, 1969) involved administration of the tracer through the food, water or via intragastric infusion; today they are given by vein, artery and/or intestine and, occasionally, via inhalation (Molnar *et al.* 1986).

Table 3. Evolution and expansion or complexity of tracer protocols or paradigms

In the past:	Single tracers: feeding or drinking water
Today:	Single or multiple tracers
	Isotopomers*: i.e. [1-^{13}C]leucine and [^{15}N]leucine
	Isotopologues†: i.e. [1-^{13}C]phenylalanine and [$^{2}H_5$-ring]phenylalanine
	Oral, intravenous or inhaled
	Continuous, bolus or staggered (overlapping)
	Blood, urine, faeces, tissues or expired air
	Kinetic studies
	Kinetics of tracer (autogenic kinetics)
	Conversion of tracer (heterogenic kinetics)
Factors:	Instrumentation, cost of isotopes, computational analysis and techniques

* Molecules of the same molecular mass and nominal elemental composition that differ from each other by virtue of the position and type of contained isotopic label.

† Molecules of the same structural type and nominal class that differ incrementally in molecular mass by virtue of the number of contained isotopic labels.

There have been a number of factors contributing to this expansion of tracer paradigms, in addition to the advances in instrumentation for isotope measurement, and these include:

(a) increased availability of specifically-labelled compounds at reasonable cost. Even when we began our studies of leucine kinetics in healthy adults about 20 years ago the tracer [1-^{13}C]leucine cost $850/g (US dollars). In 1998 terms this would be equivalent price of about $2500/g, compared with the present rate for [1-^{13}C]leucine of about $60/g. Current ^{13}C feedstock has a market price of $1650 per mol ^{13}C. If the ^{13}C should ever be produced as a commodity, as has been the case with the rare 'noble' gases, one could speculate that the price of [1-^{13}C]leucine, or any of its more-labelled isotopologues, could fall by another order of magnitude. Similarly, the cost of $^{2}H_2{}^{18}O$ is now to the point at which the doubly-labelled-water technique for measurement of whole-body energy expenditure in an adult (Wong, 1996) can be applied at an isotope cost of about US $300 per single study. When Schoeller & van Santen (1982) first applied this method to human subjects (developed earlier by Lifson *et al.* (1955) for measurement of CO_2 output in rodents) the approximate cost of the isotope might have been about US $3200 in current terms. However, strict cost comparisons are difficult to make because of the earlier assistance given via government subsidies for the development of enriched sources of isotopes;

(b) the explosion of electronically-based computational analysis techniques. In Schoenheimer's time it was necessary, in practice, to conduct experiments which required only relatively simple algebra, and to some extent this 'requirement' undoubtedly helped promote the extensive use of the continuous tracer administration

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protocols for measurement of plasma substrate fluxes (Wolfe, 1992) and whole-body turnover of proteins (Waterlow *et al.* 1978). It is now, of course, possible to go well beyond the earlier gross simplification of whole-body metabolism, and compartmental and non-compartmental models can be readily applied for their possible value in understanding the fine structure of metabolism *in vivo*, including the dynamic associations among metabolic pools that may or may not have an immediately obvious anatomical identity. Indeed, modelling is an essential and critical component in the effective use of tracer techniques and for the improved understanding of the biological and metabolic system under investigation (Clifford & Müller, 1998; Cobelli & Caumo, 1998).

Thus, using compounds or elements enriched with particular stable isotopes, the *in vivo* aspects of amino acid (Irving *et al.* 1986; Cobelli *et al.* 1991), glucose (Cobelli *et al.* 1990), ketone body (Cobelli *et al.* 1987), water- and fat-soluble vitamin (Coburn & Townsend, 1996), and mineral (Wastney *et al.* 1997) metabolism have been further explored using multi-compartmental modelling analysis. Some of the models are quite complex (ten compartments in the leucine model of Cobelli *et al.* 1991; twelve compartments in the β-carotene model of Novotny *et al.* 1995) and perhaps it is for this reason there has been a tendency to avoid their application. In consequence some of us, at least, have previously felt more comfortable with the more traditional single-pool continuous-tracer-infusion paradigm. Nevertheless, we are of the view that the concept of nutrient bioavailability, for example, would be enhanced greatly through the application of full-compartmental mathematical and kinetic models, using stable-isotope-tracer techniques which permit the safe study of specific nutrient metabolism in human subjects under different pathophysiological conditions. An outcome would be the generation of predictive information regarding quantitative kinetic aspects of nutrient utilization and interactions, and further enhancing our understanding of the roles of nutrients in biological processes *in vivo*.

In this context of modelling, we have now begun to use more elaborate modelling techniques in our studies of (a) the rates of amino acid oxidation in healthy adults and their relationships to dietary factors and nutritional requirements, and (b) the metabolism of L-5-oxoproline (which is an intermediate in the γ-glutamyl cycle of glutathione metabolism) and its urinary excretion, which has been used as a marker of glycine sufficiency (Jackson, 1991). These latter studies are still underway, but in reference to the former we have initiated studies that involve a modification of our current 24 h continuous-tracer administration–amino acid balance approach (El-Khoury *et al.* 1994), using a systems analysis paradigm. We asked ourselves whether a model fitted to data generated from a single bolus of tracer could predict the physiological outcome that is generated by our routine primed constant-tracer-infusion paradigm. Hence, we have conducted preliminary studies in healthy Indian subjects using $H^{13}CO_3^-$ and [^{13}C]acetate (the latter as an example of an oxidizable substrate relevant to use of [1-^{13}C]lysine) to obtain an initial answer to this question.

Table 4. Summary of compartmental modelling of bicarbonate kinetics in four Indian adults (AV Kurpad, A Ajami and VR Young, unpublished results)

	Present work		Saccomani *et al.* (1995)
	Fast	Fed	Fast
Percentage ^{13}C recovery	69	67	82
$k_{(1,2)}$ rapid exchange (/min)	0·0676	0·3146	0·184
$k_{(1,3)}$ slow exchange (/min)	0·0050	0·0391	0·029
Q_1 (CO_2 in central; mmol/kg)	3·56	2·61	3·50
Q_2 (CO_2 in rapid; mmol/kg)	9·67	2·93	3·0
Q_3 (CO_2 in slow; mmol/kg)	6·44	8·44	6·7
Flux (2,1) (rapid; mmol/kg per min)	0·647	0·867	0·483
Flux (3,1) (slow; mmol/kg per min)	0·032	0·324	0·1470
Flux ratio (rapid : slow)	22·6	2·6	3·29

The $H^{13}CO_3^-$ study was conducted in four well-nourished Indian adults in Bangalore, with the assistance of our collaborator, Professor A. V. Kurpad. The model used was that which has been validated by Saccomani *et al.* (1995). The data were well fitted by the model, and a summary of the relevant variables is given in Table 4. The HCO_3^- dose was 1 mg/kg, given at the mid-point of both a 12 h fast and 12 h fed period within the 24 h fed–fast protocol (El-Khoury *et al.* 1994). This dose is one-quarter of the dose that would have been dispensed by primed continuous infusion. Further, the model was then used to simulate the results of a constant infusion at the given input of tracer. As shown in Fig. 4, the model predicts well the results of a constant infusion.

Since we also plan to include a consideration of the intermediate formation of [1-^{13}C]acetate in our future single bolus [^{13}C]lysine oxidation studies, we then asked the question; 'can we combine the HCO_3^- model with the behaviour of a tracer bolus of acetate to predict the behaviour of a constant infusion of [1-^{13}C]acetate'? Hence we conducted a labelled-acetate study using a design similar to that for the HCO_3^- study. Separately, a standard constant 24 h (12 h fast–12 h fed) infusion of [^{13}C]acetate (8 μmol/kg per h) was conducted. We then sought to fit a model to the $^{13}CO_2$ data, while being constrained both by the HCO_3^- data and a more complex impulse response observed following the [^{13}C]acetate *v.* $^{13}CO_2$ bolus. Without going into detail, the key question was whether our model predicted the outcome of a primed constant tracer infusion of this oxidizable tracer. As shown in Fig. 5 the prediction is in excellent agreement with the results for the 24 h constant infusion of [^{13}C]acetate. Similarly, we might note here that Hovorka *et al.* (1997) have concluded that a constant infusion and a bolus injection of stable isotopically-labelled glucose give reproducible and comparable estimates of hepatic glucose output.

It is our hypothesis that we can now further develop the appropriate numerical (system) models for estimating leucine and lysine oxidation (irreversible oxidative disposal) based on this successful experience, and it should be noted that we do not wish at this time to develop complex models

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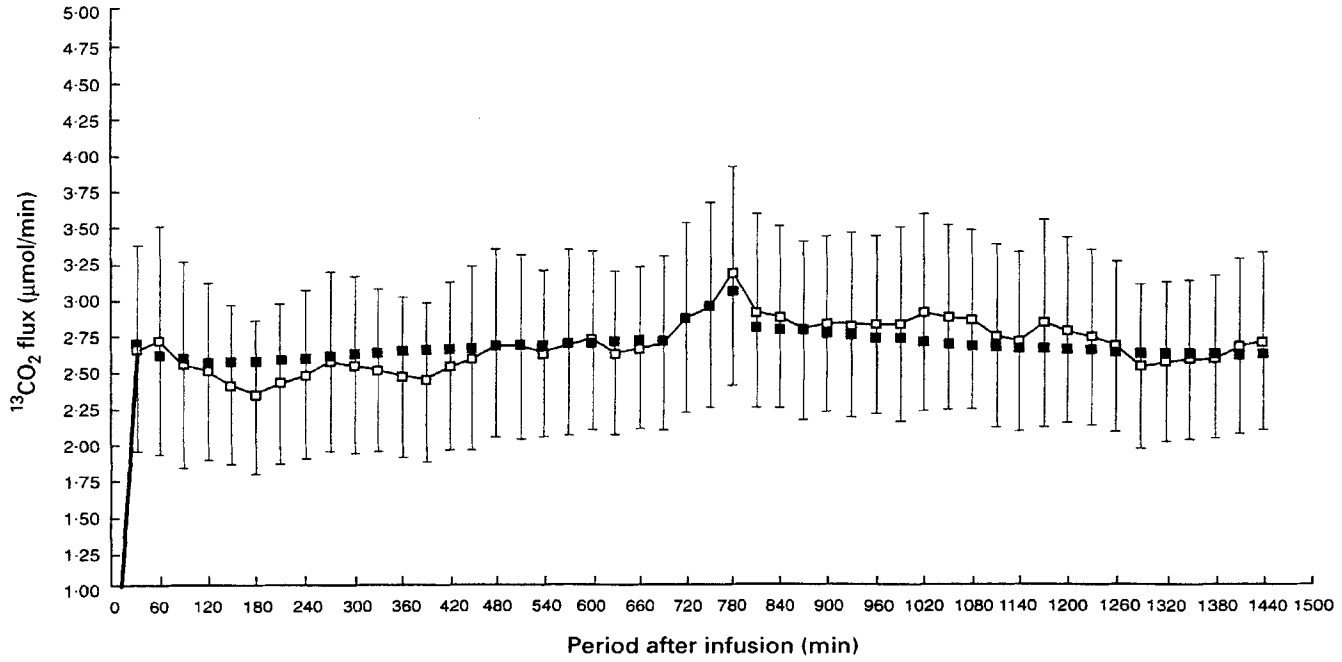

Fig. 4. The flux of expired $^{13}CO_2$ over a 24 h period as measured with a constant infusion of $H^{13}CO_3$ (prime 5·15 μmol/kg, constant 3·75 μmol/kg per h; □) and as simulated from results of a single bolus of $H^{13}CO_3$ (1 mg/kg; ■) given during the fast and fed phases of the 24 h period. Values are means and 1 SD represented by vertical bars for four subjects (AV Kurpad, A Ajami and VR young, unpublished results).

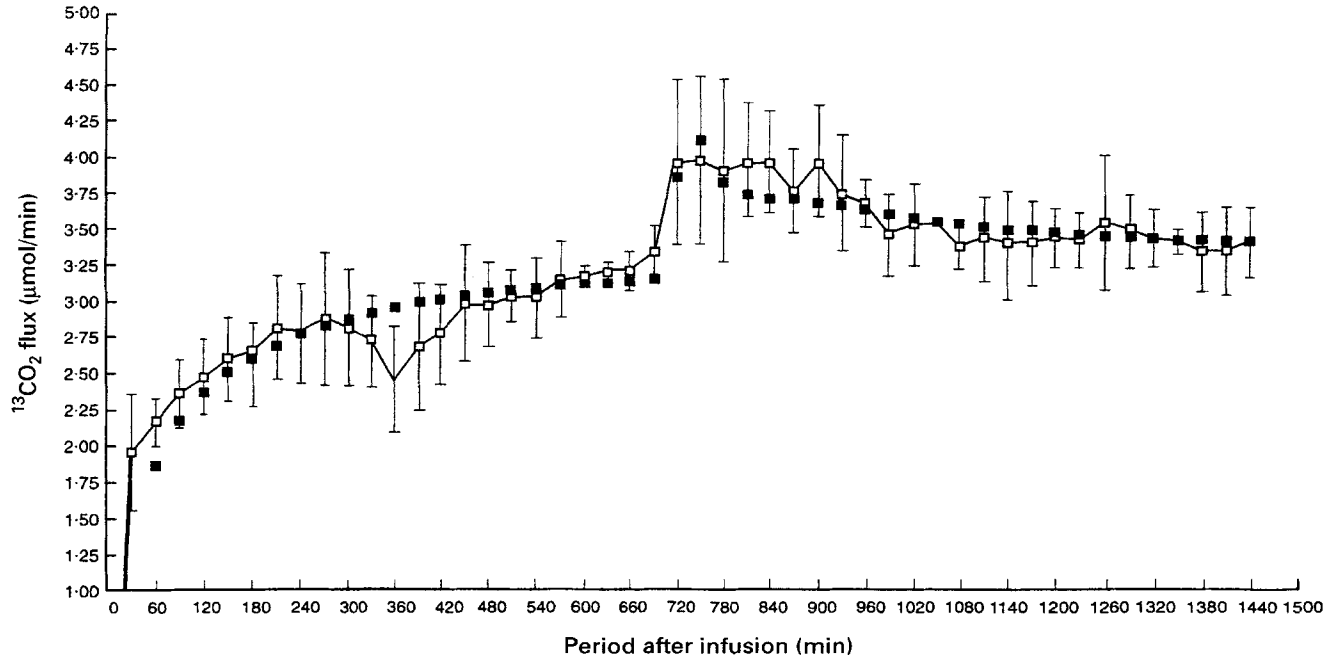

Fig. 5. The flux of expired $^{13}CO_2$ over a 24 h period as measured using a constant infusion of [1-^{13}C]acetate (prime 11 μmol/kg, constant 8 μmol/kg per h; □) and as simulated from results of a single bolus of [^{13}C]acetate given during both the fast and fed phases of the 24 h period. Values are means and 1 SD represented by vertical bars for four subjects (AV Kurpad, A Ajami and VR Young, unpublished results).

with anatomical and/or physiological concerns in mind. Our purpose is to develop minimal numerical models that can predict the behaviour of a system, in reference to altered dietary intakes of a test indispensible amino acid.

Beyond non-compartmental measures which provide a relative insight into net substrate and tracer disposal, either upper or lower limits, the more rigorous data reduction formalism available within the scope of this preliminary work consists of compartmental modelling. Bier (1981) has discussed these complex models and their interpretation, and Waterlow (1995) has pointed out some practical drawbacks, particularly with reference to the number of

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blood samples needed and the variability of the derived variables. These may indeed limit their widespread, as well as field, application but our anticipation is that compartmental and non-compartmental models will be used increasingly in nutrition research with advances in the analytical and experimental methods noted previously. Parenthetically, attempts have been made to reduce the number of samples that need to be taken for a kinetic analysis, and the first of these efforts appears to have been that by Evans *et al.* (1985) who used a technique of staggered stable-isotope-administration protocol to study phenobarbital entry into the cerebrospinal fluid of a dog. More recently, Dudley *et al.* (1998) used an overlapping (staggered) infusion of multiple stable amino acid isotopologues to estimate the fractional rate of synthesis of lactase (*EC* 3.2.1.108) in the brush border of intestinal enterocytes of piglets.

Also, with respect to some further developments in the use of isotopes in nutrition research, it has become almost routine to include more than one tracer in a given single experiment. In terms of our interests this, of course, permits study of both N and C metabolism simultaneously, as well as the importance of the position of an atom within a molecule, such as the methyl- *v.* carboxyl-C of methionine (Storch *et al.* 1988; Fukagawa *et al.* 1998) or the different roles and metabolism of the guanidino- *v.* amino-N of arginine (Castillo *et al.* 1993, 1996). As have many others, we have exploited this 'multi-tracer' opportunity, which was not easily available to Schoenheimer, although he did synthesize a number of amino acids (Table 5) containing both ^{2}H and ^{15}N. Indeed, using L-[3,4,5,5′-$^{2}H_{9}$-^{15}N]leucine as a tracer which he fed to rats, he noted the higher mobility of the N moiety of this amino acid than the ^{2}H portions (Schoenheimer *et al.* 1939*a*). This study preceded by about 42 years the initial human studies by Matthews *et al.* (1981) on the kinetics of doubly-labelled leucine, using GC–mass spectrometry, and we have similarly exploited this tracer to investigate some of the finer details of splanchnic leucine metabolism in a catheterized animal model (Yu *et al.* 1992).

We have used both isotopomers and isotopologues of arginine and related urea cycle intermediates to explore the nutritional significance of arginine and its roles in metabolism. Using L-[guanidino-^{13}C]arginine, L-[5,5-$^{2}H_{2}$]citrulline and L-[5,5,5-$^{2}H_{3}$]leucine as tracers, we have estimated the rate of conversion of labelled citrulline to arginine to be approximately 5–10 μmol/kg per h in healthy adults, and that this rate appears to be unaffected by the level of arginine intake. Thus, it appears that endogenous arginine synthesis is not markedly responsive to acute alterations in arginine intake in healthy adults, and we have proposed that arginine homeostasis is achieved via a modulation in the net rate of arginine degradation (Castillo *et al.* 1993).

An exciting technique that enhances the study of endogenous synthesis of amino acids such as arginine, and of polymeric macromolecules in particular, is that which is based, as pointed out by Bier (1997), on the value of the full isotopic information inherent within substrate mass isotopomers. This approach is called mass isotopomer distribution analysis (Hellerstein & Neese, 1992; Brunengraber *et al.* 1997), and it was applied in a simple but elegant experiment by Berthold *et al.* (1991) by feeding a laying hen uniformly-^{13}C-labelled algae hydrolysate and isolating amino acids from the egg-white protein. They confirmed the indispensable nature of phenylalanine and the nutritionally-dispensable character of glutamine and glutamate from the distribution of ^{13}C in the glutamate isolated from the egg proteins. A comparable approach has been used to estimate proline and arginine synthesis in adult female subjects (Berthold *et al.* 1995*b*) and in human neonates (Miller *et al.* 1995*b*) and premature infants (Miller *et al.* 1995*a*). Further, the principle of mass isotopomer distribution analysis has been applied successfully to quantify glycogenolysis, gluconeogenesis and lipogenesis in man (Katz & Lee, 1991; Landau *et al.* 1995; Neese *et al.* 1995; Hellerstein *et al.* 1996, 1997; Yang *et al.* 1996).

In additional multi-tracer studies with arginine and citrulline, the rate of whole-body NO synthesis was estimated by the rate of conversion of the [^{15}N]guanidino-N of arginine to plasma [^{15}N]ureido citrulline. This was then compared with the NO synthesis rate based on measurement of urinary NO_2^- : NO_3^- excretion (Castillo *et al.* 1996). The rates of NO synthesis (μmol/kg per h) in healthy adults over a period of 24 h averaged 0·96 (SD 0·1) and 0·95 (SD 0·1), for the [^{15}N]citrulline and the NO_2^- : NO_3^- methods, respectively (Table 6). Thus, the proportion of the plasma arginine flux associated with NO and also urea synthesis in healthy human subjects is small, although the plasma arginine compartment appeared to serve as a significant precursor pool (54 %) for whole-body NO formation. We hope that this tracer model, possibly modified using a compartmental modelling approach, will be useful for exploring these metabolic relationships *in vivo*, under

Table 5. Synthetic compounds containing ^{15}N (Table 5 of Schoenheimer, 1942)

Urea	DL-Ornithine (D)	Guanidoacetic acid
Glycine	L-Leucine (D)	Betaine
DL-Alanine	D-Leucine (D)	Aminoethanol
DL-Tyrosine	L-Lysine (D)	Guanine
L-Phenylalanine	D-Lysine (D)	Arginine
D-Phenylalanine	DL-Norleucine	Hydantoic acid
DL-Glutamic acid	Choline	Methylhydantoic acid
L-Glutamic acid (D)	Creatine	L-α-Aminophenylbutyric acid
D-Glutamic acid (D)	Creatinine	
DL-Aspartic acid	Sarcosine	D-α-Aminophenylbutyric acid
	Thiourea	

D, dual-labelled (i.e. ^{15}N and ^{2}H).

Table 6. Estimates of whole-body nitric oxide synthesis, based on L-[guanidino-$^{15}N_2$]arginine as a tracer

Investigation	Arginine → citrulline	Arginine → NO_3 (or NO_3 output)	Comment
Castillo *et al.* (1996)	960 nmol/kg per h	950 nmol/kg per h	36 h protocol
Macallan *et al.* (1997)		approximately 397 nmol/kg per h	12 h protocol
Lagerwerf *et al.* (1998)	220 nmol/kg per h		120 min protocol

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specific pathophysiological states where the L-arginine–NO pathway might be altered.

Some recent studies by Macallan *et al.* (1997) using L-[guanidino-$^{15}N_2$]arginine with measurement of urinary $^{15}NO_3^-$ output, and by Lagerwerf *et al.* (1998) also using this arginine tracer but with measurement of plasma [^{15}N]citrulline enrichment (by a combination of HPLC and electron-spray-ionization mass spectrometry), have reported lower rates of conversion of arginine to citrulline than those given here (Table 6). However, their findings may be due to differences in the tracer protocols as applied in our various experiments; in Macallan *et al.* (1998) account was not made of the presence of $^{15}NO_3^-$ that would have been retained in the body during the period of measurement of ^{15}N in urine. This would lead to an underestimate of the conversion rate. In the study by Lagerwerf *et al.* (1998), their 2 h tracer protocol may not have been long enough to reach a sufficient level of ^{15}N labelling in the citrulline pool, at least as based on a further analysis of our own 24 h tracer infusion protocol which was extended to 36 h for purposes of collection of urinary $^{15}NO_3$.

The point here is an obvious one, and that is that the effective use of tracers in nutrition research requires the combination of appropriate analytical methodology and experimental design, including choice of tracer and the structure of the tracer protocol used. We have shown, for example, that [^{2}H-ring]phenylalanine is not an ideal tracer to use in *in vivo* studies of phenylalanine hydroxylation and oxidation (Marchini *et al.* 1993), with possible implications, therefore, for use of this ^{2}H tracer in the estimation of the *in vivo* activity of the phenylalanine-hydroxylating system for the classification and treatment of phenylketonuria (Treacy *et al.* 1996). However, it might be an entirely suitable tracer for measurement of tissue and organ protein synthesis rates (Ballmer *et al.* 1995*a,b*; Patterson *et al.* 1997). Indeed, the choice of an appropriate tracer has quite important implications and we must be mindful of this; two relatively recent studies, one in elderly women (Pannemans *et al.* 1997) and the other in young adult women (Berthold *et al.* 1995), have shown that the effect of feeding on whole-body protein metabolism differs according to the tracer amino acid used. Clearly such findings challenge the adequacy of current models of whole-body protein turnover (see also Millward *et al.* 1991). Perhaps, they further underscore the potential of a more rigorous effort to develop and validate kinetic compartmental models, as we have discussed previously.

At this point we will turn to the last topic in our selected focus on isotopes in nutrition research. It arises really in reference to the point made previously about whole-body protein turnover, an index of body protein metabolism which John Waterlow (Waterlow *et al.* 1978) presented to many of us with the hope that it would possibly provide for the study of protein nutrition and metabolism what BMR has provided for energy metabolism and requirements. Although the relative stability of whole-body protein turnover has been commented on (Stroud *et al.* 1996), just as we now better appreciate the metabolic basis for the BMR (Rolfe & Brown, 1997) and the multiple cellular and metabolic processes that contribute to, and can account for, changes in the BMR under various pathophysiological conditions (Yu *et al.* 1998), it is now important that we try to use tracers (and other approaches) to describe and quantify more completely the metabolic and regional components of whole-body protein turnover. In his look to the future, Waterlow (1995) states; '... a limited but important objective of future work on whole-body protein turnover would be to find ways of assessing, separately and non-invasively, the responses of what might be called liver-type and muscle-type components. We are not even certain whether these two different types of response correspond to the conventional separation of rapidly and slowly turning over protein pools.' This is certainly a challenge, and among other possible experimental approaches the technique of PET could assist in meeting this goal. Hence, we turn to a brief consideration of short-lived isotopes in nutrition research.

Short-lived isotopes in nutrition research

In the review by Schoenheimer & Rittenberg (1938) it was said: 'No radio-active isotopes of the elements of C, H, N or O are known with a half-life long enough to permit their use for organic synthesis and metabolism experiments.' The C isotope ^{11}C was known then, but only relatively recently has it seemed possible to begin a serious consideration of the application of PET and ^{11}C-labelled tracers in experimental nutritional studies (Young *et al.* 1997). PET combines a high spatial resolution with a very high sensitivity, and is able to quantify the measured radioactivity in absolute terms, i.e. in Bq per pixel.

While the reader is referred to more extensive discussions for further detail (Ter-Pogassian *et al.* 1980; Hoffman & Phelps, 1984; Fowler & Wolf, 1986; Daghighian *et al.* 1990; Christian, 1994; Links, 1994), the basic principles of PET and measurement techniques are as follows. Briefly, proton-rich nuclides decay to a more stable isotope by two possible decay processes; (1) by electron capture, and (2) by positron decay, which is the particular emission of interest to us in the present paper. In this latter process a proton is converted to a neutron, positron (a positive electron), neutrino and energy.

The radionuclides employed in PET have short half-lives (^{11}C 20·4 min, ^{13}N 9·9 min, ^{15}O 2·07 min, ^{18}F 109·7 min) so they must be produced 'on site'. A small compact cyclotron is the accelerator of choice. Since a transformation of chemical elements takes place, for example ^{11}C is produced by the proton irradiation of N, the required nuclide can be obtained with a very high specific activity, i.e. activity of the nuclide (e.g. ^{11}C) : mass of the abundant stable isotope (e.g. ^{12}C). When this high specific activity is maintained during the synthesis of the tracer and it is combined with the high sensitivity of PET cameras this enables the measurement of picomolar concentrations of the tracer.

Although PET has not yet found extensive use in nutritional and metabolic research, its spatial resolution and quantitative features allow quantification of metabolic variables in volumes of tissue as small as 1·0 ml. PET techniques have been validated for measurement of regional blood flow, blood volume, pH, O_2 utilization (Huang *et al.* 1986), and applied in studies of glucose metabolism in the

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Table 7. Brain glucose utilization as estimated using positron-emission tomography, with 2-deoxy- 2[^{18}F]fluoro-D-glucose*

	Cerebral metabolic rate for glucose		
Age (years)	g/d	% TDEE	% *Ra*
0·5	34	21	52
2	70	27	66
5	144	41	90
12	127	22	58
Adult	100	14	44

TDEE, total daily energy expenditure; *Ra*, rate of plasma glucose appearance.
* This summary was developed by D. Bier, Baylor College of Medicine, Houston, TX, USA. It is based on the positron-emission tomography study by Chugani *et al.* (1987), on estimates of TDEE based on doubly-labelled water and a determination of *Ra* by isotopic methods.

brain (Phelps *et al.* 1986), for measuring blood flow (Huang *et al.* 1983; Raichle *et al.* 1983), glucose metabolism and tricarboxylic acid cycle activity in the myocardium (Huang & Phelps, 1986; Schelbert & Schwaiger, 1986), regional glucose uptake (leg, arm and heart; Nutila *et al.* 1993) and amino acid metabolism and protein synthesis (Mazoyer *et al.* 1993).

A vivid example of the valuable application of PET for the study of nutrition and regional substrate metabolism is in terms of glucose utilization by the brain. Using 2-deoxy-2[^{18}F]fluoro-D-glucose, Chugani *et al.* (1987) have quantified local rates of glucose utilization in regions of the brain of subjects, varying in age from the newborn to adults. A summary of some of these findings, which was compiled for us by D. Bier of Baylor College of Medicine, Houston, TX, USA, is presented in Table 7. Further, they are compared with (a) the rates of plasma glucose appearance, as measured using isotopic methods, and (b) the contribution that brain glucose metabolism makes to total daily energy expenditure. Perhaps a number of points might be made; first, the rate of glucose utilization increases after birth reaching adult values by about 2 years, with higher rates in the intervening years; second, the glucose utilization rate by brain accounts for about 20 % of daily energy expenditure during the first year of life and as much as 40–50 % during years 3–5, declining to about 14 % of daily energy expenditure in the adult; third, brain glucose utilization accounts for a sizable proportion of the rate of glucose production at all ages, being about 50 % in the newborn and adult and 90–100 % in the 3–5 year old.

Less-extensive studies using PET have been conducted in relation to amino acid metabolism in peripheral tissues (for example, see Hawkins *et al.* 1989; Planas *et al.* 1992), although this technique is particularly attractive because, in principle, it is possible to make sequential site-specific time-dependent physiological and biochemical measurements within the same subject. More importantly, and again due to its relatively non-invasive nature, PET measurements could be made routine for application in experiments with human subjects. Although there are several other ways to estimate *in vivo* rates of muscle protein synthesis, they tend to be more invasive since they involve either arterio–venous amino acid difference studies, requiring catheterization of an artery and deep vein (Nair *et al.* 1988; Tessari *et al.* 1995) or one or more biopsies of muscle tissue (Nair *et al.* 1988; McNurlan *et al.* 1991; Yarasheski *et al.* 1992), or both, as in the case of the model developed by Wolfe and his coworkers (Biolo *et al.* 1995). For example, in a recent study by the Galveston group (Volpi *et al.* 1998) on the dynamic status of protein synthesis in the *vastus lateralis* muscle of elderly men, three biopsies were taken over a 480 min period, together with blood sampling from peripheral arm veins and the femoral vein and artery. This model is elegant in that it provides estimates of inward and outward amino acid transport, net amino acid balance and indices of protein synthesis and of proteolysis. A less-invasive approach such as that offered by PET is, therefore, an attractive addition to these other approaches. PET offers the opportunity to 'view' protein synthesis not only in skeletal muscle but also, theoretically at least (by realigning the camera), regions such as the brain and liver could also be examined in the same experiment. It is also possible to use a selection of different tracers such as L-[1-^{11}C]tyrosine or L-[1-^{13}C]leucine (Paans *et al.* 1996), although not simultaneously. In the case of a [^{11}C]leucine PET protocol it might be useful to combine it with a simultaneous infusion of L-[1-^{13}C, ^{15}N]leucine so that account might be taken of the conversion of the ^{11}C from leucine to α-ketoisocaproate.

L-[methyl-^{11}C]methionine has been found to be a useful tracer with PET for evaluation of amino acid kinetics (for example, see Stalnacke *et al.* 1982) *in vivo* and for detection of tumours (Kubota *et al.* 1985; Bustany *et al.* 1986; LaFrance *et al.* 1987; Mosskin *et al.* 1987). Thus, with our collaborators at the Massachusetts General Hospital we have explored how L-[methyl-^{11}C]methionine might be used to estimate the rate of mixed protein synthesis in skeletal muscle.

Our initial studies in a canine skeletal muscle model (Hsu *et al.* 1996) suggested that PET with L-[methyl-^{11}C]methionine could be used along with arterial blood sampling and a kinetic model to provide a less-invasive, as well as a regional, assessment of the protein synthetic rate. The protein synthetic rate determined by PET was similar to the results of simultaneous stable-isotope measurements using L-[1-^{13}C-methyl-$^{2}H_3$]methionine. We (Fischman *et al.* 1998) have now extended and refined this model in an investigation with six healthy adult volunteers studied in the post-absorptive state. They received approximately 925 MBq L-[methyl-^{11}C]methionine by intravenous injection, and serial PET images over the anterior and posterior regions of the two thighs, and arterial blood samples were acquired over 90 min. The tissue and metabolite corrected arterial blood time *v.* activity curves were fitted to a three-compartment model, and protein synthetic rate was calculated from the fitted variable values and plasma methionine concentrations, assuming that the rates of protein synthesis and degradation were equal over the 90 min period of study (Table 8). The protein synthetic rate (nmol methionine/min per g muscle tissue) was 0·50 (SE 0·044). When converted to a fractional protein synthesis rate for mixed proteins in muscle the value is 0·125 (SE 0·01) %/h.

Strict comparisons of these results with published values for fractional protein synthesis rate in healthy adults, as obtained from direct tracer-incorporation methods, should be made with caution because of differences due to subject

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Table 1. Human muscle (thigh) protein synthesis assessed using positron-emission tomography with L-[methyl-^{11}C] methionine and fitted to a three compartment model
(Mean values with their standard errors for ten determinations)

	Anterior		Posterior	
Variable	Mean	SE	Mean	SE
$K_{1,2}$ (ml/min per g)	0·0265	0·004	0·0262	0.004
$k_{2,1}$ (/min)	0·0589	0·006	0·0668	0·006
$k_{2,3}$ (/min)	0·0594	0·0002	0·05914	0·0003
PSR (nmol/min per g)	0·531	0·063	0·465	0·059
FSR (%/h)	0·135	0·02	0·116	0·02

K, flux; *k*, exchange rate; PSR, protein synthesis rate; FSR, fractional protein synthesis rate.

characteristics, metabolic state, muscle protein fraction, type and location, the precise analytical measures and assumptions made about the isotopic enrichment of the amino acid precursor. Nevertheless, this fractional protein synthetic rate of 0·125 %/h based on PET compares well with estimates involving direct incorporation of stable-isotope tracers, ranging from about 0·05 %/h (Nair *et al.* 1988; McNurlan *et al.* 1991; Yarasheski *et al.* 1992; Biolo *et al.* 1995) to about 0·1 %/h (Rennie *et al.* 1982; Welle *et al.* 1994; Patterson *et al.* 1997). It seems, therefore, that we have made a reasonable estimation of muscle protein synthesis in healthy adults. It now remains for us to carry out additional studies in well-defined groups of human subjects, such as elderly, malnourished and stressed patients during acute illness and recovery, so that a coherent picture of the status of muscle protein synthesis rates in different muscles and regions and the quantitative impact of various factors on these rates can be assembled.

Finally, with reference to PET, and possibly to imaging techniques as a more general analytical or technical category (see Table 2), we anticipate that this offers new opportunities for understanding (a) how diet and nutritional factors affect central nervous system metabolism and function, and (b) on the other side of the coin, the neurophysiological basis for food choices and eating behaviour. Indeed, this would be an exciting and much needed expanded, if not new, application of 'isotopes in nutrition research'.

Summary and conclusion

Although Schoenheimer had deduced from his early studies of sterol balance and tissue concentration that there was turnover and metabolic conversion of macromolecules, it was his early use of ^{2}H and ^{15}N tracers that gave an unprecedented boost to the study of mammalian intermediary metabolism. These two stable-isotope tracers, as well as ^{13}C and ^{18}O, have contributed enormously to the advances of nutrition research, just as electrophoresis and the polymerase chain reaction, for example, have done for modern molecular biology. Further, not only have elements and compounds intentionally enriched with specific non-radioactive isotopes been invaluable in nutrition research, but the determination of the natural enrichment of biological samples also permits a historical exploration of interesting nutritional and metabolic issues (for example, Boutton *et al.* 1991; Larsen *et al.* 1992; White & Armelagos, 1997). These nuclides are being used increasingly in clinical studies for diagnosis and treatment evaluation; the development of safe stable-isotope-based methods for measuring cell proliferation (Chan & Abramson, 1998; Macallan *et al.* 1998) is an exciting example of one such recent development. Further, Stanley *et al.* (1998) concluded recently, through molecular and *in vitro* studies as well as from theoretical considerations of leucine, glutamate and insulin interactions and urea-cycle metabolism, that a new form of a congenital hyperinsulinism–hyperammonaemia syndrome is caused by mutations in the glutamate dehydrogenase (*EC* 1.4.1.2) gene that impair the control of enzyme activity. Clearly, tracer studies of glutamate, leucine and N metabolism would measurably enhance and extend the interpretation and significance of the molecular and *in vitro* focus of this clinical investigation.

Recently, Koshland (1998) proposed that knowledge of metabolic processes in cells can be roughly divided into three eras, i.e. (1) those of pathway identification (1890–1950), (2) pathway regulation (1950–1980) and (3) pathway quantification (1980–?); he identifies a number of the major scientific contributors to each of these eras. We find this interesting, in the sense that it would be hard to identify the appropriate era for Schoenheimer, since his enduring research contributions have significance within all three of these eras. It is truly difficult to overestimate the impact of Schoenheimer's research on the evolution of biological science.

Finally, it is also difficult to predict the future of isotopes in nutrition research. Winston Churchill (1874–1965) said 'It's a mistake to look too far ahead. Only one link in the chain of destiny can be handled at one time'. In this spirit, therefore, we will not overindulge. However, it is safe to state that the future uses of isotopes in nutrition research and their capacity to help unravel metabolic–physiological–functional aspects of diet and well-being will be different from those in the past and present, because it (the future) has not yet occurred!

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References

Abrams SA (1994) Clinical studies of mineral metabolism in children using stable isotopes. *Journal of Pediatric Gastroenterology and Nutrition* **19**, 151–163.

Aggett PJ (1997) Iron, copper and zinc absorption and turnover; the use of stable isotopes. *European Journal of Pediatrics* **156**, S29–S34.

Arquint P, Kovdelka-Hep M, van der Schoot BH, van der Wal P & de Rooij NF (1994) Micromachined analyzers on a silicon chip. *Clinical Chemistry* **40**, 1805–1809.

Ballmer PE, McNurlan MA, Essen P, Anderson SE & Garlick PJ (1995*a*) Albumin synthesis rates measured with [$^{2}H_{5}$-ring]phenylalanine are not responsive to short-term intravenous nutrients in healthy humans. *Journal of Nutrition* **125**, 512–519.

Ballmer PE, McNurlan MA, Hulter HN, Anderson SE, Garlick PJ & Krapf R (1995*b*) Chronic metabolic acidosis decreases albumin synthesis and induces negative nitrogen balance in humans. *Journal of Clinical Investigation* **95**, 39–45.

Berthold HK, Hachey DL, Reeds PJ, Thomas OP, Hoeksema S & Klein PD (1991) Uniformly ^{13}C-labeled algae protein used to determine amino acid essentiality *in vivo*. *Proceedings of the National Academy of Sciences USA* **88**, 8091–8095.

Berthold HK, Jahoor F, Klein PD & Reeds PR (1995*a*) Estimates of the effect of feeding on whole-body protein degradation in women vary with the amino acid used as tracer. *Journal of Nutrition* **125**, 2516–2527.

Berthold HK & Klein PD (1991) Zum 50 Todestag von Rudolf Schoenheimer. Er revolutionerte die Kenntnisse vom Stoffwechsel (On the 50th anniversary of Rudolf Schoenheimer's death. He revolutionized the knowledge of metabolism). *Deutsches Arzteblatt-Arztliche Mitteilungen* **68**, 3058–3060.

Berthold HK, Reeds PJ & Klein PD (1995*b*) Isotopic evidence for the differential regulation of arginine and proline synthesis in man. *Metabolism* **44**, 466–473.

Beylot M (1994) The use of stable isotopes and mass spectrometry in studying lipid metabolism. *Proceedings of the Nutrition Society* **53**, 355–362.

Bier DM (1981) Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. *Diabetes/Metabolism Reviews* **5**, 111–132.

Bier DM (1987) The use of stable isotopes in metabolic investigation. *Ballière's Clinical Endocrinology and Metabolism* **1**, 817–836.

Bier DM (1997) Stable isotopes in biosciences, their measurement and models of amino acid metabolism. *European Journal of Pediatrics* **156**, S2–S8.

Biolo G, Flemming RY, Moggi SP & Wolfe RR (1995) Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *American Journal of Physiology* **268**, E75–E84.

Bloom FE (1997) Breakthroughs 1997. *Science* **278**, 2029.

Boutton TW, Lynott MJ & Bumsted MP (1991) Stable carbon isotopes and the study of prehistoric human diet. *Critical Reviews in Food Science and Nutrition* **30**, 373–385.

Brunengraber H, Kelleher JK & des Rosiers C (1997) Applications of mass isotomer analysis to nutrition research. *Annual Reviews of Nutrition* **17**, 559–596.

Buckley WT (1988) The use of stable isotopes in studies of mineral metabolism. *Proceedings of the Nutrition Society* **47**, 407–416.

Bustany P, Chatel M, Derlon JM, Sgouropoulos P, Soussaline F & Syrota A (1986) Brain tumor protein synthesis and histological grades; a study by positron emission tomography (PET) with C-11-L-methionine. *Journal of Neuro-oncology* **3**, 397–404.

Castillo L, Beaumier L, Ajami AM & Young VR (1996) Whole body nitric oxide synthesis in healthy men determined from [^{15}N]arginine-to-[^{15}N]citrulline labeling. *Proceedings of the National Academy of Sciences USA* **93**, 11460–11465.

Castillo L, Chapman TE, Sànchez M, Yu Y-M, Burke JF, Ajami AM, Vogt J & Young VR (1993) Plasma arginine and citrulline kinetics in adults given adequate and arginine-free diets. *Proceedings of the National Academy of Sciences USA* **90**, 7749–7753.

Chan P & Abramson FP (1998) Measuring DNA synthesis with [1-^{13}C]glycine. *Analytical Chemistry* **70**, 1664–1669.

Christian PE (1994) Physics of nuclear medicine. In *Nuclear Medicine. Technology and Techniques,* 3rd ed., pp. 35–52 [DR Bernier, PE Christian and JK Langan, editors]. St Louis, MO: Mosby.

Chugani HT, Phelps ME & Mazziotta JC (1987) Positron emission tomography study of human brain functional development. *Annals of Neurology* **22**, 487–497.

Ciechanover A & Schwartz AL (1998) The ubiquitin-proteasome pathway: the complexity and myriad functions of proteins death. *Proceedings of the National Academy of Sciences USA* **95**, 2727–2730.

Clifford AA & Müller H-G (editors) (1998) Mathematical modeling in experimental nutrition. New York: Plenum Press.

Cline GW, Jucker BM, Trajanoski Z, Rennings AJM & Shulman GI (1998) A novel ^{13}C NMR method to assess intracellular glucose concentration in muscle, in vivo. *American Journal of Physiology* **274**, E381–E389.

Cobelli C, Bier DM & Ferannini E (1990) Modelling glucose metabolism: theory and practice. *Hormone and Metabolic Research* **24**, Suppl., 1–10.

Cobelli C & Caumo A (1998) Using what is accessible to that which is not: necessity of model of systems. *Metabolism* **47**, 1009–1035.

Cobelli C, Saccomoni MP, Tessari P, Biolo G, Luzi L & Matthews DE (1991) Compartmental model of leucine kinetics in humans. *American Journal of Physiology* **261**, E539–E550.

Cobelli C, Toffolo G, Bier DM & Nosadini R (1987) Models to interpret kinetic data in stable isotope tracer studies. *American Journal of Physiology* **253**, E551–E564.

Coburn SP & Townsend DW (editors) (1996) Mathematical modeling in experimental nutrition: vitamins, proteins, methods. *Advances in Food and Nutrition Research* **40**, 1–362.

Cohn M (1995) Some early tracer experiments with stable isotopes. *Protein Science* **4**, 2444–2447.

Crews HM, Ducros V, Eagles J, Mellon FA, Kastenmayer P, Luten JB & McGaw BA (1994) Mass spectrometric methods for studying nutrient mineral and trace element absorption and metabolism in humans using stable isotopes. A review. *Analyst* **119**, 2491–2514.

Daghighian F, Sumida R & Phelps ME (1990) PET imaging: an overview and instrumentation. *Journal of Nuclear Medicine Techniques* **18**, 5–15.

Demmelmair H, Sauerwold T, Koletzko B & Richter T (1997) New insights into lipid and fatty acid metabolism via stable isotopes. *European Journal of Pediatrics* **156**, S70–S74.

Drexler HCA (1997) Activation of the cell death program by inhibition of proteasome function. *Proceedings of the National Academy of Sciences USA* **94**, 855–860.

Dudley MA, Burren DG, Wykes LJ, Toffolo G, Cobelli C, Nichols BL, Rosenberger J, Jahoor F & Reeds PJ (1998) Protein kinetics determined in vivo with a multi-tracer, single-sample protocol: application to lactase synthesis. *American Journal of Physiology* **274**, G591–G598.

Effenhauser CS & Manz A (1994) Miniaturizing a whole analytical laboratory down to chip size. *American Laboratory* September, 15–18.

El-Khoury AE, Fukagawa NK, Sànchez M, Tsay RH, Gleason RE, Chapman TE & Young VR (1994) Validation of the tracer-

balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-^{13}C]leucine and [^{15}N-^{15}N]urea. *American Journal of Clinical Nutrition* **59**, 1000–1011.

Evans JE, Browne TR, Kasdon DL, Szabo GK, Evans BA & Greenblatt DJ (1985) Staggered stable isotope technique for study of drug distribution. *Journal of Clinical Pharmacology* **25**, 309–312.

Fischman AJ, Yu Y-M, Livini E, Bobech JW, Young VR, Alpert NM & Tompkins RG (1998) Muscle protein synthesis by positron emission tomography with L-[methyl-^{11}C]methionine in adult humans. *Proceedings of the National Academy of Sciences USA* **95**, 12793–12798.

Fitzpatrick SM, Hetherington HP, Behar KL & Shulman RG (1990) The flux from glucose to glutamate in the rat brain in vivo as determined by ^{2}H-observed, ^{13}C-edited NMR spectroscopy. *Journal of Cerebral Blood Flow and Metabolism* **10**, 170–179.

Flatt JP (1995) McCollum award lecture 1995: Diet, lifestyle and weight maintenance. *American Journal of Clinical Nutrition* **62**, 820–836.

Folin O (1905) A theory of protein metabolism. *American Journal of Physiology* **13**, 117–138.

Fowler JS & Wolf AP (1986) Positron emitter-labeled compounds: priorities and problems. In *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain,* pp. 391–450 [ME Phelps, TC Mazziotta and HR Schelbert, editors]. New York: Raven Press.

Fukagawa N, Yu Y-M & Young VR (1998) Methionine-cysteine kinetics at different intakes of methionine and cystine in elderly men and women. *American Journal of Clinical Nutrition* (In the Press).

Garlick PJ & Millward DJ (1972) An appraisal of techniques for the determination of protein turnover in vivo. *Proceedings of the Nutrition Society* **31**, 249–255.

Ghislain M, Udvardy A & Mann C (1993) *S. cerevisiae* 26S protease mutants arrest cell division in G2/metaphase. *Nature* **366**, 358–362.

Grindstaff PD, Kreider R, Bishop R, Wilson M, Wood L, Alexander C & Almada A (1997) Effects of creatine supplementation on respective sprint performance and body composition in competitive swimmers. *International Journal of Sports Nutrition* **7**, 330–346.

Hadd AG, Raymond DE, Halliwell JW, Jacobson SC & Ramsey JM (1997) Microchip device for performing enzyme assays. *Analytical Chemistry* **69**, 3407–3412.

Halliday D & Read WW (1981) Mass spectrometric assay of stable isotopic enrichment for the estimation of protein turnover in man. *Proceedings of the Nutrition Society* **40**, 321–324.

Halliday D & Rennie MJ (1982) The use of stable isotopes for diagnosis and clinical research. *Clinical Science* **63**, 485–496.

Harding CV, France J, Song R, Farah JM, Chatterjee S, Igbal M & Siman R (1995) Novel dipeptide aldehydes are proteasome inhibitors and block the MHC-1 antigen-processing pathway. *Journal of Immunology* **155**, 1767–1775.

Hawkins RA, Huang S-C, Barrio JR, Keen RE, Feng D, Massiotta JC & Phelps ME (1989) Estimation of local cerebral protein synthesis rates with 1-[1-^{11}C]leucine and PET: Methods, modal and results in animals and humans. *Journal of Cerebral Blood Flow and Metabolism* **9**, 446–460.

Hellerstein MK & Neese RA (1992) Mass isotomer distribution analysis: A technique for measuring biosynthesis and turnover of polymers. *American Journal of Physiology* **263**, E998–E1001.

Hellerstein MK, Neese RA, Linfort P, Christiansen M, Turner S & Letscher A (1997) Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans: a stable isotope study. *Journal of Clinical Investigation* **100**, 1305–1319.

Hellerstein MK, Schwarz JM & Neese R (1996) Regulation of hepatic de novo lipogenesis in humans. *Annual Reviews of Nutrition* **16**, 523–527.

Hevesey G (1923) The absorption and translocation of lead by plants. *Biochemical Journal* **17**, 439–445.

Hoffman EJ & Phelps ME (1986) Positron emission tomography: Principles and quantitation. In *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain,* pp. 237–286 [ME Phelps, TC Mazziotta and HR Schelbert, editors]. New York: Raven Press.

Hovorka R, Eckland DJA, Halliday D, Lettis S, Robinson C, Bannister P, Young MA & Bye A (1997) Constant infusion and bolus injection of stable-label tracer given reproducible and comparable fasting HGO. *American Journal of Physiology* **273**, E192–E201.

Hsieh S & Jorgensen JW (1997) Determination of enzyme activity in single bovine adrenal medullary cells by separation of isotopically labeled catecholamines. *Analytical Chemistry* **69**, 3907–3914.

Hsu H, Yu YM, Babich JW, Burke JF, Livini E, Tompkins RG, Young VR, Alpert NM & Fischman AJ (1996) Measurement of muscle protein synthesis by positron emission tomography with L-[*methyl*-^{11}C]methionine. *Proceedings of the National Academy of Sciences USA* **93**, 1841–1846.

Huang SC, Carson RE, Hoffman EJ, Carson J, MacDonald N, Barrio JR & Phelps ME (1983) Quantitative measurement of local cerebral blood flow in humans by positron computed tomography and O-15 water. *Journal of Cerebral Blood Flow and Metabolism* **3**, 141–153.

Huang SC, Feng DG & Phelps ME (1986) Model dependency and estimation reliability in measurement of cerebral oxygen utilization rate with oxygen-15 and dynamic positron emission tomography. *Journal of Cerebral Blood Flow and Metabolism* **6**, 105–119.

Huang SC & Phelps ME (1986) Principles of tracer kinetic modeling in positron emission tomography and autoradiography. In *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain,* pp. 287–346 [ME Phelps, TC Mazziotta and HR Schelbert, editors]. New York: Raven Press.

Irving CS, Thomas MR, Malphus EW, Marks L, Wong WW, Boutton TW & Klein PD (1986) Lysine metabolism and protein metabolism in young women. *Journal of Clinical Investigation* **77**, 1321–1331.

Jackson AA (1991) The glycine story. *European Journal of Clinical Nutrition* **45**, 59–65.

Janghorbani M (1984) Stable isotopes in nutrition and food science. *Progress in Food and Nutrition Science* **8**, 303–332.

Janghorbani M & Young VR (1982) Advances in the use of stable isotopes in minerals in human studies. *Federation Proceedings* **41**, 2702–2708.

Kaehler T (1994) Nanotechnology: basic concepts and definitions. *Clinical Chemistry* **40**, 1797–1799.

Kamen M (1963) The early history of carbon-14. *Journal of Chemical Education* **40**, 234–242.

Katz J & Lee WNP (1991) Application of mass isotomer analysis for determination of pathways of glycogen synthesis. *American Journal of Physiology* **261**, E332–E336.

Kohler RE Jr (1977) Rudolf Schoenheimer, isotopic tracers and biochemistry in the 1930's. In *Historical Studies in the Physical Sciences,* vol. 8, pp. 257–298 [R McCormmach and L Pyenson, editors]. Baltimore, MD: Johns Hopkins University Press.

Koshland DE Jr (1992) The molecule of the year. *Science* **258**, 1861.

Koshland DE Jr (1998) The era of pathway quantification. *Science* **280**, 852–853.

Kreider RB, Ferreira M, Wilson M, Grindstaff P, Plisk S, Reinardy J, Cantler E & Almada AL (1998) Effects of creatine supplementation on body composition, strength and sprint performance. *Medicine and Science in Sports and Exercise* **30**, 73–82.

Kubota K, Matsuzawa T, Ito M, Ito K, Fujiwara T, Abe Y, Toshioka S, Fukuda H, Hatazawa J, Iwata R, Watanuki S & Ido T (1985) Lung tumor imaging by positron emission tomography using C-11-L-methionine. *Journal of Nuclear Medicine* **26**, 37–42.

LaFrance ND, O'Tauma L, Villemagne V, Williams J, Douglas K, Dannals RF, Ravert H, Wilson A, Drew H, Links J, Wong D, Carson B, Brem H, Strauss L & Wagner HN (1987) Quantitative imaging and follow-up experience of C-11-L-methionine accumulation in brain tumors with positron emission tomography. *Journal of Nuclear Medicine* **26**, 37–42.

Lagerwerf FM, Wever RMF, van Rijn HJM, Versluis C, Herrma W, Haverkamp J, Koomans HA, Robelink TJ & Boer P (1998) Assessment of nitric oxide production by measurement of [^{15}N]citrulline enrichment in human plasma using high performance liquid chromatography-mass spectrometry. *Analytical Biochemistry* **257**, 45–52.

Landau BR, Chandramouli V, Schumann WC, Ekberg K, Kumaran K, Kalhan SC & Wahren J (1995) Estimates of Krebs cycle activity and contributions of gluconeogenesis to hepatic glucose production in fasting healthy subjects and IDDM patients. *Diabetologia* **38**, 831–838.

Larsen CS, Schoeninger MJ, van der Merwe NJ, Moore KM & Lee-Throp JA (1992) Carbon and nitrogen isotopic signatures of human dietary change in the Georgia Bight. *American Journal of Physical Anthropology* **89**, 197–214.

Lifson N, Gordon GB & McClintock R (1955) Measurement of total carbon dioxide by means of $D_2{}^{18}O$. *Journal of Applied Physiology* **7**, 704–710.

Links JM (1994) Instrumentation. In *Nuclear Medicine. Technology and Techniques,* 3rd ed., pp. 53–85 [DR Bernier, PR Christian and JK Langan, editors]. St Louis, MO: Mosby.

Macallan DC, Fullerton CA, Neese RA, Haddock K, Park SS & Hellerstein MK (1998) Measurement of cell proliferation by labelling of DNA with stable isotope-labeled glucose: Studies *in vitro*, in animals, and in humans. *Proceedings of the National Academy of Sciences USA* **95**, 708–713.

Macallan DC, Smith LM, Ferber J, Milne E, Griffin GE, Benjamin N & McNurlan M (1997) Measurement of NO synthesis in humans by L-[$^{15}N_2$]arginine: application to the response to vaccination. *American Journal of Physiology* **272**, R1888–R1896.

McEuen PL (1998) Carbon-based electronics. *Nature* **393**, 15–17.

McNurlan MA, Essen P, Heys SD, Buchan V, Garlick PJ & Wernerman J (1991) Measurement of protein synthesis in human skeletal muscle: further investigation of the flooding technique. *Clinical Science* **81**, 557–564.

Marchini JS, Castillo L, Chapman TE, Vogt J, Ajami A & Young VR (1993) Phenylalanine conversion to tyrosine: Comparative determination with L-[ring-$^2H_5$]phenylalanine and L[l-^{13}C]phenylalanine as tracers in man. *Metabolism* **42**, 1316–1322.

Matthews DE & Bier DM (1983) Stable isotope methods for nutritional investigation. *Annual Reviews of Nutrition* **3**, 309–339.

Matthews DE, Bier DM, Rennie MJ, Edwards RHT & Halliday D (1981) Regulation of leucine metabolism in man: a stable isotope study. *Science* **214**, 1129–1131.

Mazoyer BM, Heiss WD & Comar D (1993) *PET Studies on Amino Acid Metabolism and Protein Synthesis.* Dordrecht, The Netherlands: Kluwer Academic Publishers.

Mbikay M, Todros H, Isheda N, Lerner CP, Lamirande E, de Chen A, El-Alfy M, Clermont Y, Seidah NG, Chrétien M, Gagnon C & Simpson EM (1997) Impaired fertility in mice deficient for the testicular germ-cell protease PC4. *Proceedings of the National Academy of Sciences USA* **94**, 6842–6846.

Miller RJ, Jahoor F & Jaksic T (1995*a*) Decreased cysteine and proline synthesis in parenterally-fed, premature infants. *Journal of Pediatric Surgery* **30**, 953–957.

Miller RJ, Jahoor F, Reeds PJ, Heird WC & Jaksic T (1995*b*) A new stable isotope technique to assess human neonatal amino acid synthesis. *Journal of Pediatric Surgery* **30**, 1325–1329.

Millward DJ, Price G, Pacy PJH & Halliday D (1991) Whole body protein and amino acid turnover in man: what can we measure with confidence? *Proceedings of the Nutrition Society* **50**, 197–216.

Molnar JA, Alpert NM, Burke JF & Young VR (1986) Synthesis and degradation rates of collagen in vivo in whole skin of rats, studied with $^{18}O_2$ labelling. *Biochemical Journal* **240**, 431–435.

Mosskin M, von Hjolst H, Bergstrom M, Collins VP, Ericksson L, Johnstrom P & Norén G (1987) Positron emission tomography with ^{11}C-methionine and computed tomography of intracranial tumors compared with histopathologies examination of multiple biopsies. *Acta Radiologa* **28**, 673–681.

Nair KS, Welle SL, Halliday D & Campbell RG (1988) Effect of β-hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. *Journal of Clinical Investigation* **82**, 198–205.

Nuutila P, Knuuti J, Ruotsalainen U, Koivisto VA, Eronen E, Teräs M, Bergman J, Haaparanta M, Voipio-Pulkka L-M, Viikari J, Rönnemaa T, Wegelius U & Yki-Järvomem J (1993) Insulin resistance is localized to skeletal but not heart muscle in type I diabetes. *American Journal of Physiology* **264**, E756–E763.

Neese RA, Schwartz JM, Faix D, Turnver S, Letscher A, Vu D & Hellerstein MK (1995) Gluconeogenesis and intrahepatic triose phosphate flux in response to fasting or substrate loads. Application of the mass isotomer distribution analysis technique with testing of assumptions and potential problems. *Journal of Biological Chemistry* **27**, 14452–14463.

Novotny JA, Dueker SR, Zech LA & Clifford AJ (1995) Compartmental analysis of the dynamics of β-carotene metabolism in an adult volunteer. *Journal of Lipid Research* **36**, 1825–1838.

Olson RE (1997) The dynamic state of body constituents (Schoenheimer, 1939). *Journal of Nutrition* **127**, 1041S–1043S.

Paans AM, Prium J, van Waarde A, Willemsen AT & Vaalburg W (1996) Radiolabeled tyrosine for the measurement of protein synthesis rate in vivo by positron emission tomography. *Ballière's Clinical Endocrinology and Metabolism* **10**, 497–510.

Palombella VJ, Rando OJ, Goldberg AL & Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NK-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* **78**, 773–785.

Pannemans DL, Wagenmakers AJ, Westerterp KR, Schaafima G & Halliday D (1997) The effect of an increase of protein intake on whole-body protein turnover in elderly women is tracer dependent. *Journal of Nutrition* **127**, 1788–1794.

Patterson BW (1997) Use of stable isotopically labeled tracers for studies of metabolic kinetics: An overview. *Metabolism* **46**, 322–329.

Patterson BW, Zhang XJ, Chen Y, Klein S & Wolfe RR (1997) Measurement of very low stable isotope enrichments by gas chromatography/mass spectrometry: application to measurement of muscle protein synthesis. *Metabolism* **46**, 943–948.

Phelps ME, Massiotta JC & Schelbert HR (editors) (1986) In *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain and Heart.* New York: Raven Press.

Picou D & Taylor-Roberts T (1969) The measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amounts of dietary protein. *Clinical Science* **36**, 283–296.

Picou D, Taylor-Roberts T & Waterlow JC (1969) The measurement of total protein synthesis and nitrogen flux in man by constant infusion of ^{15}N-glycine. *Proceedings of the Physiological Society* **200**, 52P–53P.

Planas AM, Prenant C, Mazoyer BM, Comar D & DiGiamberardino L (1992) Regional cerebral L-[^{14}C-*Methyl*]methionine incorporation into proteins: Evidence for methionine recycling in the rat brain. *Journal of Cerebral Blood Flow and Metabolism* **12**, 603–612.

Raichle ME, Martin WRW, Herscovitch P, Mintum MA & Markham J (1983) Brain blood flow measured with intravenous $H_2{}^{15}O$. II. Implementation and validation. *Journal of Nuclear Medicine* **24**, 790–798.

Ratner S (1979) The dynamic state of body proteins. *Annals of the New York Academy of Sciences* **325**, 189–209.

Rennie MJ, Edwards RH, Halliday D, Matthews DE, Wolman SL & Millward DJ (1982) Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clinical Science* **63**, 519–523.

Rittenberg D, Keston AS, Rosebury F & Schoenheimer R (1939) Studies in protein metabolism. II. The determination of nitrogen isotopes in organic compounds. *Journal of Biological Chemistry* **127**, 291–299.

Rittenberg D & Schoenheimer R (1935) Deuterium as an indicator in the study of intermediary metabolism. II. Methods. *Journal of Biological Chemistry* **111**, 169–174.

Rittenberg D & Schoenheimer R (1939) Studies in protein metabolism. VI. Hippuric acid formation studied with the aid of the nitrogen isotope. *Journal of Biological Chemistry* **127**, 329–331.

Robertson JS (editor) (1983) Historical Development. In *The Compartmental Distribution of Radiotracers,* pp. 1–9. Boca Raton, FL: CRC Press.

Rolfe DFS & Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological Reviews* **77**, 731–758.

Roth E (1997) Critical evaluation of the rise and analysis of stable isotopes (Technical Report). *Pure and Applied Chemistry* **69**, 1753–1828.

Rousell R, Cartier PG, Robert J-J, Velho G & Block G (1998) ^{13}C/^{31}P NMR studies of glucose transport in human skeletal muscle. *Proceedings of the National Academy of Sciences USA* **95**, 1313–1318.

Saccomani MP, Bonadonna RC, Caveggion E, DeFranzo RA & Cobelli C (1995) Bicarbonate kinetics in humans: identification and validation of a three compartment model. *American Journal of Physiology* **269**, E183–E192.

Schelbert RR & Schwaiger M (1986) PET studies of the heart. In *Positron Emission Tomography and Autoradiography. Principles and Applications for the Brain and Heart,* pp. 581–661 [ME Phelps, TC Mazziotta and HR Schelbert, editors]. New York: Raven Press.

Schoeller DA & van Santen E (1982) Measurement of energy expenditure in humans by doubly-labeled water method. *Journal of Applied Physiology* **53**, 955–959.

Schoenheimer R (1931) New contributions in sterol metabolism. *Science* **74**, 579–584.

Schoenheimer R (1936–7) The investigation of intermediary metabolism with the aid of heavy hydrogen. *The Harvey Lectures,* series 32, pp. 122–144. Baltimore, MD: Williams & Wilkins Co.

Schoenheimer R (1942) *The Dynamic State of Body Constituents.* Cambridge, MA: Harvard University Press.

Schoenheimer R & Ratner S (1939) Studies in protein metabolism. III. Synthesis of amino acids containing isotopic nitrogen. *Journal of Biological Chemistry* **127**, 301–313.

Schoenheimer R, Ratner S & Rittenberg D (1939*a*) Studies in protein metabolism. X. The metabolic activity of body proteins investigated with (-)-leucine containing two isotopes. *Journal of Biological Chemistry* **130**, 703–732.

Schoenheimer R, Ratner S & Rittenberg D (1939*b*) Studies in protein metabolism. VII. The metabolism of tyrosine. *Journal of Biological Chemistry* **127**, 334–344.

Schoenheimer R & Rittenberg D (1935) Deuterium as an indicator in the study of intermediary metabolism. III. The role of the fat tissues. *Journal of Biological Chemistry* **111**, 175–181.

Schoenheimer R & Rittenberg D (1938) The application of isotopes to the study of intermediary metabolism. *Science* **87**, 221–226.

Seidell JC (1998) Dietary fat and obesity: an epidemiologic perspective. *American Journal of Clinical Nutrition* **67**, Suppl., 546S–550S.

Shulman GI (1997) Nuclear magnetic resonance studies of liver and muscle glycogen metabolism in humans. In *Emerging Technologies for Nutrition Research,* pp. 259–272 [S Carlson-Newberry and RB Costello, editors]. Washington, DC: National Academy Press.

Sibson NR, Dhankar A, Masson GF, Behar KL, Rothman DL & Shulman RG (1997) In vivo ^{13}C NMR measurements of cerebral glutamine synthesis as evidence for glutamate-glutamine cycling. *Proceedings of the National Academy of Sciences USA* **94**, 2699–2704.

Stalnacke C-G, Jones B, Langstron B, Lundquist H, Malmborg P, Sjorberg S & Larsson B (1982) Short-lived radionuclides in nutritional physiology. A model study with L-[*Me*-^{11}C]methionine in the pig. *British Journal of Nutrition* **47**, 537–545.

Stanley CA, Lieu YK, Hsu BYL, Burlina BB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E & Poncz M (1998) Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *New England Journal of Medicine* **338**, 1352–1357.

Storch KJ, Wagner DA, Burke JF & Young VR (1988) Quantitative study in vivo of methionine cycle in humans using [methyl-$^2H_2$] and [1-^{13}C]methionine. *American Journal of Physiology* **255**, E322–E331.

Stroud MA, Jackson AA & Waterlow JC (1996) Protein turnover rates of two human subjects during an unassisted crossing of Antarctica. *British Journal of Nutrition* **76**, 165–174.

Ter-Pogossian MM, Raichle ME & Sobel BE (1980) Positron-emission tomography. *Scientific American* **243**, 171–181.

Tessari P, Inchiostro S, Zanetti M & Barazzoni R (1995) A model of skeletal muscle leucine kinetics measured across the forearm. *American Journal of Physiology* **269**, E127–E136.

Treacy E, Pitt JJ, Seller K, Thompson GN, Ramus J & Cotton RG (1996) In vivo disposal of phenylalanine in phenylketonuria: a study of two siblings. *Journal of Inherited Metabolic Disease* **19**, 595–602.

Urey HC, Brickwedde FG & Murphy GM (1932) A hydrogen isotope of mass 2. *Physical Reviews* **40**, 1–15.

Volek JS, Kraemer WJ, Bush JA, Boetes M, Incledan T, Clark KL & Lynch JM (1997) Creatine supplementation enhances muscular performance during high-intensity resistance exercise. *Journal of the American Dietetic Association* **97**, 765–770.

Volpi E, Ferrando AA, Yeckel CW, Tipton KD & Wolfe RR (1998) Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *Journal of Clinical Investigation* **101**, 2000–2007.

Wastney ME, Subramanian S, Broering N & Boston R (1997) Using models to explore whole-body metabolism and accessing models through a model library. *Metabolism* **46**, 330–332.

Waterlow JC (1995) Whole-body protein turnover in humans – past, present, and future. *Annual Reviews of Nutrition* **15**, 57–92.
Waterlow JC, Garlick PJ & Millward DJ (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: North Holland Publishing Company.
Welle S, Thornton C, Statt M & McHenry B (1994) Postprandial myofibrillar and whole body protein synthesis in young and old subjects. *American Journal of Physiology* **267**, E599–E604.
White CD & Armelagos GJ (1997) Osteopenia and stable isotope ratios in bone collagen of Nubian female mummies. *American Journal of Physical Anthropology* **103**, 185–189.
Wiechert W & de Graaf AA (1996) In vivo stationary flux analysis by ^{13}C labelling experiments. *Advances in Biochemical Engineering* **54**, 109–154.
Willett WC (1998) Is dietary fat a major determinant of body fat? *American Journal of Clinical Nutrition* **67**, Suppl., 556S–562S.
Wilson EK (1998) Instant DNA detection. *Chemical Engineering and News* **76**, 47–49.
Wolfe RR (1992) *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York: Wiley-Liss.
Wong WW (1996) Total energy expenditure of free-living humans can be estimated with the doubly labeled water method. *Advances in Food and Nutrition Research* **40**, 171–180.
Woolley AT, Lao K, Glazer AN & Mothies RA (1998) Capillary electrophoresis chips with integrated electrochemical detection. *Analytical Chemistry* **70**, 684–688.
Yang L-Y, Kuksis A, Myher JJ & Steiner G (1996) Contributions of de novo fatty acid synthesis to very low density lipoprotein triacylglycerols: evidence from mass isotopomer distribution analysis of fatty acids synthesized from [$^2H_6$]ethanol. *Journal of Lipid Research* **37**, 262–274.
Yarasheski KE, Campbell JA, Smith K, Rennie MJ, Holloszy JO & Bier DM (1992) Effect of growth hormone and resistance exercise on muscle growth in young men. *American Journal of Physiology* **262**, E261–E267.
Young VR, Yu YM, Hsu H, Babich JW, Alpert N, Tompkins RG & Fischman AJ (1997) Combined stable isotope-positron emission tomography for in vivo assessment of protein metabolism. In *Emerging Technologies for Nutrition Research*, pp. 231–257 [S Carlson-Newberry and RB Costello, editors]. Washington, DC: National Academy Press.
Yu Y-M, Burke JF, Vogt JA, Chambers L & Young VR (1992) Splanchnic and whole body L-[1-^{13}C, ^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. *American Journal of Physiology* **262**, E687–E694.
Yu Y-M, Tompkins RG, Ryan CM & Young VR (1998) The metabolic basis for the rise in energy expenditure in severely burned patients. *Journal of Parenteral and Enteral Nutrition* (In the Press).