The use of stable isotopes in studies of mineral metabolism

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In recent years stable isotopes of metallic and semi-metallic elements have become popular as tracers in studies of nutrition and metabolism. There are several recent reviews on the use of stable isotopes of the mineral elements (Janghorbani & Young, 1980; Janghorbani *et al.* 1985). The orientation of these reviews has tended toward neutron-activation analysis in conjunction with the faecal isotope balance (faecal-monitoring) technique. The present discussion will be orientated toward mass spectrometry and the use of stable isotopes in techniques other than faecal isotope balance.

Stable isotopes have been used in studies of nutrition and metabolism since Schoenheimer & Rittenberg (1935) published their first paper on deuterated stearic acid. Since that time, the vast majority of stable-isotope research has utilized the isotopes of hydrogen, carbon, nitrogen and oxygen, with at least 2400 papers using ^{2}H, ^{13}C, ^{15}N and ^{18}O being published between 1971 and 1978 (Klein & Klein, 1979). The natural ratios (rare isotope:abundant isotope) are usually smaller for H, C, N and O than the natural ratios for the metallic and semi-metallic elements. The low natural ratios combined with relatively large sample sizes and the high efficiency of gas-isotope-ratio mass spectrometry (GIRMS) yield analytical precisions (2 × relative SD) varying from 10^{-3} to 10^{-2}% for $^{2}H:^{1}H$, $^{13}C:^{12}C$, $^{15}N:^{14}N$ and $^{18}O:^{16}O$ (Hachey *et al.* 1987). In contrast, analytical precision for isotope-ratio analysis of the mineral elements in recent biological-tracer studies varied from about 0·1 to 10% (from Table 1). Analytical precision is an important factor in the design of an experiment because it determines the number of times a tracer may be diluted before it can no longer be detected. Nevertheless, the less favourable analytical precision available for the mineral elements is adequate for many studies and may be offset by opportunities for multiple-isotope-tracer experiments.

Analytical techniques

A variety of mass-spectrometry techniques have been utilized for stable-isotope-tracer analysis (Hachey *et al.* 1987). Techniques which have been utilized recently in tracer studies of mineral nutrition or metabolism have been listed in order of increasing SD for isotope-ratio determination (Table 1). The minimum ratio above natural abundance which can be detected is determined by the SD for the ratio (usually 2 × SD).

In order to compare analytical techniques *per se*, a normalized SD was calculated and included in Table 1. The variance of the number of ions colliding with the detector of a mass spectrometer per unit time is, according to the Poisson probability function, equal to the mean number of ions. Consequently, small isotope ratios can be determined with a smaller SD than large isotope ratios and, also, the SD of an isotope ratio is a function of the total number of ions analysed. Thus:

$$\text{normalized SD} = \frac{\text{observed SD}}{\{R(N_V + N_W)/N_W^2\}^{0.5}} \quad (1)$$

where the denominator of eqn (1) is the theoretical SD based on the Poisson probability function and estimated by a standard method for non-linear combinations of variables

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Table 1. *Analytical precision obtained with mass spectrometric analysis of stable-isotope ratios in metabolism studies**

Method	Isotope ratio	Natural ratio	SD	Normalized SD†	Source
FABMS	$^{42}Ca:^{40}Ca$	0·0067	0·000016	0·43	Smith *et al.* (1985)
TIMS	$^{70}Zn:^{68}Zn$	0·033	0·000043	0·51	Janghorbani *et al.* (1981)
ICPMS	$^{70}Zn:^{68}Zn$	0·033	0·00047	5·6	Serfass *et al.* (1986)
ICPMS	$^{82}Se:^{78}Se$	0·391	0·00058	1·5	W. Buckley, K. Koenig and D. Godfrey (unpublished results)
ICPMS	$^{77}Se:^{78}Se$	0·322	0·00059	1·8	W. Buckley, K. Koenig and D. Godfrey (unpublished results)
TIMS	$^{65}Cu:^{63}Cu$	0·447	0·00081	1·8	Turnlund *et al.* (1985)
ICPMS	$^{67}Zn:^{68}Zn$	0·221	0·0013	5·1	Serfass *et al.* (1986)
ICPMS	$^{58}Fe:^{57}Fe$	0·151	0·0015	7·5	Ting & Janghorbani (1986)
EIMS	$^{67}Zn:^{64}Zn$	0·084	0·0020	14	Johnson (1982)
EIMS	$^{54}Fe:^{56}Fe$	0·063	0·0024	20	Johnson (1982)
EIMS	$^{65}Cu:^{63}Cu$	0·447	0·0039	9·0	Buckley *et al.* (1982)
GCMS	$^{76}Se:^{80}Se$	0·181	0·0051	23	K. Koenig, W. Buckley and J. Shelford (unpublished results)
EIMS	$^{65}Cu:^{63}Cu$	0·447	0·0085	20	Johnson (1982)

FABMS, fast-atom-bombardment mass spectrometry; TIMS, thermal-ionization mass spectrometry; ICPMS, inductively-coupled-plasma mass spectrometry; EIMS, electron-impact-ionization mass spectrometry with sample introduction by solid probe; GCMS, gas chromatography–mass spectrometry.

*When SD was not provided in the references cited or when SD referred to ion-intensity ratio for complex ions, SD was calculated from residual SD and the natural-isotope ratio.

†For description, see p. 407.

(Kendall & Stuart, 1977); R is the molar-isotope ratio, N_V and N_W are the number of ions of the tracer isotope (V) and the reference isotope (W). The value of $(N_V + N_W)$ was taken as 10^7 for all cases in Table 1.

The highest degree of precision reported in Table 1 was determined by fast-atom-bombardment mass spectrometry (FABMS) for $^{42}Ca:^{40}Ca$ (Smith *et al.* 1985). This level of precision may be possible only with the macroelements because of the relatively large amount of element available for analysis. FABMS is similar to secondary-ion mass spectrometry (SIMS) which has been proposed as an analytical technique for magnesium stable-isotope-ratio determination (Ramseyer *et al.* 1984). A major advantage of FABMS and SIMS is that little or no sample preparation may be required for the macroelements. However, FABMS and SIMS require high-resolution mass spectrometers in order to overcome interferences from multiple ions derived from the sample matrix.

Thermal-ionization mass spectrometry (TIMS) appears to be the most precise technique for the trace elements (normalized SD 0·51 and 1·8, Table 1). However, TIMS analysis requires extensive sample preparation and relatively long analysis *time per* sample. Analytical precision obtained with inductively-coupled-plasma mass spectrometry (ICPMS) may be competitive with TIMS in some applications. ICPMS has a number of advantages including relatively facile sample preparation, and rapid sample analysis.

Electron-impact-ionization mass spectrometry (EIMS) with sample introduction by solid probe and gas chromatography–mass spectrometry (GCMS) tend to be less precise techniques, although they have been utilized successfully in a number of tracer

procedures. GCMS has been used mainly for selenium stable-isotope determination by a technique originally developed by Reamer & Veillon (1981). Both EIMS and GCMS require preparation of volatile complexes for isotope-ratio analysis, but EIMS with solid-probe sample introduction requires purification of the metal complex.

Expression of stable isotope enrichment

For the present discussion, 'tracee' is defined as a naturally occurring element, and 'tracer' is the same element which has been enriched in one of its stable isotopes. A tracer, when obtained from the supplier, is always contaminated with other stable isotopes of the same element. To distinguish between a pure stable isotope and a tracer, different notations have been used. Thus, 'Se-82' designates the enriched isotope as purchased from the supplier, while '^{82}Se' is the standard chemical notation for a pure isotope (nuclide).

The most commonly used notation for expressing stable-isotope enrichment of C, H, N or O in biological-tracer experiments is atom percent excess (Halliday & Read, 1981). Another method of expressing stable-isotope enrichment is 100 multiplied by mass of tracer divided by the mass of the tracee (tracer:tracee mass percentage; TTMP) (Buckley *et al.* 1985*a*), which is based on an equation originally proposed by Hintenberger (1956). Turnlund (1984) and Veillon (1984) have used variations of the Hintenberger equation to determine the mass of tracer in single- and double-isotope-tracer experiments with mineral elements.

The relation between TTMP and atom percent excess is shown in Table 2. TTMP is consistent among elements and among isotopes within elements. TTMP also directly reflects an important physiological quantity—the amount of tracer dose relative to pool size. Atom percent excess does not possess these properties. In Table 2, values of TTMP are exactly doubled when the quantity of tracer is doubled, whereas values of atom percent excess are not exactly doubled. The main reason for this discrepancy is that atom percent excess is not a linear function of the quantity of tracer (Buckley *et al.* 1985*a*).

The error associated with the non-linearity of atom percent excess is relatively insignificant for the stable isotope ratios of H, C, N, and O when analysed by GIRMS. However, the error in using atom percent excess with the mineral elements can be significant. The error arises from the requirement to use relatively large tracer doses, which is dictated by the precision of isotope-ratio analysis. As a result of utilizing atom percent excess, errors up to 6% in estimation of first-order rate-constant and pool size were obtained in single-pool model systems into which a tracer dose of 10% of the pool size was introduced (Buckley *et al.* 1985*a*).

Table 2. *Relation between two notations for stable isotope enrichment*

Isotope ratio	Mass tracee (ng)	Mass tracer (ng)	TTMP	Atom percent excess*
^{65}Cu:^{63}Cu	100	1	1·000	0·667
	100	2	2·000	1·320
^{77}Se:^{78}Se	100	1	1·000	0·855
	100	2	2·000	1·692

TTMP, tracer:tracee mass percentage.

*Specifications for stable isotopes supplied by Oak Ridge National Laboratory (Oak Ridge, TN) were used in the calculations: Cu-65 was 99·61% ^{65}Cu (atom percent), 0·39% ^{63}Cu; Se-77 was 94·75% ^{77}Se, 0·03% ^{74}Se, 1·2% ^{76}Se, 2·37% ^{78}Se, 1·49% ^{80}Se and 0·16% ^{82}Se.

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In order to perform tracer studies with the mineral elements, an expression of stable-isotope enrichment which differs from that typically used for C, H, N and O is required. The notation should permit multiple-isotope studies to be performed and it should allow for the use of tracers which are contaminated with other stable isotopes. The TTMP notation satisfies these requirements.

The algebraic calculations required to utilize TTMP are cumbersome when multiple-isotope experiments are performed. However, the equations for TTMP, once derived, apply to any element and isotope combination. Equations for TTMP apply to polyatomic ions (with the added complication of heavy isotopes of C, H, N and O) as well as atomic ions of the mineral elements. The equations for TTMP for the solution of up to three simultaneously-enriched isotopes of the same element have not previously been published and are included here.

Calculation of TTMP

In the following equations V, W, X, and Y refer to stable isotopes of an element by either the mass: charge ratio (m/z) of the isotope ion or the nominal mass of the isotope. A distinction between m/z and nominal mass is made because in EIMS and GCMS analysis of polyatomic complexes, m/z differs from the isotope nominal mass. When V, W, X, and Y are in parentheses, they refer to the tracer enriched in the indicated stable isotope. Isotope W is always the reference isotope; that is, the isotope ion intensity which is the denominator in a ratio of ion intensities.

Hintenberger (1956) proposed an equation for stable-isotope-dilution analysis utilizing one enriched isotope:

$$\Theta_{V(m)} = \frac{(bH_{V(n)} + dH_{V(V)})}{(bH_{W(n)} + dH_{W(V)})}, \tag{2}$$

where subscript m designates a mixture of natural element and tracer and subscript n designates the natural element; Θ is the ion-intensity ratio determined for the substance indicated in parentheses (e.g. $\Theta_{V(m)}$ is the ion intensity at m/z for isotope V divided by ion intensity at m/z for isotope W determined for a mixture of natural element and tracer); b is the tracee content (mol); H is the ion abundance determined for the substance indicated in parentheses (e.g., $H_{V(V)}$ is the ion intensity at m/z for isotope V divided by total ion intensity for the tracer enriched in isotope V); d is the tracer content (mol).

Sets of simultaneous equations for two and three enriched isotopes were developed on the same principle as the Hintenberger equation. Molar quantities were converted to mass, and the equations were solved for TTMP.

One enriched isotope (V):

$$\mathrm{TTMP}_V = 100\frac{M_{(V)}H_{W(n)}r}{M_{(n)}H_{W(V)}s}. \tag{3}$$

Two enriched isotopes (V and X):

$$\mathrm{TTMP}_V = 100\frac{M_{(V)}H_{W(n)}\,(jp + ur)}{M_{(n)}H_{W(V)}\,(us - hp)}, \tag{4}$$

$$\mathrm{TTMP}_X = 100\frac{M_{(X)}H_{W(n)}\,(rh + js)}{M_{(n)}H_{W(X)}\,(us - hp)}. \tag{5}$$

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Three enriched isotopes (V, X and Y):

$$\mathrm{TTMP}_V = 100\frac{M_{(V)}H_{W(n)}\,(eip + gjp + cjq + equ + gru - cir)}{M_{(n)}H_{W(V)}\,(gsu - aqu - cis - ghp - chq - aip)}, \quad (6)$$

$$\mathrm{TTMP}_X = 100\frac{M_{(X)}H_{W(n)}\,(ehq + ghr + air + eis - gjs - ajq)}{M_{(n)}H_{W(X)}\,(gsu - aqu - cis - ghp - chq - aip)}, \quad (7)$$

$$\mathrm{TTMP}_Y = 100\frac{M_{(Y)}H_{W(n)}\,(ajp + aru + chr + cjs + esu - ehp)}{M_{(n)}H_{W(Y)}\,(gsu - aqu - cis - ghp - chq - aip)}, \quad (8)$$

where M is the exact atomic mass for the substance indicated in parentheses, and:

$$\begin{aligned} a &= \Theta_{Y(V)} - \Theta_{Y(m)}, & c &= \Theta_{Y(X)} - \Theta_{Y(m)}, \\ e &= \Theta_{Y(n)} - \Theta_{Y(m)}, & g &= \Theta_{Y(m)} - \Theta_{Y(Y)}, \\ h &= \Theta_{X(V)} - \Theta_{X(m)}, & i &= \Theta_{X(Y)} - \Theta_{X(m)}, \\ j &= \Theta_{X(n)} - \Theta_{X(m)}, & p &= \Theta_{V(X)} - \Theta_{V(m)}, \\ q &= \Theta_{V(Y)} - \Theta_{V(m)}, & r &= \Theta_{V(n)} - \Theta_{V(m)}, \\ s &= \Theta_{V(m)} - \Theta_{V(V)}, & u &= \Theta_{X(m)} - \Theta_{X(X)}. \end{aligned}$$

$\Theta_{V(m)}$, $\Theta_{V(n)}$, $\Theta_{X(m)}$, $\Theta_{X(n)}$, $\Theta_{Y(m)}$ and $\Theta_{Y(n)}$ are obtained from mass-spectrometric analysis after subtraction of reagent blank or background ion intensities if necessary. The remaining factors in the equations are calculated from specifications supplied with purchased isotopes and from standard reference texts. It can readily be shown with eqn (3) that minor errors in the values used to calculate $\Theta_{V(V)}$, $M_{(V)}$, $M_{(n)}$, $H_{W(n)}$ and $H_{W(V)}$ are insignificant because the calculated factors tend to cancel each other. Once TTMP has been calculated (and corrected for instrumental bias if necessary) it can be used in calculations in the same manner as one would use specific activity in a radiotracer experiment.

Applications

The most frequent application of stable isotopes of the mineral elements in studies of nutrition and metabolism is the estimation of dietary mineral availability or absorption. The most frequently used tracer technique is the faecal isotope-balance (faecal-monitoring) procedure, which has been evaluated in detail for both neutron-activation analysis (Janghorbani *et al.* 1985) and mass-spectrometry analysis (Turnlund, 1984). The technique consists of determining the difference between a dose of isotope fed and the amount of isotope recovered in the faeces after passage of the meal. An important assumption is that the rate of absorption of the tracer, which is usually administered in a simple inorganic form, is equal to the rate of absorption of the natural form of the element in the diet. Since this may not be true, some investigators have included the isotope dose as an intrinsic part of the diet by growing a diet component in the presence of the tracer. In our work with dairy cows, however, intrinsic labelling of feed would not be practical, nor were we willing to make the assumption that tracer and natural element would be absorbed at the same rate. Consequently, we have explored other methods for measuring mineral absorption.

In studies with copper and Se stable isotopes in dairy cows the uniformity of isotope distribution achieved in the tissues of the body was an important factor in the choice of

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Table 3. *Mean Cu-65 enrichment in tissues of two lactating dairy cows 46 d after intravenous administration of 70 mg Cu-65*

Tissue	No. of sites*	TTMP†	Pooled SD‡	Estimated total Cu (mg)§
Bone	3	—	—	147
Heart	1	4·2	—	7
Hide	1	—	—	10
Intestine	2	3·1	0·45	4
Kidney	1	4·9	—	4
Liver	5	5·3	0·22	535
Lung	1	5·0	—	5
Skeletal muscle	6	3·4	0·66	111
Stomach				
Smooth muscle	4	4·0	0·81	—
Epithelium	4	3·8	0·39	—
Udder	1	3·9	—	14

TTMP, tracer:tracee mass percentage.

* Sample sites: femur, humerus and rib (bone); small and large intestine; left and right ventral, left and right dorsal and caudal lobes (liver); biceps femoris, deep pectoral, longissimus dorsi, semimembranosus, semitendinosus and superficial pectoral (skeletal muscle); rumen, reticulum, omasum and abomasum (stomach).

† Analysis was by electron-impact-ionization mass spectrometry.

‡ SD for sample sites.

§ Estimates of total tissue Cu were obtained from Cu analysis, from anatomical information for Holstein cows (Matthews *et al.* 1975) adjusted for body-weights and from estimates of gross body composition (R. J. Forrest, personal communication: dressing percentage 52, percentage bone in carcass 20, percentage muscle in carcass 55).

tracer strategy. The tissue distribution of Cu-65 and Se-76 administered either intravenously (Cu-65) or orally (Se-76) was examined with dairy cows in separate experiments. The variation in enrichment of Cu-65 throughout the tissues examined was relatively small at slaughter 46 d after isotope administration (Table 3, W. T. Buckley, S. N. Huckin and G. K. Eigendorf, unpublished results). The lowest enrichment, which was found in the intestine, was 58% of the highest enrichment, which was found in the liver. Cu-65 enrichment of skeletal muscle was 64% that of liver. Liver represented the largest store of body Cu, while bone and skeletal muscle were also quantitatively important.

A different pattern of isotope enrichment was found with Se. At 26 d after oral administration of Se-76 (selenite), enrichment was greatest in serum, liver, kidney, intestine, udder and epithelial lining of the rumen, reticulum, omasum and abomasum (Table 4, Koenig *et al.* 1986). However, the tissue representing the largest store of body Se, skeletal muscle, was only enriched about 16% as much as liver.

Because of the different patterns of distribution of Se-76 and Cu-65 in dairy cows, tracer strategies for determining dietary absorption of the elements have also differed. We have used two methods for determining absorption of dietary Cu and one method for Se.

The first method of measuring Cu absorption was based on determining whole-body turnover of Cu. Whole-body Cu was assumed to behave like a single pool, and rate of Cu absorption (F) was determined by the following equation:

$$F = B/T, \quad (9)$$

where B is the whole-body Cu pool size and T is the turnover time of whole-body Cu. Following intravenous (jugular) administration of Cu-65 equivalent to 5–10% of the whole-body Cu pool size, liver biopsy samples were taken over a period of about 150 d (Buckley *et al.* 1985*b*). Both B and T were calculated from liver Cu-65 enrichment using standard compartment analysis procedures for tracer kinetics (Shipley & Clark, 1972) with corrections for non-steady-state conditions. The assumption that whole-body Cu behaved like a single pool which could be sampled from the liver was supported by correlation coefficients of –0·999 and –0·988 obtained for the linear regression of the natural logarithm of tracer enrichment *v.* time in two trials with six dairy cows each. In addition, the majority of body Cu was expected to be in the liver (Table 3). The error due to the lower enrichment in skeletal muscle and other tissues could not be determined exactly, but rate of Cu absorption may have been underestimated by 10–15%. The error would probably be larger in Cu-deficient animals which would have low liver Cu concentrations; however, the error was considered acceptable for cows of normal liver Cu concentration.

The second method of determining Cu absorption in dairy cows utilized a metabolic sink for Cu. Based on preliminary observations, it appeared that milk Cu could be divided approximately into two artificial components: milk Cu derived directly from the diet, and milk Cu derived from the whole-body Cu pool. The fraction (β) of absorbed dietary Cu transferred directly to the milk was estimated from the fraction of an injection (intravenous) of 5 mg Cu-65 which appeared in the milk within 36 h. The fraction (γ) of milk Cu derived directly from the diet (i.e. without recycling through the body pool) was given by:

Table 4. *Mean Se-76 enrichment in tissues of two dry dairy cows 26 d after oral administration of 20 mg Se-76*

Tissue	No. of sites*	TTMP†	Pooled SD‡	Estimated total Se (mg)§
Bone	3	<DL	—	1·4
Heart	1	9·1	—	0·6
Hide	1	7·2	—	2·4
Intestine	2	11·7	0·30	1·2
Kidney	2	12·7	0·66	1·1
Liver	5	12·8	0·33	2·1
Lung	1	10·6	—	0·9
Pancreas	1	10·1	—	0·2
Serum	1	13·1	—	0·6
Skeletal muscle	5–6	2·1	0·52	21·5
Stomach				
Smooth muscle	4	7·1	0·99	1·4
Epithelium	4	11·2	1·1	—
Udder	1	11·9	—	1·6

TTMP, tracer:tracee mass percentage; DL, detection limit (2 × SD) for TTMP 0·55%.

* Sample sites as described in Table 3 except for cortex and medulla (kidney).

† Analysis was by gas chromatography–mass spectrometry.

‡ SD for sample sites.

§ Estimates of total tissue Se were obtained from Se analysis, and from anatomical information and body composition estimates as described in Table 3. A packed cell volume of 0·40 was assumed.

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$$\gamma = 1 - \frac{\text{milk Cu enrichment}}{\text{non-direct reacting Cu enrichment}}, \quad (10)$$

where nine milk and plasma samples were obtained between 24 and 42 d after a second injection of Cu-65 (80 mg), and non-direct reacting Cu is an empirically-defined fraction of plasma Cu (predominantly the cuproprotein, ferroxidase (*EC* 1.16.3.1) which is synthesized in the liver). The rate of Cu absorption (F) was expressed by:

$$F = G\gamma/\beta, \quad (11)$$

where G is the mean daily secretion of Cu in milk from 24 to 42 d after Cu-65 administration.

In order to test the assumptions in the two methods of determining dietary Cu absorption, both methods were performed concurrently in the same six cows (Buckley *et al.* 1985*b*). Good agreement between the two methods was obtained (Table 5), which was taken as evidence that the procedures are valid.

A method different from either of the two Cu procedures was required for determining dietary Se absorption. The whole-body-turnover method was unlikely to be successful for Se because of the relatively large variation in Se enrichment found in body tissues (Table 4). The metabolic-sink method also was considered as a poor prospect because the determination of β depends on administering the tracer intravenously in the same chemical form as it is absorbed. The method of determining Se absorption was based on conventional balance techniques utilized in conjunction with an estimate of endogenous faecal excretion. This concept has been used with radioisotopes in rats (Evans *et al.* 1979) and stable isotopes in humans (Jackson *et al.* 1984) for determining absorption of dietary zinc. It was not possible to evaluate this approach for Cu studies because analysis of Cu-isotope ratio was by EIMS which did not have sufficient precision to measure the relatively small amount of endogenous faecal Cu. On the other hand TIMS (and probably ICPMS) analysis for Cu and GCMS and ICPMS analysis for Se isotope ratios do provide sufficient precision.

All the tissues and fluids which might represent sources of endogenous faecal Se were found to be equally enriched 26 d after an oral dose of Se-76 (Table 4). Thus, it appeared that serum or liver samples could be used to determine the stable-isotope enrichment of endogenously excreted faecal Se.

An experiment was performed to evaluate the effect of two routes of tracer administration and the choice of tissue sampled on estimation of Se absorption and endogenous faecal excretion. Approximately 4·6 mg of Se-77 (selenite) was administered

Table 5. *Comparison of whole-body turnover and metabolic sink methods of determining dietary copper absorption in six lactating dairy cows*

(Mean values with their standard errors)

	Whole-body turnover method		Metabolic sink method	
Cu absorption	Mean	SEM	Mean	SEM
mg/d	11·0	1·1	11·6	2·0
Per cent of intake	8·9	0·9	9·5	1·6

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Table 6. *Effect of route of administration of tracer and choice of tissue analysed on estimation of endogenous faecal selenium excretion and Se absorption in eleven dairy cows*

(Mean values with their standard errors)

		Endogenous faecal Se (μg/d)		Se absorption			
				μg/d		% of intake	
Route	Tissue	Mean	SEM	Mean	SEM	Mean	SEM
Oral (Se-77)	Liver	235	14	268	32	10·4	1·1
	Serum	262	18	290	34	11·3	1·1
Jugular (Se-82)	Liver	197	12	230	32	8·9	1·1
	Serum	241	15	274	34	10·6	1·2

orally and 1·3 mg Se-82 was given intravenously (jugular) to each of eleven dairy cows fed only on orchardgrass hay with supplemental Se (183–193 μg Se/kg dry matter). Total faeces samples were collected from 20 to 24 d after Se-stable-isotope administration. Serum samples were collected every second day during the faeces collection period and a liver biopsy was performed on day 26. Each sample was spiked with Se-76 and analysed for enrichment of Se-76, Se-77 and Se-82 by hydride generation–ICPMS (Buckley *et al.* 1988). The addition of Se-76 internal standard was required for the quantitative determination of Se by the method of stable-isotope-dilution analysis. Endogenous faecal excretion (E) of Se was determined by:

$$E = \frac{\text{TTMP}_{(\text{faeces})}}{\text{TTMP}_{(\text{serum})}} \times \text{daily natural Se in faeces.} \quad (12)$$

Se absorption was calculated as follows:

$$\text{Se absorption} = \text{Se intake} - \text{total faecal excretion} + E. \quad (13)$$

The oral (Se-77) route of administration with liver or serum analysis and the jugular route of administration (Se-82) with serum analysis yielded comparable results and should be acceptable for routine work (Table 6; K. M. Koenig, W. T. Buckley and J. A. Shelford, unpublished results). The jugular route of administration is a significant economic advantage over the oral route because of the smaller quantity of tracer required for the experiment.

Concluding remarks

As analytical precision has improved, the versatility of tracer techniques with stable isotopes of the mineral elements has increased. A reliable notation of stable-isotope enrichment, such as TTMP, is required for effective use of stable isotopes in many procedures, particularly multiple-isotope studies. Stable isotopes of the mineral elements have numerous applications in studies of nutrition and metabolism, although emphasis up to now has been on studies of dietary absorption.

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