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DETERMINATION OF INTESTINAL UPTAKE OF ESSENTIAL TRACE ELEMENTS USING STABLE ISOTOPIC TRACERS AND RARE EARTH MARKERS

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By

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I declare that this thesis embodies the results of my own special work, that it has been composed by myself and that it does not include work forming part of a thesis presented for a degree in this or another university. The sources of information have been specifically acknowledged.

Ulvi ULUSOY

To my parents, my wife Hatice and to my daughter Seher

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DEFINITION AND CONVENTION

Absorption of a constituent of diet is the process in which it is taken up by the intestine and transferred to the body.

Bioavailability is the efficiency with which a nutrient is absorbed from a diet and utilised by the body.

The net luminal disappearance of constituent of diet is the difference between input and output, and it is determined by the use of isotopic tracers and is synonymous with luminal disappearance throughout this study.

Stable isotopes are indicated with the mass number as a preceding superscript *e.g.* ^{58}Fe

Radioactive isotopes are indicated with the mass number as a following script *e.g.* Fe-59

Subject identities: In the measurement of net luminal disappearance with rare earth markers, three investigations are described: Recovery of markers determined by INAA, recovery of markers by CNAA, and an investigation of the net luminal disappearance of Fe and Zn with account of marker recovery. In compilation of results, subjects in three studies are identified with prefixes A, B, and C.

ABBREVIATIONS

$_{Z}$RE: Rare earth (elements)

$_{57}$**La**: Lanthanum	$_{58}$**Ce**: Cerium	$_{59}$**Pr**: Praseodymium	$_{60}$**Nd**: Neodymium
$_{62}$**Sm**: Samarium	$_{63}$**Eu**: Europium	$_{64}$**Gd**: Gadolinium	$_{65}$**Tb**:Terbium
$_{66}$**Dy**: Dysprosium	$_{67}$**Ho**: Holmium	$_{68}$**Er**: Erbium	$_{69}$**Tm**: Thulium
$_{70}$**Yb**: Ytterbium	$_{71}$**Lu**: Lutetium	$_{61}$**Pm**: Promethium (Radioactive)	

NAA: Nuclear activation analysis

INAA: Instrumental NAA

CNAA: NAA with chemical separation before or after irradiation

HPGe: High purity germanium

ICP-MS: Inductively coupled plasma mass spectrometry

AAS: Atomic absorption spectrophotometry

SUMMARY

Previous studies for determination of intestinal uptake of trace elements by faecal monitoring using enriched stable isotopic tracers have required extended collection of faecal output to ensure quantitative recovery of excreted tracer. This requirement can lead to underestimation of 'absorption' since the contribution of 're-excreted' isotopic tracer initially taken up can not be identified. The use of non-absorbable dietary markers can enable account to be taken of faecal recoveries achieved in a reduced period of collection, removing the inconvenience of long-term collection of faeces and extending the potential applications of such investigations.

Faecal recoveries of rare earths and their excretion kinetics have been investigated to confirm that they meet the required criteria for nutritional markers. Particular attention is given to the extent to which markers duplicate the behaviour of tracers. Determination of intestinal uptake of iron and zinc by combined use of enriched stable isotopic tracers and rare earths as markers is then considered.

La, Sm, Eu, Tb, and Yb were administered to two healthy subjects with their lunch. Faecal samples were collected for 1 day before (baseline) and their concentrations in faecal output were determined by instrumental neutron activation analysis. The recovery investigation was then extended to six subjects with the rare earth contents of samples being separated by ion-exchange before irradiation, to minimize radiation exposure during analysis. This reduced the time required for analysis and improved limits of detection. Faecal recoveries of rare earths are interpreted in terms of profiles of excretion as a function of time and kinetic parameters.

Having established that recoveries of rare earths in faeces for a week after consumption were nearly quantitative, their application to determine intestinal uptake of iron and zinc is considered. Procedures for determination of

intestinal uptake from a standard drink containing ferrous sulphate labelled with ^{57}Fe tracer and Sm marker, and from a wheat flour based meal containing ferric chloride and zinc labelled with ^{58}Fe and ^{70}Zn tracers and Yb marker were established.

Seven healthy subjects consumed the standard solution and meal at a 3 day interval. Faecal samples were collected for 10 days starting from day before the first administration. Urine samples were collected for 24 h before the first and for 24 h after each administrations. Blood samples also were taken at the end of collection period to establish if there is any relation between intestinal uptake of iron and ferritin concentrations in blood serum.

Following ion exchange column separation, concentrations of Sm and Yb markers, and ^{58}Fe and ^{70}Zn tracers were determined by neutron activation analysis. ^{57}Fe and natural background levels of rare earths in faeces and urine were determined by inductively coupled plasma mass spectrometry. Total iron and zinc contents of faecal samples were determined by atomic absorption spectrophotometry, to enable account to be taken of the isotopic contribution of iron and zinc of natural composition to enrichment in the faecal pool.

Faecal recoveries are interpreted in terms of the intestinal kinetic behaviour of markers and tracers and Intestinal uptake of tracers are derived from recoveries of markers and tracers for individual samples and for composites of sequential faecal outputs.

Recovery profiles of the rare earths show that they appear within 24 hours after consumption, and reach a maximum in 48 hours. The major fraction of recovery is with the first two samples, and recoveries with the three samples are not significantly different from recoveries with the first four or five samples.

For iron consumed with a standard solution, intestinal uptakes obtained for composites of the first two and three samples are significantly different from those for composites of the first four samples and total collection. Correlation between serum ferritin levels and intestinal uptakes obtained for composites of the first two samples, and total collection suggest that iron is bioavailable in the ferrous state, taken up by the intestine and transferred into circulating blood at a slow rate, whilst a significant fraction is excreted with exfoliated epithelial cells.

Intestinal uptakes of iron consumed with the farina meal are more consistent and there is no significant differences between composites of the first two, three, and four samples, but not for composites of the following samples. This can be explained by the contribution of retarded excretion of iron intially taken up becoming significant in the later collections.

Intestinal uptakes of zinc, obtained from composites of the first to five samples are not significantly different. Excretion of endogenous zinc is approximately equivalent to its daily absorption.

For determination of intestinal uptake of essential trace elements with reduced faecal collection by the combined use of enriched stable isotopic tracers and rare earths as markers has been established for iron and zinc for specified conditions. It has been shown that rare earths enable such investigations with multi-isotopes/elements. It is anticipated that the method developed should be widely applicable to unconfined situations and vulnerable groups of subjects such as children and pregnant women.

I. INTRODUCTION

Investigations to evaluate the adequacy of dietary intake are of increasing concern because of the effects of nutrition on health. The increased incidence of obesity, cardiovascular diseases, and some cancers etc is related to over nutrition with immense social and health care costs. In countries where some 2 000 million suffer from anaemia, growth failure, delayed motor and mental development, impaired immunocompetence, and high risks of complications and death from infectious disease, 40 000 children under the age of five die every day from conditions related to under nutrition or inadequate food quality or variety (leading to deficiencies of vitamins and essential minerals (Parr and Fjeld, 1994).

Iron and zinc are recognized as essential trace elements for life competency. Although much is known about the functions and required amounts of these elements, further investigation is required to reveal their interactions with the constituents of dietary intake, and to optimize dietary bioavailability, so enabling efficient use of resources. Investigations are also required for development of methodology to extend the knowledge of the absorption mechanism of essential trace elements and their utilization.

Trace element absorption is frequently measured by the difference between the element content of intake and output (balance), but this is not precise enough to measure low absorption, does not take account of the contribution of endogenously excreted elements to faecal losses, and requires reliable cooperation of subjects, usually involving confinement in a metabolic unit for an extended period of at least a week. Furthermore this technique does not measure 'absorption', it measures luminal disappearance and one can not presume that the difference between oral intake and faecal loss represents the amount transfered to the body, particularly when applied to trace elements. Isotopic tracer techniques, using either radioactive or enriched stable isotopes are highly suitable for trace element research. Investigations

involving radioactive tracers are prohibited for vulnerable population groups such as pregnant women and young children. Current opinion is opposed to any use unless clinically necessary. In applications with stable isotopic tracers, the major limitation is that enriched isotopes are expensive, so the number of subjects in an investigation may be limited.

Methods of determining absorption from a dietary source labelled with isotopic tracer involve measurement of the incorporated enrichment in systemic tissues (appearance method) or the difference between the amounts of isotopic tracer in input and in faecal output (disappearance method) or a combination of both methods. The disappearance method is non-invasive and potentially applicable to investigations with unconfined subjects provided that faecal collections are quantitative or account taken of incomplete recoveries.

Measurement of luminal disappearance with stable isotopic tracers requires an appropriate period of collection to ensure recovery of all excreted tracer. Extended periods of collection result in the inclusion of excreted endogenous isotope. The use of dietary non-absorbable markers would enable measurements from limited sample collection and also to take account of losses during sample collection.

I.1 ELEMENTAL COMPOSITION OF THE HUMAN BODY AND ESSENTIAL TRACE ELEMENTS

In recent decades, increasing attention has been paid to the biochemical behaviour of the elements, particularly at trace levels. The role of essential elements in nutrition and in biochemical processes in the body of man, both in health and disease is receiving continued attention.

Hamilton (1981) provides following useful guidelines in considering relationships between man and the elements ;

1. All the elements of the Periodic Table are available to enter man at some level of exposure and bioavailibility.
2. Above a defined level, in which the balance of elements is correct in terms of body requirements, all the elements are toxic.
3. When some essential elements are below a defined level, there results a state of primary deficiency per se, or secondary disease.
4. Substitution of one element for another sometimes occurs naturally, often related to their mutual relationships in the periodic table, without jeopardising health.
5. An abnormal concentration of an element at a disease site is not always an indication that the element is the primary cause of the disease.

I.1.1 CLASSIFICATION OF ELEMENTS IN THE HUMAN BODY

Chemical elements present in the body can be basically grouped into three categories:

1. **Major elements**: These elements consist of carbon, hydrogen, nitrogen, and oxygen which comprise 99% of body weight.

2. **Minor elements**: These elements maintain the electrolyte balance and include *e.g.* sodium, potassium, calcium, magnesium, chlorine.

3. **Trace elements**: The term 'trace elements' evolved when the elements could not be precisely quantified using classical methods. Although this usage continues, improvements in analytical sensitivity have led to the current definition as elements which constitutes less then one per cent of the wet weight of biological material, and whose concentration levels fall below a few part per million (Bowen, 1988 and Iyengar, 1989). A classification of the elements derived from Iyengar (1989), Parr (1987) and Heydorn (1984) is provided in **Table-1**.

Trace elements may also be classified two groups; essential and toxic (**Table-2**). The definition and functions of the former will be discussed in the following section. Others are detected in human systems but no specific functions for them has yet been established. Elements such as Cd, Hg, Pb, and Tl are known to be inherently toxic, and are therefore classified as major environmental contaminants. There is occasional overlap in classification for elements such as As, F, and Se which are beneficial to health but which are toxic in excessive amounts.

I.1.2 ESSENTIAL TRACE ELEMENTS

An element is considered essential if an animal species deprived of it develops reproducible features which can be reversed or prevented by its intake. The nine trace elements, Fe, Zn, Cu, Mn, Se, Mo, Cr, I and Co are accepted as essential for optimum human health. Their biological functions, deficiency signs and occurrence of imbalance are summarized in **Table-3**, from Aggett (1985) and Parr (1987).

Table-1: Classification of elements found in human tissues, and their total body content (Iyengar, 1989 and Parr, 1987)

MAJOR ELEMENTS (Structural)			**TRACE ELEMENTS**		
Element	Amount, g	% of body	Element	Amount, g	% of body
Oxygen	43000	61	Silicon	18	0.026
Carbon	16000	23	Iron	4.2	0.006
Hydrogen	7000	10	Fluorine	2.6	0.0037
Nitrogen	1800	2.6	Zinc	2.3	0.0033
Calcium	1000	1.4	Rubidium	0.32	0.00046
Phosphorus	780	1.1	Strontium	0.32	0.00046
Sulphur	140	0.2	Bromine	0.2	0.00029
			Lead	0.12	0.00017
			Copper	0.072	0.0001
MINOR ELEMENTS (Electrolyte)			Aluminium	0.061	0.00009
Element	Amount, g	% of body	Cadmium	0.05	0.00007
Calcium	1000	1.4	Boron	0.048	0.00007
Potassium	140	0.2	Barium	0.022	0.00003
Sodium	100	0.14	Tin	0.017	0.00002
Chlorine	95	0.12	Manganese	0.012	0.00002
Magnesium	19	0.027	Iodine	0.013	0.00002
			Nickel	0.01	0.00001
			Gold	0.01	0.00001
			Molybdenum	0.0093	0.00001
			Chromium	0.0018	0.000003
			Caesium	0.0015	0.000002
			Cobalt	0.0015	0.000002
			Uranium	0.00009	1.00e-07
			Beryllium	0.000036	
			Radium	3.10e-11	

Table-2: Classification of trace elements found in human tissues (Heydorn, 1984)

Biologically important		**Potentially Toxic**
Essential	Possibly essential	
Cobalt	Arsenic	Arsenic
Chromium	Fluorine	Beryllium
Copper	Nickel	Cadmium
Iron	Silicon	Mercury
Iodine	Tin	Lead
Manganese	Vanadium	Selenium
Molybdenum		Thallium
Selenium		
Zinc		

Table-3: Biological functions, deficiency signs, and occurence of imbalance of the nine essential trace elements

ELEMENT	FUNCTION	DEFICIENCY SIGNS	OCCURRENCE OF IMBALANCE
Iron	Oxygen transport and storage, cytochromes, oxidative phosphorylation, redox enzymes, antioxidant	Anaemia	Deficiencies widespread; exces dangerous in haemochromatosis; acute poisoning
Copper	Oxidases, oxidative phosphorylation, antioxidant, fatty acid metabolism, connective tissue synthesis	Anaemia, changes of ossification; possibly elevated serum cholesterol	Deficiencies in the malnourished, and those receiving total intravenous feeding
Zinc	Catalytic, structural and regulatory roles in numerous enzymes, gene expression, macromolecular and polymeric structures	Growth depression, sexual immaturity, skin lesions, depression of immunocompetence, change of taste acuity	Deficiencies in Iran, Egypt, in total intravenous feeding, genetic diseases, malnutrition syndromes
Manganese	Antioxidant, sterol synthesis, glycosylation, carbohydrate metabolism	Vitamin K deficiency; decreased serum concentrations of cholesterol, triglycerides, and phospholipids	One possible case resulting from experimental feeding
Chromium	Optimizes glucose tolerance	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids	Deficiency known in malnutrition, aging, total intravenous feeding
Selenium	Antioxidant, micromosal mixed function oxidases, sperm integrity	Endemic cardiomyopathy (Keshan disease), conditioned by selenium deficiency	Deficiency and excess in areas of China
Molybdenum	Purine and sulphur metabolism	Hypermethionaemia	One case resulting from inravenous feeding
Iodine	Constituent of thyroid hormones	Goitre, depression of thyroid function, cretinism	Deficiencies widespread; excessive intakes may lead to thyrotoxicosis
Cobalt	Part of vitamin B_{12}	Vitamin B_{12} deficiency	Inability to absorb vitamin B_{12}; low B_{12} intake from vegetarian diets

Dietary deficiencies for essential elements have been reported in general populations for Fe, Zn, Se, I, and Ca but not for Cu, Mn, Mo, Cr, and Mg. Deficiencies of the latter minerals may also occur but only under special circumstances such as during total parenteral nutrition or the feeding of low birth weight infants (Hurrell *et. al.*, 1992).

Iron and zinc are the main concern of this investigation. Although their biochemical importance is well known, their bioavailibility from different foods, factors influencing their absorption and metabolism, and their homeostasis in health and disease requires further investigation. Optimized dietary intakes will enable avoidance of worldwide essential element deficiencies. Fe deficiency for example affects about one billion people. The incidence of Fe deficiency is higher in developing countries than in developed countries but overall the risk of Fe deficiency is greatest in populations with rapid growth, such as infants, young children, and in women (Turnlund *et al.*, 1990). On the other hand there might be an increased risk of cancer with high levels of tissue iron (Knekt et al., 1994 and Stevens et al., 1994).

Zinc supplementation increases weight gain and linear growth in children who are zinc deficient and adolescents. Severe diseases have been known to be predisposing factors for Zn deficiency and such deficiency is common in middle east countries where high phytic acid levels in cereals and legumes constitute significant portion of diets consumed (Prasad, 1984). Zinc plays a fundamental role in expression of genetic potential; the synthesis, repair and structural integrity of nucleic acids require Zn (Fairweather-Tait, 1988).

Since methodological development is the subject of this investigation, a brief description of the absorption mechanism of Fe and Zn is provided below.

Iron absorption

Heme iron is absorbed from food more efficiently than inorganic iron. Heme iron enters the intestinal absorptive cell as an intact metalloporphyrin.

Subsequently, the iron is released from porphyrin in the intestinal mucosa by heme oxygenase and it then enters the same absorptive pathway as for inorganic iron.

Unlike inorganic iron, heme is soluble in alkaline solutions and is precipitated in an acidic medium, making chelation less important in facilitating solubility. Many substances that either enhance or inhibit absorption of inorganic iron have no effect on the absorption of heme iron because they do not chelate in this chemical form (Conrad and Umbreit, 1993).

"Dietary inorganic iron is solubilized at the acid pH of the stomach. It then combines with mucin which make the iron soluble and available for absorption with increasing pH in the small bowel. Integrins on the surface of the absorptive cell facilitate transfer of iron through the cell membrane for binding mobilferrin. Mobilferrin serves as the shuttle protein within the absorptive cell. If there is excess iron in the cell, apoferritin synthesis is stimulated and iron is deposited in ferritin to prevent oxidative damage to the cell from ionic iron. Transferrin receptors located on the basoletaral membranes of the absorptive cell probably act to permit iron to enter the cell from plasma similar to cells in other organs. In the gut, this keeps absorptive cells attuned to the state of iron repletion in the body and aids in regulation of mucosal uptake of iron by binding or lack of binding of the proteins." (Conrad and Umbreit, 1993) Further discussion of iron absorption are provided by Charlton and Bothwell (1983), and Cook *et al.* (1993).

Zinc absorption

The process of zinc absorption can be divided into two processes; uptake of zinc from the lumen into the cell and zinc transport from the cell into circulatory system. Uptake or cellular entry of Zn is carrier mediated. A small portion of Zn uptake may also occur through simple diffusion. Subsequently, the receptor releases Zn intracellularly.

The capacity of the small intestine for Zn transport depends on the body's zinc requirement. Affinity for Zn is not affected by previous dietary Zn intakes. Thus, the increase in transport rate at low dietary Zn intakes is due only to increase in number of enterocyte receptors for free Zn or Zn bound to a low molecular weight ligand. Further discussion of the biochemistry and metabolism of zinc is provided by Fairweather-Tait (1988) and (Swinkels *et al.*, 1994).

I.2 METHODS FOR MEASUREMENT OF TRACE ELEMENT ABSORPTION

In this section the concepts and approaches to investigating determination of absorption are described. Applications of isotopic tracers techniques in those approaches are considered with particular reference to iron and zinc.

I.2.1 TRACE ELEMENT ABSORPTION AND BIOAVAILIBILITY

There is a need for mineral absorption and bioavailibility studies to investigate the dietary and physiological factors influencing mineral absorption and to understand the homeostatic mechanisms for adaptation to high and low intakes. In this way requirements for health and disease can be better defined and strategies can be developed so that requirements are met (Hurrell *et al.*, 1994).

The process of mineral metabolism can be considered as follows; "When a mineral is consumed as a constituent of food, a fraction is absorbed and passes into the blood stream, while the non-absorbed fraction passes to the stools. From the blood stream, some of the absorbed mineral will pass into body tissues and some will be excreted in urine. A second excretory pathway is from the body tissues back into the gut via the bile or gut secretions. This could include a small fraction of the dietary mineral recently absorbed, but is mostly mineral already present in the body. Although some of this endogenous mineral can be re-absorbed, the larger fraction passes into the stools" (Hurrell *et. al.*, 1994) (**Figure-1**).

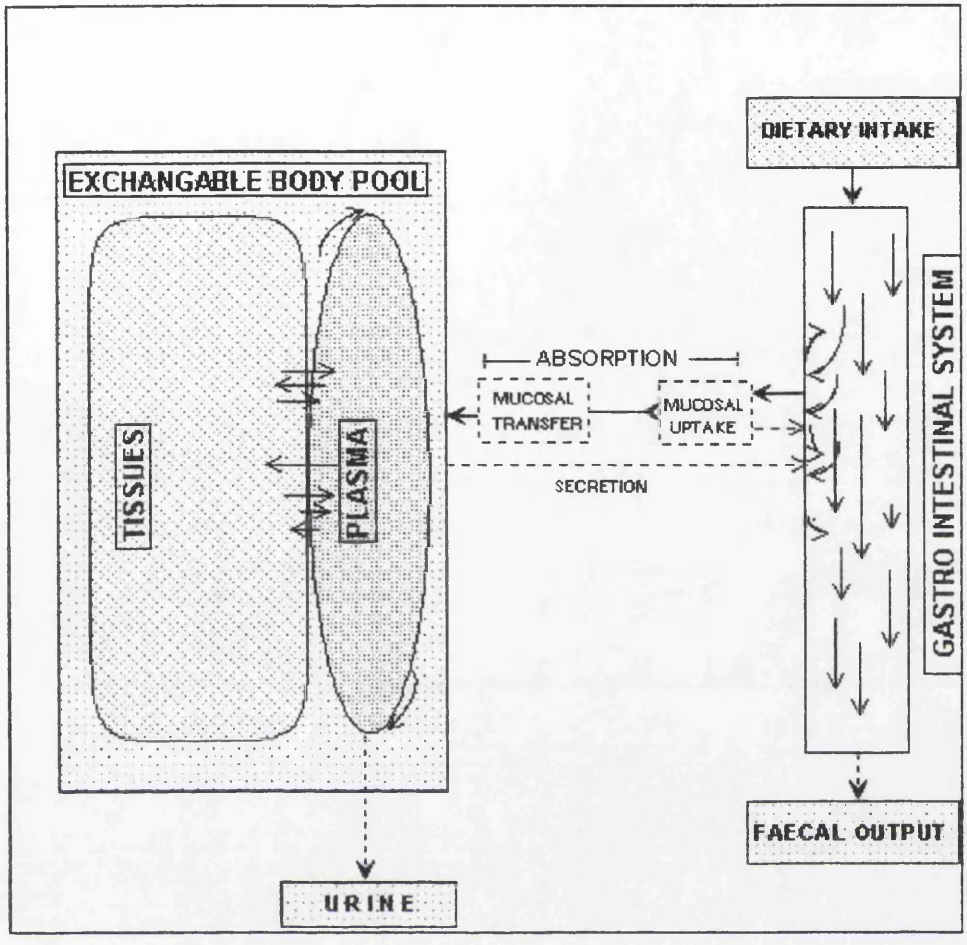


Figure-1: Schematic diagram of mineral metabolism

Bioavailibility is the fraction of any dietary nutrient consumed which is ultimately utilized in the body. Although bioavailibility is an important consideration for all nutrients, it is particularly pertinent for trace elements because so many factors affect their intestinal absorption and systematic use (**Table-4**).

Table-4: Factors influencing the intestinal uptake and transfer of trace elements (Aggett and Whitley, 1994)

SYSTEMATIC FACTORS
Anabolic demands
Growth in infancy and childhood
Pregnancy and lactation
Post-catabolic states
Endocrine effects
Infection and stress
Specific systematic reserves of metal
Genetic influence, inborn errors of metabolism
Nutritional status for other nutrients
LUMINAL AND DIETARY FACTORS
Chemical form and oxidation state of element in the diet
Presence of:
Antagonistic ligands (phosphate, carbonate, tannates, polyphenols, oxalate)
Facilitatory ligands (ascorbate for iron; carboxylic acids, some sugars, amino acids, fatty acids)
Competing metals
Intestinal redox state
Luminal redox state

In practice bioavailibility is taken to be as the proportion of intake that is absorbed or retained in the body. Measurement of true bioavailibility requires determination of the appearance of minerals in specific body pools, which is not always practical or ethical, or is restricted usually to plasma and blood cells and the excretion of the nutrient in urine, so that experimental measurements frequently measure uptake to a specified stage (*e.g.* luminal disappearance or erythrocyte incorporation).

Approaches to investigate trace element absorption can be classified as follows;

I. Chemical balance methods

Measurement of disappearance from the dietary pool by faecal monitoring

II. Isotopic tracer methods

Measurement of disappearance from the dietary pool by faecal monitoring

Measurement of retention in the body by monitoring plasma appearance or tissue incorporation.

Simultaneous oral and intravenous isotope administration

Compartmental modelling and kinetic analysis

III.*In-vitro* procedures

I.2.2 CHEMICAL BALANCE METHODS

Mineral absorption by faecal monitoring has been frequently investigated by chemical balance. The difference between intake and faecal output of nutrient provides **"net apparent absorption"** (Same definition is also given for net luminal disappearance, when 'absorption' is measured by using isotopic tracers, see section I.2.3.3) given . If urinary excretion is subtracted from the intake **"maximum apparent retention"** is determined, since no allowance is made for additional losses through skin, sweat, hair etc.

The major disadvantage in the chemical balance approach is that true absorption can not be determined as it is not possible to distinguish between faecal output of non-absorbed elements of dietary origin and the same elements of endogenous origin. (Sandstrom et. al., 1994, Aggett and Whitley, 1994). Measurement of absorption by chemical balance is only applicable to studies of total diet. Measurements for elements which are not well absorbed can lead to large errors (Fairweather-Tait, 1992).

In spite of its various drawbacks, the chemical balance technique offers one of the most definitive estimates of bioavailibility in adult man if performed carefully and with intakes near or below the minimal requirement (O'Dell, 1984). The following recent investigations demonstrate the importance of the

chemical balance techniques in studies which require monitoring of the effects of a diet for a long period of time.

Mason *et. al.*, 1990 investigated the effect of moderately increased intakes of complex carbohydrates on iron and zinc balance in a study lasting 12 weeks. By monitoring faecal, urine and blood samples they found that there were no significant changes in Fe or Zn balance.

Such investigations as performed by Milne *et al.* (1983) and Srikumar *et al.* (1992) are probably only possible with chemical balance methods. Milne *et al.* investigated the effect of dietary zinc on whole body surface loss of zinc on 8 volunteers who lived for 6 months in a research environment under close supervision. The losses with whole body sweat were in a range of 0.24-0.62 mg d^{-1}, depending on the levels of input, suggesting that these losses reduced apparent balances by 12-84%, and need to be considered when evaluating zinc retention. Srikumar *et al.* (1992) measured trace element levels in faecal, urine, blood and hair samples from 26 subjects, switching from a mixed to a lactovegeterian diet for 48 months. The changes in trace element status was less optimal than a mixed diet with regard to Zn, Se and Cu but not for Mg. The investigation also revealed that drastic decrease in consumption of fish products (source of Hg) and alcoholic beverages (a major source of Pb) caused significant reduction in concentration of Hg and Pb in hair, while decreasing Cd concentration in hair was attributed to the higher intake of Ca from lactovegeterian diet depressing Cd absorption.

As an alternative to a long period of faecal monitoring for the chemical balance approach, a whole lavage technique has been proposed by Zheng *et al.* (1993). It was demonstrated that zinc absorption can be measured in humans from single foods containing a physiological dose of zinc by using a whole-gut lavage and that zinc bioavailibility from beef is about fourfold greater than from a single high-fibre breakfast cereal.

I.2.3 ISOTOPIC TRACER METHODS

The metabolic fate of minerals can be followed by the use isotopic tracers. The absorbed fraction of a tracer dose can be determined either by monitoring the amount appearing in the body (body, plasma or tissue monitoring) or that disappearing from the input (faecal monitoring). For enriched stable isotopic tracers, enrichment over natural abundance can be determined in serum, tissue, or faecal sample as required. For radioactive tracers, radioactivity can be measured in the same samples or by whole body counting. The advantages and disadvantages of these techniques are summarized in **Table-5**.

I.2.3.1 THE RADIOACTIVE TRACER TECHNIQUE

Approaches for determination of absorption were established with radioactive tracers until stable isotopic tracers became available. Moore *et al.* (1944) and Hahn *et al.* (1945) determined absorption of iron by measurement of tissue incorporation. This approach is still valid and enables determination of less than 1% absorption of iron from foods from which it is poorly available (Cook *et al.*, 1995). The metabolic fate of iron absorbed from a dose administered intravenously was investigated extensively with determination of losses through skin, urine, and luminal excretion by Dubach *et al.* (1955) and Bothwell *et al.* (1955). The determination of iron absorption by use of simultaneous oral and intravenous administration of two radioactive iron isotopes was established by Saylor and Finch (1953). The rate of entry of the isotope from the intestine into the plasma, and of the rate of disappearance from the plasma pool of the isotope caused by its uptake into the body tissue were measured. The same approach was also applied for plasma appearance and disappearance deconvoluation for Zn-69m by Molokhia et al. (1980).

Table-5: Advantages and disadvantages of the stable and radioisotopic tracers

STABLE ISOTOPIC TRACERS	RADIOISOTOPIC TRACERS
Advantages	**Advantages**
1.They are not radioactive; -No internal or external radiation -Especially useful for studies in vulnerable subjects (infants and women in reproductive years), -Natural constituents of foods, -Samples can be processed when convenient.	1.External monitoring by whole body counting is a reference method for measurement of mineral retention
2.They permit multiple-labelling studies; -Mineral-mineral interaction studies are possible, - Multiple isotopic studies are possible, -Longitudinal studies in the same individual are possible.	2.Multiple-labelling studies are possible -Mineral-mineral interaction studies are possible, - Multiple isotopic studies are possible,
3.They permit studies related to group or field comparison studies	3.Isotope can be added as a true tracer without changing the total element content of the diet
4.They can be measured (quantitative or isotopic ratio) with good sensitivity and precision by mainly TIMS and ICP-MS	4.They can be measured by relatively simple methods with easy detection in blood, urine or faeces
	5.Relatively cheap so that a greater number of subjects can be studied
Disadvantages	**Disadvantages**
1.Application to a substantial number of subjects is limited because of the high cost	1.They are a potential hazard of ionizing radiation, so are not applicable to vulnerable subjects
2.Analysis techniques are expensive usually MS and NAA. In MS chemical separation is often required to eliminate isobaric interferences	2.Multi-labelling studies are limited with radiation dose accumulation
3.The amount of stable isotope to be added may not represent usual dietary levels or the chemical form may differ from that found in food	3.They require expensive measurement equipment
	4.The time required for such studies is limited by the half-life of radioisotope(s)

Whole body counting enables determination of trace element absorption by external monitoring and is accepted as a reference method. The fraction of a single dose retained can be measured in a whole body or a specific part of it. Examples of investigations concerned with iron and zinc metabolism are provided by Bothwell *et al.* (1955) and Will and Boddy (1967) for iron and Graig and Siegel (1960) and Spencer *et al.* (1965) for zinc.

Lunn *et al.* (1967) compared absorption of iron obtained from whole body counting with that obtained from faecal monitoring and found poor agreement The discrepancy was attributed to incomplete stool collection, suggesting the requirement for faecal marker as a yield indicator. Measurement of absorption with reduced faecal collection by the use of radioactive markers was confirmed by comparing results for absorption derived from whole body and faecal monitoring. Examples of investigations which compare the 2 approaches are provided by Jasani *et al.* (1971) and Jasani & Fletcher (1972) using Fe-59 tracer and Ba-131 marker, and Payton *et al.* (1982) and Flanagan *et al.* (1985) using Zn-65 tracer and Cr-51 marker.

The main disadvantage of using radioactive tracers is the hazard associated with radiation exposure. This has partly been overcome with developments in radiation detection systems. Lykken (1983) used Zn-65 and Fe-59 radioisotopes to monitor absorption and metabolism by whole body counting for 418 days. The radiation dose received was less than that received from naturally occurring radionuclides present in the body over the period of the study.

Although the applications of radioactive tracers in measurement of absorption are limited on subjects in reproductive age and vulnerable groups, investigations with radioactive tracers and their determination by whole body counting has been shown to be a reliable standard. An investigation for determination of zinc absorption by whole body counting with the use of radioactive Zn-65 tracer performed by Hunt *et al.* (1995) on post menopausal

women is an example of a recent application. For monoisotopic elements, absorption investigations with the isotopic tracer approach are only possible with the use of their radioactive isotopes. The study for measurement of manganese (^{55}Mn with 100% abundance) absorption (by whole body counting) by using Mn-54 involving men and women subjects aged 22-30 years (Davidsson *et al.*, 1995) is a typical example for such elements.

I.2.3.2 THE ENRICHED STABLE ISOTOPIC TRACER TECHNIQUE

The basic principle of the enriched stable isotope approach is that an enriched stable isotope of an element is contained in an intake as a tracer and absorption is derived from enrichment determined in blood, urine, or faecal samples. An element must possess more than a single stable isotope to be amenable to the stable isotope approach. Of the 9 essential trace elements Mn, Co, and I are monoisotopic and thus are not amenable to the stable isotope approach (**Table-6**).

Table-6: Stable isotope constitution of the 9 essential trace elements

Element	*Isotopes and natural abundance, %
Cr	^{50}Cr (4.34), ^{52}Cr (83.79), ^{53}Cr (9.50), ^{54}Cr (2.37)
Mn	^{55}Mn (100)
Fe	^{54}Fe (5.8), ^{56}Fe (91.72), ^{57}Fe (2.2), ^{58}Fe (0.28)
Co	^{59}Co (100)
Cu	^{63}Cu (69.17), ^{65}Cu (30.83)
Zn	^{64}Zn (48.6), ^{66}Zn (27.9), ^{67}Zn (4.1), ^{68}Zn (18.8), ^{70}Zn (0.6)
Se	^{74}Se (0.89), ^{76}Se (9.36), ^{77}Se (7.63), ^{78}Se (23.78), ^{80}Se (49.61), ^{82}Se (8.73)
Mo	^{92}Mo (14.84), ^{94}Mo (9.25), ^{95}Mo (15.92), ^{96}Mo (16.68), ^{97}Mo (9.55), ^{98}Mo (24.13), ^{100}Mo (9.63)
I	^{127}I (100)

* Commission of atomic weights and isotopic abundances (1991)

The application of stable isotopic tracers in studies of human mineral absorption and metabolism was first described by Lowman and Krivit (1963). The study involved a comparison of plasma clearance rates of intravenously injected radioactive Fe-59 and stable ^{58}Fe tracers. A stable isotope tracer was used to measure iron absorption by its incorporation in red blood cell by Dyer and Brill (1972). The authors measured the percentage transfer from the maternal plasma to infant's circulating blood at birth. Simultaneously injected ^{50}Cr, to measure blood volume, and ^{58}Fe as a tracer were determined by NAA. Effects of oral contraceptives on mineral absorption from a daily intake labelled with ^{58}Fe, ^{70}Zn, and ^{65}Cu with faecal monitoring and analysis by NAA has been described by King *et al.* (1978).

Extrinsic and intrinsic labelling

An isotopic tracer can be inserted into the diet in an extrinsic or intrinsic form. For extrinsic labelling the tracer is added to the diet as a chemical element. For intrinsic labelling isotopic tracer is biologically incorporated in the diet. For intrinsic labelling the most desirable method since the tracer incorporated with a nutrient represents natural food constituents.

Production of intrinsic tracers can be achieved by hydroponic cultivation, by direct injection to leaves of plants, by oral feeding, or by direct injection to animals. Labelling with these methods give low yields (Janghorbani and Young 1982, and Fox *et al.* 1991) so that it is expensive. Comparative absorption studies for foodstuffs intrinsically and extrinsically labelled with radioactive tracers (Fe-55 and Fe-59) have shown that absorption results obtained from both types of labelling do not differ significantly (Layrisse et al., 1969 and Cook et al., 1972). A comparison with stable isotopic tracers has not been made for iron. However they would be expected to behave similarly to radioactive iron tags (Hurrell *et al.*, 1994). In this context, it is worth mentioning that Barrett *et al.* (1994) found similar iron absorption from a meat meal labelled with 2.8 mg ^{54}Fe and 10 ng of radioactive Fe-59.

Janghorbani *et al.* (1982) compared the two labelling methods in an investigation of zinc absorption and found no difference between intrinsic and extrinsic labelling. Serfass *et al.* (1989) showed the applicability of extrinsic tagging for formula based infants diet. However Fairweather-Tait *et al.* (1991) emphasized the need for caution when using extrinsic labels for studies of Zn bioavailibility. Insignificant differences in absorption from intrinsically and extrinsically labelled nutrients have been reported for magnesium, by Schwartz *et al.* (1980) and for copper, by Johnson *et al.* (1988).

When an investigation is planned to determine absorption from a nutrient by extrinsic labelling, care should be taken that the level of element added to the diet should not perturb the physiological requirements and the mineral status of subjects. The chemical form and solubility of an element in natural constituents and the form added should also be taken into account. This is particularly important for iron in view of its solubility (Forbes *et al.*, 1989) and for selenium regarding its chemical form (Janghorbani and Young, 1982).

I.2.3.3 APPROACHES TO INVESTIGATING 'ABSORPTION' BY USE OF THE STABLE ISOTOPIC TRACER TECHNIQUE

Measurement of disappearance from the dietary pool by faecal monitoring

When enriched isotopic tracers are used In determination of absorption by faecal monitoring, the difference between tracer recovered with faecal samples and that added to the diet provides the "net luminal disappearance" (NLD). NLD includes mucosal uptake and transfer of the mineral (i.e. absorption), and mucosal surface adsorption. It makes no assumption that all or any of the amount which has disappeared has been transfered to the body. The amount excreted includes the fraction initially absorbed and then re-excreted into the gut lumen.

Determination of luminal disappearance by faecal monitoring is particularly applicable to the elements Zn, Ca, Se, Mg, and Cu for which excretion from the intestinal system is significant. Measurement of absorption by this approach has been widely used for human subjects since it is not invasive. Examples of applications to infants are provided by Gordon *et al.* (1982) for Zn and Se, Ehrenkranz *et al.* (1984) and Ehrenkranz *et al.* (1989) for Zn and Cu, and Whitley and Aggett (1986) for Fe. Recent examples of applications to adults include Couzy *et al.* (1993), Johnson *et al.* (1988), and Turnlund (1990) for Cu, Mason *et al.* (1990) and Turnlund *et al.* (1990) for Fe, and Fairweather-Tait *et al.* (1992) and Couzy *et al.* (1993) for Zn.

A disadvantage of determination of luminal disappearance by faecal monitoring is that the contribution to output of isotopic tracer which was initially taken up and then re-excreted can not be identified. This can be significant for Ca, Cu, Se, and Zn and underestimate the fraction taken up. This effect was considered by Turnlund (1990) for Cu and Fairweather-Tait et al. (1992) for Zn and can be minimized by reducing the period of faecal collection after dose administration. With the use of markers, to account for recoveries in limited faecal collections, the effects of re-excretion may be significantly reduced.

Measurement of retention in the body by tissue incorporation or monitoring plasma appearance

Absorption can also be determined by measuring the extent of tissue incorporation of the element of interest. This approach has been frequently used in studies of absorption of iron. Usually an oral dose is given to subjects and the quantity incorporated in blood determined 2 weeks after administration. Iron incorporation into erythrocytes has been determined by the use of both stable and radioisotopic measurements (Skikne and Cook, 1992, Barrett *et al.*, 1992).

Determination of iron absorption by this approach, with the application of a double isotopic technique, eliminates variation of absorption amongst subjects depending on their iron status. Practically, a reference meal or a reference solution, and a test meal, labelled with different isotopes are administered to subjects on consecutive days and absorption from the test meal adjusted with reference to the reference meal or standard solution (Forbes *et al.* 1989). Application of this approach to adult subjects with the use of stable isotopic tracers is limited since the provision of a determinable enrichment 14 days after administration requires a high isotopic input. The approach has been shown to be practical for studies on infants. Kastenmayer *et al.* (1994) determined absorption from an infant formula which was administered twice in a day, each labelled with different tracers with the amounts of 2.5 mg of ^{57}Fe and 0.6 mg of ^{58}Fe compared with the required dose (for adults) of 10 mg of ^{57}Fe (in orange juice) for provision of a measurable enrichment when 10% of the dose was absorbed (Barrett *et al.*, 1992).

Monitoring the appearance of nutrients in plasma after an oral dose is a semi-quantitative approach to assessing the absorption since the orally given nutrient myy be taken up by the liver before entering the systematic circulation. The method is not applicable to most food sources of trace elements without giving pharmacological doses. Principally a sufficient oral dose is administered to enable observation of plasma appearance kinetics (Fairweather-Tait, 1992).

Monitoring plasma disappearance following intravenous administration of a tracer is an alternative approach which can provide information on the magnitude of endogenous losses via intestinal lumen, size of exchangeable pool, body turnover time, and biological half-life and has been used for Zn (Jackson *et al.*, 1984 and Jackson *et al.*, 1988).

Simultaneous oral and intravenous isotope administration

Following simultaneous oral and intravenous administration of two isotopic tracers, isotopic enrichments are measured with reference to a third isotope in urine or plasma samples as a function of time. Absorption the administered isotope is calculated with reference to the time-integrals of enrichment ratios.

Measurement of the absorption of iron by monitoring enrichments in plasma samples by this approach has been described by Whittaker *et al.* (1991) and Barrett *et al.* (1994) for Fe, and by Friel *et al.* (1992) for Zn. By measurement of enrichment in urine, Hillman *et al.* (1988) and Yergey *et al.* (1990) determined the absorption of Ca.

In determination of absorption by simultaneous oral and intravenous administration, both of the isotopes are assumed to be similarly metabolised. This may not be the case, because orally administered elements may be bound to different carriers in the plasma than those with which the intravenous isotope binds, and because orally given isotopes are more likely to be processed or taken up by the liver before entering the systematic circulation (Aggett and Whitley, 1994).

Compartmental modelling and kinetic analysis

With administration of more than one stable and/or radioactive isotopic tracers and their determination in the appropriate samples, it is possible to monitor the fate of absorbed trace elements. With this approach, compartmental and kinetic models have been developed and investigated.

Investigations by compartmental modelling and kinetic analysis have been carried out for zinc metabolism and kinetics by Foster *et al.* (1979), using radioactive Zn-69m, Wastney *et al.* (1986) with radioactive Zn-65, Wastney *et al.* (1991) with radioactive Zn-65 and stable ^{70}Zn isotopes by simultaneous

oral administration, and by Fairweather-Tait *et al.* (1993) to measure the size of exchangeable pools for zinc with ^{70}Zn. Selenite metabolism has also been examined with the same approach, with the use of stable ^{74}Se tracer (Patterson *et al.*, 1989). Theoretical modelling for these approaches is provided in Cobelli *et al.* (1987).

***In-vitro* procedures**

The composite effects of minerals in different diet or foods on bioavailibility and specific steps in absorption or metabolism processes have also been investigated by *in-vitro* procedures which simulate release of elements from foodstuffs and related processes. Such approaches have been applied to animal and human investigations.

The following investigations are examples of *in-vitro* studies; estimation of available dietary iron (Monsen *et al.*, 1978), review of the use of laboratory animals in zinc bioavailibility studies with references for *in-vitro* applications (Forbes, 1984), the effect of picolinic acid on metal translocation across lipid bilayers (Aggett *et al.*, 1989), soluble and ionizable iron determination in foods (Turnlund *et al.*, 1990), absorption and subsequent metabolism of metal amino acid chelates and inorganic salts (Ashmead, 1991), selective extraction of iron species present in diet and rat gastrointestinal tract contents (Simpson *et al.*, 1992), investigations related to bioavailibility of different form of ferric phosphates (Willis and Montgomery, 1994).

I.3 THE USE OF MARKERS IN MEASUREMENT OF TRACE ELEMENT ABSORPTION

The use of stable or radioactive tracers to measure faecal output enables the determination of net luminal disappearance (net intestinal uptake). Measurements based on faecal analysis requires reliable faecal collection over several days, until all unabsorbed tracer is excreted. Faecal collection for a longer period than required can cause low estimation of luminal disappearance, due to re-excretion of tracer initially absorbed. Re-excretion can be significant for elements for which homeostasis is maintained by intestinal excretion, depending on the amount of intake and state of health, *e.g.* Zn, Cu. Incomplete collection of faecal samples (accidental rejection, lack of care due to illness, or with toilet paper) can result in overestimation of luminal disappearance. By the use of markers completeness of faecal collection can be measured and the endogenous contribution can be minimized by determination of luminal disappearance with reduced sample collection.

1.3.1 CLASSIFICATION OF NUTRITIONAL MARKERS

The marker must be neither adsorbed nor absorbed, and must be either soluble or completely dispersed in water.The desirable properties of nutritional markers are summarized in **Table-7** from Morgan (1986), Kotb and Luckey (1972), and Fordtran (1966).

Lack of digestibility, complete recovery, and ease of measurement have been the characteristics of major concern in the search for ideal nutritional markers (Kotb and Luckey, 1972). A marker which successfully meets the requirements of one application might not be useful in other applications. In most balance studies (particularly short-term studies) two type of faecal markers have been used: intermittent markers such as charcoal or carmine administered at the beginning and end of an experimental period to distinguish

faeces related to the period of feeding, and continuous markers such as PEG or $BaSO_4$, which define the quantity of food, assuming that the marker and nutrient studied have similar transit times through the alimentary tract (Sharpe and Robinson, 1970, Bacon, 1980).

Table-7: Criteria for effective nutritional markers

An ideal marker

A. Should

1. Mix intimately with the usual food and remain uniformly distributed in the digesta, and pass through gastro-intestinal tract,
2. Enable an estimate of faecal irregularities in excretion and losses,
3. Be soluble and have diffusion space similar to test substance,
4. Be well defined chemically,
5. Be accurately determined at low concentrations.

B. Should not

1. Have toxic physiological affects,
2. Strictly be absorbable (slowly absorbed markers slightly underestimate absorption rate),
3. Be trapped by mucus or other intestinal content,
4. Influence digestion of the test substance,
5. Influence gut flora activity, or influence intestinal motility,
6. Be degraded.

In applications of chemical balance techniques, markers have been used to indicate whether or not a steady state was obtained. After a period of continuous administration of intake tagged with a marker, absorption was determined by the difference between daily intake and output when a steady state was reached (Rose, 1964 and Davignon *et al.*, 1968).

Radioactive and stable isotopic tracers enable determination of absorption from a single input. Radioactive tracers were used by Najean and Ardaillou (1962), Boender and Verloop (1969), and Payton *et al.* (1982), and stable isotopic tracers by King *et al.* (1978). In the latter case, subjects were fed a

controlled diet containing a constant amount of trace elements of interest on daily basis, and the isotopic tracers of Fe, Cu, and Zn were administered with 2 g of polyethylene glycol (PEG) to measure completeness of faecal collection. Classification of the markers used in absorption investigations is provided in **Table- 8** derived from Kotb and Luckey (1972) and Morgan (1986).

Table-8: Classification of markers [derived from Kotb and Luckey (1972) and Morgan (1986)]

A. Compounds

1. Organic; natural (carmine, chromogen) and synthetic dyes (methylene blue, anthraquinone violet, etc.), Polyethylene glycol (PEG)
2. Inorganic
    a. Metal oxides (Cr_2O_3, TiO_2)
    b. Mineral salts ($BaSO_4$, CuSCN, $CrCl_3$)
    c. Rare earths
3. Other (cellulose, plant sterols)

B. Natural, or artificial radioactive isotopes (^{40}K, ^{51}Cr, ^{131}Ba, ^{140}La, ..)

C. Particulates

1. Radio-opaque pellets
2. Polymers (glass beads, rubber)
3. Cells, yeast, bacteria

Considerations and applicability in terms of toxicity and practicality are provided below for markers which are commonly used in investigations of trace element absorption. Although metabolic balance studies are not the concern for this investigation, some of the important characteristics of markers for such investigations are also considered, since recent investigations have involved the combined use of continuous input of a trace element with a dietary input (until a steady state is maintained) and a single input of the nutrient labelled with tracer isotope.

1.3.1.1 COMMONLY USED MARKERS

Dyes

Intermittent faecal dye markers are best used in conjunction with continuous markers as the advantages of two types will then combine. Continuous markers assess the extent of excretion of dietary substances, while intermittent markers indicate transit time and identify separate study periods, especially where new dietary or therapeutic regimes are introduced. This combination gives better accuracy than a marker alone and increases the reliability of the metabolic balance study, particularly where time is limited. Dye markers are observed by faecal colour (Bacon, 1980).

A noticeable effect of giving any dye is the tendency for it to spread out in the intestinal contents over several days. It has been shown that in a standard meal containing phenol red, dissociation occurs in the stomach to a solid phase rich in fat and poor in phenol marker, and a liquid phase poor in fat and rich in phenol red marker, which was preferentially emptied from the stomach. This and variation in transit time show that these are unsatisfactory markers for balance studies (Rose, 1964, Morgan, 1986).

Typical applications of intermittent dye markers have been described by Jackson *et al.* (1984), Mason *et al.* (1990), Fairweather-Tait *et al.* (1992) for carmine; Couzy *et al.* (1993) for brilliant blue together with a marker (radio-opaque pellets, CuSCN etc.) to measure completeness of stool collection on adult subjects. Dye markers have been widely used in studies of absorption on premature infants to mark the time interval for faecal collection, as described in studies performed by Ehrenkranz *et al.* (1984), Whitley and Aggett (1986), Ehrenkranz *et al.* (1989), Ziegler et al. (1989) using carmine red. The doses used are usually 500 mg for adults and 100 mg for infants.

Metal oxides and mineral salts

Chromium compounds

Chromium is one of the least toxic of the essential trace metals on the basis of the ratio of essential to toxic dose. The mammalian body tolerates about 100-200 times its total body content of chromium without showing effect. Rose (1964) suggested that Cr_2O_3 capsules were tolerated well by patients, whether adults or children, and no side effects were observed when 500 mg per day of Cr_2O_3 was administered to patients in a balance study. The required dose of Cr_2O_3 was proposed to be 300 mg per day by Davignon et al. (1968). For these high amounts of marker input, toxic effects have been occasionally reported, one development of pelvic appendicitis occuring in an investigation carried out by Whitby and Lang (1960).

In a study comparing the applicability of PEG and Cr_2O_3, it was found that Cr_2O_3 was excreted slightly more slowly than PEG. PEG was recovered quantitatively in 5 days after marker administration while Cr_2O_3 was still being excreted on day 7. Cr_2O_3 was recovered even 42 days after feeding ceased. The absence of Cr_2O_3 in some of the intervening samples during this time was explained by sedimentation of the relatively dense Cr_2O_3 (Allen *et al.*, 1979). Late excretion could be significant when intestinal mobility was abnormal, as in steatorrhoea (Bacon, 1980).

Hinton *et al.* (1969) showed that chromium chromate (labelled with Cr-51) was not an ideal marker since significant losses occurred due to absorption. In attempts to determine trace element absorption with single input by the use of radioactive Cr salts as marker, $CrCl_3$ (labelled with Cr-51) has been used in several investigations *e.g.* Powell *et al.* (1970) and Marx (1979). Gibson *et al.* (1988) found that the absorption of $CrCl_3$ was 23-33% in pigs, compared with levels of up to 4% in humans.

Barium sulphate

Figueroa *et al.* (1968) recommended that barium sulphate could be a more ideal marker on the basis of its specific gravity of being 1.5 in comparison with chromic oxide being 5.2.

In studies of iron absorption, Najean and Ardalillou (1962), Boender and Verloop (1969), Jasani *et al.* (1971), and Jasani and Fletcher (1972) used radioactive tracer (Fe-59) and $BaSO_4$ (labelled with Ba-131) as marker. Recoveries were shown to be nearly quantitative, and the level in samples provided a week after administration were below detection.

Copper thiocyanate

Copper thiocyanate has limited use due to the absorption of SCN radicals (Morgan, 1986). Daily adult doses of less than 500 mg over a limited period appear to be harmless (Kotb and Luckey, 1972 and Bacon, 1980). Jackson *et al.* (1984) used copper thiocyanate in the investigation of Zn absorption with stable isotopic tracer, but no details were provided.

Particulates

Radio-opaque pellet (ROP)

It has been shown that the rate of passage of various substances (*e.g.* cotton seeds, glass beads, rubber, cells etc.) along the gastro intestinal tract relate to their specific gravity and particle size. Morgan (1986) considered that ROP to be a satisfactory inert marker.

From an investigation of fat absorption by the use three markers (ROP, PEG, and Cr_2O_3), recoveries were found to be 98.1% for ROP, 93.8% for PEG, and 100.2% for Cr_2O_3 (Simpson *et al.*, 1979). The authors concluded that ROPs are suitable as non-absorbable markers for faecal fat studies, and offer several distinct advantages over other markers. Transit time of the ROP was faster than that of Cr_2O_3, and the tailing effect of ROP shorter than that of

Cr_2O_3. They are limited in use for infants and patients with gastro-intestinal obstructions (Morgan, 1986). The recovery of ROP's is determined by counting them in x-rays of stools. A recent application of ROP in absorption studies with stable isotopic tracers is provided by Taylor *et al.* (1991).

Polyethylene glycol (PEG)

Polyethylene glycol has become the most popular marker in absorption investigations following investigations carried out by Wilkinson (1971) on human subjects. Allen *et al.* (1979) reported an average cumulative recovery of 93.1±8.6% for 6 subjects. Low cumulative recovery was explained by losses due to absorption of small amounts of PEG by the intestine, or destruction of small amounts of PEG by bacterial or intestinal enzymes.

Lentner *et al.* (1975) showed that 99% recovery was obtainable with three markers, which were PEG, Cr_2O_3 determined chemically and by radioactive marker. Results from single and pooled stool specimens were identical. The results suggested that for practical reasons Cr_2O_3 was preferable to PEG. When intestinal mobility is abnormal, PEG, being water soluble and less liable to sediment than insoluble Cr_2O_3 or $BaSO_4$ provides advantage in its choice (Wilkinson, 1971).

PEG has been used as marker in investigations of trace element absorption with stable isotopic tracers by King *et al.* (1978), Swanson *et al.* (1983), and Turnlund *et al.* (1991).

1.3.2 RARE EARTHS AS MARKERS

I.3.2.1 CHEMICAL PROPERTIES OF THE RARE EARTHS

The rare earths are the elements; La, Ce, Pr, Nd, Pm (artificial), Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu and these are all in Group III of the Periodic Table. Their chemical properties, involvement in biochemical systems, and metabolism are similar.

La is the most basic and Lu is the least basic. Within the group, the aqueous solubility of metal salts decreases with increase in atomic number. The toxicity of rare earth salts increases with their aqueous solubility. The solubility products of rare earth hydroxides range from 10^{-15} to 10^{-24}. Poor solubility confers adsorptive capacity since adsorption to other materials is inversely related to the solubility product (Venugopal and Luckey, 1974).

The most characteristic properties of rare earths in aqueous solution are the high stability of the +3 oxidation state and the ionic nature of bonding complexing groups. The +4 oxidation state for Ce is stable. As the result of electronic configuration being a half-filled f-subshell with 7 electrons, the +2 state is an exception for Eu. Europium in the +2 oxidation state may replace Ca^{+2} in view of the similarity of their coordination geometry. These specific characteristics of Ce and Eu reflect anomalies in natural occurrence (Gijbels, 1987). The comparable properties of $(RE)^{+3}$ and Ca^{+2} are of biochemical relevance, with their similarities in their sizes, bonding, coordination geometry, and donor atom preference (Evans, 1990).

The solubility of rare earths in natural waters is very low. The concentrations have been reported to be below $\mu g\ mL^{-1}$ (Goldstein and Jacobsen, 1988, Laul *et al.* 1988, and Esser *et al.*, 1994). In view of the low solubility of the rare earths, fractional transfer from soil to plant is expected to be low. However investigations of rare earth uptake by plants grown on a natural soil showed

that the depletion factor (plant to soil concentration ratio) was 10^{-4} (Laul and Gosselin, 1989), 10^{-2}-10^{-3} (Miekeley *et al.* 1994), and 10^{-1} (Ichihashi *et al.* 1992). Concentrations for La and Yb reported by Sun *et al.* (1994) being 379 and 13.1 ng g^{-1} in leaves, and 3.19 and 0.21 ng g^{-1} in grains of wheat from plants grown in soils where the corresponding concentrations were 47800 and 2500 ng g^{-1}. These levels provide depletion factors of ~10^{-2} for leaves and ~10^{-5} for grains.

With considering the concentration range for La in geological formations in Scotland being 10-50 µg g^{-1} (British Geological Survey, 1991), and a depletion factor ranging 10^{-1} -10^{-5}, the levels of La enter to people via drinking water and food should be very low.

I.3.2.2 TOXICOLOGY AND INTESTINAL ABSORBABILITY OF THE RARE EARTHS

Investigations of the pharmacology, toxicology, and clinical applications of rare earths indicate that they have a low to moderate acute toxicity and cause very little change in animals when fed for several months (Venugopal and Luckey, 1975). Single or repeated injections of chlorides of La, Ce, Pr, and Nd into rabbits caused considerable change in haemoglobin, leucocyte count, erythrocyte count whereas oral administration was without effect. Rare earths caused no internal organ damage when fed at levels of 0.01, 0.1 and 1 % of diet for 90 days. None of rare earths had an effect on growth or on the haematological data (Haley, 1965).

In summarising previous investigations, Kyker (1961) concluded that there is essentially no absorption of rare earths from the gastrointestinal tract. Absorption of orally administered rare earths is typically less than 0.05% for mammals. Toxicity studies comparing oral and intraperitonial routes in rats have shown that oral doses exceeding 2 g RE kg^{-1} are tolerated without adverse effects.

In preparation for administration of rare earth markers to humans, Hutcheson *et al.* (1975) investigated the effect of rare earth oxides on three generation of mice. It was found that, there were no apparent effects as measured by growth, reproduction, haematology, and gross necropsy observations. Feeding monkeys with a diet containing rare earth oxides for 63 days Hutcheson *et al.* (1974) observed no effect and found no rare earths in tissues. **Table-9** provides the LD_{50} doses of rare earths (chloride and nitrate where available) and those of essential element compounds for comparison.

Table-9: Acute lethal dose for rare earth compounds (LD_{50}) for oral administration in rat and mouse (Bruce *et al.*, 1963 and Venugopal and Luckey, 1974)

	Dose (LD_{50}), mg kg^{-1}			
Rare earths/Compounds→	Chloride	Nitrate	Oxide	Sulphate
La	2370	4500	8500	2450
Ce	1200	1355		
Pr	1700	800		
Nd	2125	905		
Sm	840	960		
Eu	2075	1740		
Tb	2175	1750		
Dy	3290	1100		
Ho	3140	1078		
Er	2700	NA		
Tm	2635	NA		
Yb	2995	1195		
Lu	4400	NA		
Iron chloride	186 (lethal)			
Zinc sulphate	500			
Copper chloride	66			
Sodium selenate	4			

Whilst primarily interested in histological, morphological, and biochemical effects of $LaCl_3$ as an agent, Kramsch *et al.* (1980) included absorption measurements were also included. 40 mg kg^{-1} d^{-1} $LaCl_3$ was administered to rabbits for a period of 8 weeks and the same amounts were provided to monkeys for 10 months. After 8 weeks of treatment about 33 µg La^{+3} were found in the whole liver of rabbits, which was 0.0006% of the total intake and no La was detected in bone. The results for monkeys showed that small amounts (average 64.5 µg) were contained in urine collected for 24 h. An average of 17.1 µg $(100\ mL)^{-1}$ were found in circulating whole blood, but none was detected after 12 h. It was concluded that even the largest dosage of La administered orally did not impair general well being, and the normal growth and the development of growing animals. No physiological changes were observed in heart, haemopoietic, and nervous systems, which appeared to have retained their normal function. Structural Ca and P were unchanged.

Robinowitz *et al.* (1988) investigated the distribution of orally administered La in rat tissues. Radioactive La-140 tracer with additional $LaCl_3$ solution in drinking water was administered for 3 days at 120 mg kg^{-1} d^{-1} (37.5 mg d^{-1}) and the La content of the tissues was measured radiometrically by liquid scintillation counting. Absorption of La was found to be less than 1% of the total dose, with no toxic effect.

In an extensive review of biochemistry of rare earths, it is emphasized that nearly all authors agree that almost no absorption occurs through the gastrointestinal tracts of adult vertebrates and that there is nearly no transfer of rare earths from mother to fetus (Evans, 1990).

In the context absorbability, it is worth mentioning the levels of rare earths determined in human tissues by different authors (**Table-10**).

Table-10: La and Yb concentrations in various human tissues

	Concentrations, ng g^{-1} or ng mL^{-1}		
Tissue	La	Yb	Author
Plasma	4.49	2.04	Esposito *et al.* (1986)
Plasma	6	4	Collechi *et al.* (1987)
Plasma and urine	<0.3	<0.3	Allain *et al.* (1990)
Plasma	3.4	1.9	Esposito *et al.* (1993)
Whole blood	5.8-16.8	-	Mosulishvili *et al.* (1985)
Human liver	3.5-190	<0.02-0.71	Tijoe *et al.* (1983)
Human heart	0.25-8.71	<0.03-<0.15	"
Human cortex	0.44-2.39	<0.03-<0.13	"
Human lung	1.5-75	0.25-6.00	Pietra *et al.* (1985)
Kidney stones	17-79	6.4-219	Koeberl and Bayer (1992)
Brain tissue	46	24	"
Cataracts	45-2266	-	Stverak *et al.* (1988)

As can be seen from the table, concentrations of La and Yb in various tissues are very low, which indicate low accumulation in the body. Using a liver weight of 1.8 kg for standard man results in total contents of 6.3-342 µg La and <0.04-1.28 µg Yb.

I.3.2.3 THE USE OF RARE EARTHS AS MARKERS

Applications of rare earth markers to animal studies have been concerned with investigations of transit in ruminants, where the rare earths have been adsorbed on forage (Miller *et al.*, 1966, Ellis and Huston, 1967, Ellis, 1968, Huston and Ellis 1968, Olbrich *et al.*, 1971, Hartnell and Satter, 1979, Crooker *et al.*, 1982, Turnbull and Thomas, 1987) and with the studies of digesta kinetic modelling (Ellis *et al.*, 1979, Poore *et al.*, 1991 and Moore *et al.*, 1992).

In preparation for the application of rare earth markers to studies of nutrient

uptake by humans, Luckey *et al.* (1975) investigated the use such markers in rats, using nitrate salts. To investigate recovery after a single dose, inputs of 27 µg of Tb, Yb, and Lu were added to diet, and virtually complete recovery (99%) was achieved after 3 days. For stabilisation of continuous input and determination of apparent absorption, daily inputs of the markers were 5 µg Yb, 7 µg Tb, and 4 µg Ce. A steady state excretion rate was achieved in 2 days. Apparent absorptions of kcal, Ca, K, and Fe determined directly (balance) and indirectly (marker correction) showed some variability, but generally agreed within ±5%. Anomalies are possibly explained by counting statistics associated with the nuclear characteristics and the levels at which some markers were used. It was concluded that some heavy metal salts could be used for a variety of purposes in studies of nutrient absorption.

In a study of monkeys, primarily concerned with safety aspects of the use of rare earth markers, Hutcheson *et al.* (1974) added oxides to diet to provide daily inputs of approximately 1.3 mg of Sm, Dy, and Tb, 0.7 mg for La, 0.1 mg for Yb, and 0.04 mg for Eu. In two balance periods with constant input, recoveries were 101.6±6%, excluding Tb, where low yields were attributed to procedural problems. It was concluded that oxides of the metals used could be safely used as nutritional markers.

The first multiple marker application to humans was described by Luckey *et al.* (1977). Rare earth oxides were administered in a capsule, with addition of methyl cellose, at 100 times the levels intended for daily marker use, thereby allowing quantitative determination of 0.1% of the total marker input several days after administration. For a single input, the amounts of rare earths ranged from 0.06 mg (for Dy) to 60 mg (for La). Recoveries were 91.5±10% for La, 84.7±10% for Sm, 95.7±11% for Eu, 88.5±8% for Tb,and 96.9±12% for Yb. There were no differences in faecal excretion patterns despite the fact that metals differed in atomic weight and specific gravity. In four subjects, differences were found in the rate of excretion. 95% of the marker appeared in 2 days for two subjects, and 3 and 4 days for the others. Recoveries also

apparently correlated with rate of throughput, 2 day excretion providing a yield of 96±10%, with 3-4 day 87±6%.

Hutcheson *et al.* (1979) applied rare earth markers to determine absorption of 4 nutrients (energy, fat, protein, and calcium), each nutrient being associated with a different marker. For 7 days, the daily marker inputs were 120 µg for Sm (for energy), 0.5 mg for Eu (for protein), 850 µg Yb (for Ca), and 1.3 mg Sc (for fat). The total doses received were therefore 0.84 mg of Sm, 3.5 mg of Eu, 6 mg of Yb, and 9.1 mg of Sc. Metal oxides were added to food before cooking. Tb was used as a marker of total intake with a daily input of 1.2 mg. Apparent absorption by balance and marker correction showed good agreement, as can be seen in **Table-11**.

Table-11: Comparison of absorption determined by balance and marker correction (Hutcheson *et al.*, 1979)

	Absorption, %	
Nutrient (marker)	Balance method	Marker method
Energy (Sm)	94.1±1.2	94.8±2.5
Protein (Eu)	93.4±0.9	94.7±1.4
Ca (Yb)	34.7±5.0	34.5±7.4
Fat (Sc)	96.0±1.4	95.9±0.6

With trace element uptake being determined by measurement of output after a single dose, the use of rare earth markers may enable account to be taken of recoveries in limited sample collection, this being less demanding of subjects, and potentially applicable in unconfined situations.

I.4 CALCULATION OF ABSORPTION

Calculation of absorption in faecal monitoring

Some of the approaches used in calculation of absorption by faecal monitoring, using marker correction for quantitative, limited, and/or single sample collections are provided below. For ease of comparison, a standard notation has been applied, with fractional "absorption" being indicated by F and the fractions of nutrient and marker in faeces and diet by $\mathbf{I}_f$ and $\mathbf{I}_d$, and $\mathbf{M}_f$ and $\mathbf{M}_d$ respectively..

Irwin and Crampton (1951) investigated the apparent absorption of dietary dry matter in man, using Cr_2O_3 as an inert marker for yield correction, where total faecal collection was considered.

Seife (1962) considered that the combined use of a radioactive inert-indicator and a radioactively labelled nutrient could eliminate the need for quantitative feeding and stool collection. The absorption by dogs from a single input of fat labelled with I-131 was measured with the use of $BaSO_4$ (labelled with Ba-131) as an inert marker. The formula used in the calculations for fractional absorption, **F** is given by,

$$F = 1 - \frac{I_f M_d}{I_d M_f}$$

Boender and Verloop (1969), and Jasani and Fletcher (1972) applied the same approach using the radioactive marker technique associated with radioactive tracer for limited and/or single sample collection. They used $BaSO_4$ (labelled with Ba-131) as marker and Fe-59 as tracer for measurement of iron absorption, and measured the fractional "absorption" for limited sample collection by using marker yield correction. The same approach was also applied by Luckey *et al.* (1975) and Hutcheson *et al.* (1979) using the rare earths as marker for determination of macronutrient "absorption" (by chemical

balance method) with limited and/or single faecal sampling when steady state had been reached for animals and human subjects respectively.

Payton *et al.* (1982) measured the fractional absorption of zinc, using radioactive tracer and marker, Zn-65 and $CrCl_3$ (labelled with Cr-51). They postulated that fractional absorption can be determined by retention measurement by whole body counting or recovery measurement with limited faecal collection. Fractional absorption, **F** was determined from:

$$F=\frac{(1-I_\beta)-(1-M_\beta)}{M_f}$$

The formula was also applied to whole body counting, since the retention of tracer and marker within the body could be determined after defeacation.

Calculation of luminal disappearance

When mineral absorption is determined with stable isotopic tracers, net luminal disappearance is provided by the difference between input and faecal output. In such calculations account must be taken of elemental output containing the natural abundance of the enriching isotope (endogenous contribution). The time dependent appearance in faeces of marker, isotopic tracer, and the endogenous contribution of the isotope are provided in **Figure-2.**

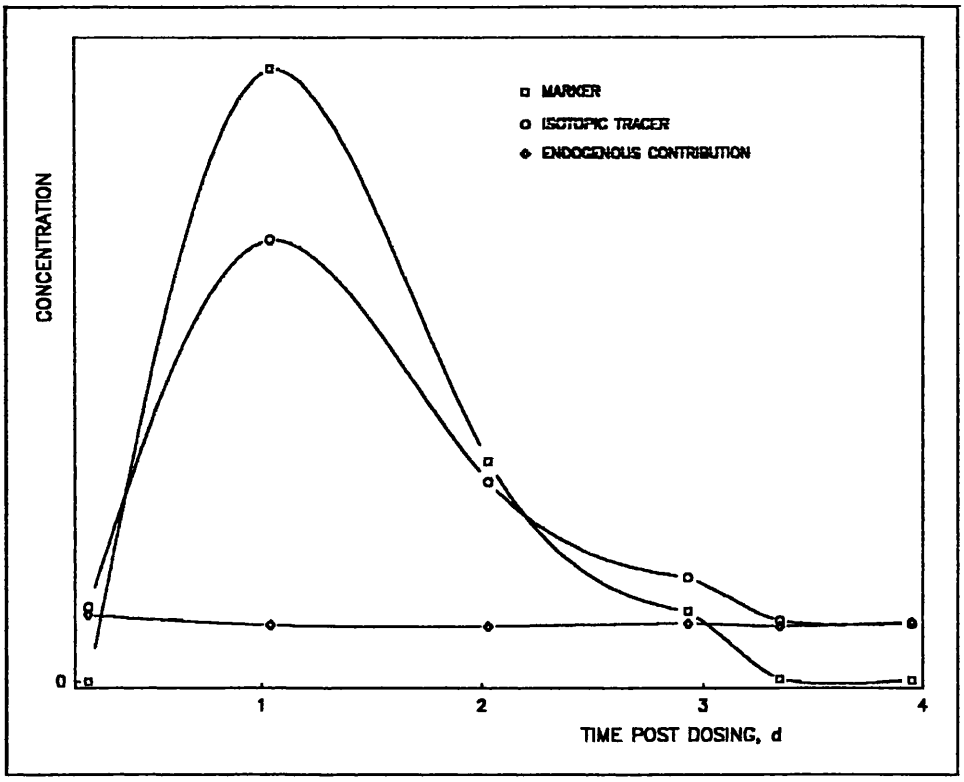


Figure-2: Profiles of recoveries of marker, isotopic tracer, and endogenous contribution of the isotope in faeces, after a single dose

Notation,

E_f : Amount of element in faeces, including unabsorbed enriched isotopic tracer,

E_n : Amount of element in faeces which consists of the fraction of total element of natural composition,

I_f : Total isotopic content of faeces, including endogenous excretion and unabsorbed isotopic tracer,

I_u : Amount of unabsorbed isotopic tracer in faeces,

I_e : Endogenous contribution of the enriching isotope to the total isotopic contents of faeces,

R_e : Natural abundance of the enriching isotope,

M : Fractional recovery of marker

I_d : Amount of enriched isotopic tracer provided with the dose

F: Fractional net luminal disappearance

a. The total amount of the element of natural composition in a faecal sample,

$$E_n = E_f - I_u \qquad 1\text{-a}$$

b. The amount of unabsorbed isotopic tracer in a faecal sample,

$$I_u = I_f - I_e \qquad 1\text{-b}$$

c. The endogenous isotopic contribution of a faecal sample in natural composition,

$$I_e = R_e E_n \qquad 1\text{-c}$$

d. For a recovery **M** (as indicated by marker), at a fractional luminal disappearance of **F**, I_u is given by

$$I_u = M(1-F)I_d \qquad 2\text{-a}$$

so **F** can be calculated from:

$$F = \frac{MI_d - I_u}{MI_d} \qquad 2\text{-b}$$

e. Combining equations 1-a and 2-a gives

$$E_n = E_f - M(1-F)I_d \qquad 3\text{-a}$$

f. From equations 3-a and 1-c, I_e is given by

$$I_e = R_e[E_f - M(1-F)I_d] \qquad 3\text{-b}$$

g. Substituting I_e from equation 3-b in equation 1-b gives

$$I_u = I_f - R_e[E_f - M(1-F)I_d] \qquad 3\text{-c}$$

h. Substituting I_u from equation 3-c in equation 2-b gives

$$F = \frac{MI_d - [I_f - R_e[E_f - M(1-F)I_d]]}{MI_d} \qquad 4$$

a. The total amount of the element of natural composition in a faecal sample,

$$E_n = E_f - I_u \qquad 1\text{-a}$$

b. The amount of unabsorbed isotopic tracer in a faecal sample,

$$I_u = I_f - I_e \qquad 1\text{-b}$$

c. The endogenous isotopic contribution of a faecal sample in natural composition,

$$I_e = R_e E_n \qquad 1\text{-c}$$

d. For a recovery **M** (as indicated by marker), at a fractional luminal disappearance of **F**, I_u is given by

$$I_u = M(1-F)I_d \qquad 2\text{-a}$$

so **F** can be calculated from:

$$F = \frac{MI_d - I_u}{MI_d} \qquad 2\text{-b}$$

e. Combining equations 1-a and 2-a gives

$$E_n = E_f - M(1-F)I_d \qquad 3\text{-a}$$

f. From equations 3-a and 1-c, I_e is given by

$$I_e = R_e[E_f - M(1-F)I_d] \qquad 3\text{-b}$$

g. Substituting I_e from equation 3-b in equation 1-b gives

$$I_u = I_f - R_e[E_f - M(1-F)I_d] \qquad 3\text{-c}$$

h. Substituting I_u from equation 3-c in equation 2-b gives

$$F = \frac{MI_d - [I_f - R_e[E_f - M(1-F)I_d]]}{MI_d} \qquad 4$$

I.5 METHODS OF ANALYSIS

The required dose of a stable isotopic tracer depends on the limit of detection and precision of the analytical method employed to determine isotopic enrichment in the analytical medium, and the naturally occurring levels of the isotope.

Determination of isotopic tracers by NAA is only possible if the irradiation product of an isotope has suitable decay characteristics for its determination. Enrichment by a factor of 5-10 over natural level for a specified isotope in a diet has been reported to be satisfactory for studies of mineral absorption, based on a single input of the enriched source (Janghorbani and Young, 1982). Enrichment in faecal samples caused by the unabsorbed fraction of 1 mg orally administered ^{70}Zn (0.6% natural abundance) could be determined with 1-5% precision by neutron activation analysis (NAA), Janghorbani *et al.* (1980). The precision for determination of the enrichment of the same isotope in plasma following an oral administration of 3.2 mg of ^{70}Zn was 5-8% (Janghorbani *et al.*, 1981-a). Precision for determination of ^{46}Ca in faecal, plasma, and urine samples has been reported in a range of 1-5% by Janghorbani *et al.* (1981-b), whilst the precision for ^{58}Fe is typically 1%. Examples of applications of NAA in absorption investigations with stable isotopic tracers are provided by King *et al.* (1978), Solomons *et al.* (1982), Gordon *et* al. (1982), Whitley and Aggett (1986), Gökmen et al. (1989), and Bang-Fa Ni *et al.* (1991).

In principal all isotopes of an element can be measured by mass spectrometric (MS) methods. Although NAA and MS have comparable limits of detection, MS provides better precision in a range of 0.1-1%. ICP-MS can achieve better than 1% precision when applied to quantification of an isotope or isotopic ratio measurements. Applications of ICP-MS in measurements of absorption studies have been described by Ting and Janghorbani (1986) for Fe, Ting and Janghorbani (1987) for Cu, Zn, and Fe, and Schuette *et al.*

(1988) for Mg isotopes. Janghorbani and Ting (1989) have surveyed the use of ICP-MS in investigations of absorption.

In the current investigation, NAA was employed for determination of the rare earths used as markers and also to determine the enrichment of ^{58}Fe and ^{70}Zn tracers. For determination ^{57}Fe tracer, which is not amenable to NAA, and rare earth baseline levels, ICP-MS was used. The total element content of samples, required to take into account the natural isotopic abundance of the element were determined by AAS. The principles of analytical methods employed are summarized below. Further details are available in De Soete *et al.*, 1972 (for NAA), Jarvis *et al.*, 1992 (for ICP-MS), and Haswell, 1991 (for AAS).

I.5.1 NEUTRON ACTIVATION ANALYSIS

In activation analysis elements are determined by measurement of the radioactivity induced when they are irradiated with nuclear particles. Nuclear activation with neutrons is called 'neutron activation analysis ' (NAA). Thermal neutrons are the most widely used of all irradiating particles in view of:

a.The availability of large fluxes of thermal neutrons in nuclear reactors.

b.The large volumes of uniform neutron flux intensity.

d.The large cross sections existing for radiative capture of thermal neutrons.

e.The avoidance of many radionuclides produced through endoergic reactions

The absolute activity at the end of irradiation A_0 of a radionuclide Y generated in a mass m of an element X by activation in a flux of Φ thermal neutrons per cm^2 s^{-1} is given by:

$$A_0 = mNR_e A^{-1} \Phi \sigma (1 - e^{-\lambda t}) (e^{\lambda t_d})$$

where

N= Avogadro's number,

R_e= The fractional abundance of parent nuclide ^{A}X in element X,

σ= The cross section for the reaction ($^{A}X + n \rightarrow Y$) in cm^2 per nucleus,

A= The atomic weight of element X,

λ= The decay constant of radionuclide Y,

t= The period of exposure to activating neutrons,

t_d= The period of decay between activation and counting, and

$1-e^{-\lambda t}$ is the saturation factor for the length of irradiation.

The activity produced tends asymptotically to a maximum as the period of activation increases, and little is to be gained by increasing the time of irradiation beyond ten half-lives.

The equation provides the absolute activity at the end of irradiation for a specified stable isotope, but requires input of parameters which are not always accurately known (*e.g.* neutron flux, reaction cross-section). This equation can be applied for absolute analysis, but analyses are usually carried out by comparing the activities produced in samples with those produced in known amounts of the elements of interest (assuming natural isotopic composition). However, since measured activation products arise from specific stable isotopes, NAA can also be applied to determine isotopic enrichment. The mass of the required isotope is provided by

$$m_1 = m_2 \frac{A_1}{A_2}$$

where

m_1 and A_1 are mass and activity of a standard, and m_2, A_2 are mass and activity of the sample respectively.

The measured count rate for a sample or a standard, **C** is given by

$$C = k\epsilon A_0 e^{\lambda t_d}$$

where $\mathbf{A_o}$ is the absolute activity at the end of irradiation, $\boldsymbol{\varepsilon}$ is the efficiency of detection, and **k** is a factor accounting for chemical processing yield, and flux correction. High detection efficiency is desirable for minimum limits of

detection.

Selection of a suitable procedure for an NAA application requires consideration of the following:

1.The choice of target isotope,

2. The irradiation facility,

3. Requirements for chemical processing,

4.Time of irradiation and the conditions,

5. Activity measurement,

6. Data processing,

7.Limit of detection, desired precision and accuracy.

1.The choice of target isotope: Most elements consist of several stable isotopes, which on irradiation yield different activation products. In selecting an appropriate target isotope (stable), it is necessary to take account of isotopic abundance, reaction probability (cross-section), and half life and decay scheme of the activation product.

2. The irradiation facility: The study was performed at the Scottish Universities Research and Reactor Centre (S.U.R.R.C.). The nuclear reactor at the centre operates at up to a maximum power of 300 kW, providing thermal neutron fluxes in excess of 10^{12} n cm^{-2} s^{-1}. The capacities of irradiation facilities and available neutron fluxes are summarized in **Table-12**.

3.Requirement for chemical processing: When compared with other methods of analysis, additional considerations for NAA are radiation exposure (which can be controlled), and time factors related to the half-life(s) of the isotopes(s) measured, i.e. activation and decay time, time of chemical separation (where required), and counting time

Table-12: Irradiation positions and the thermal neutron fluxes available in the UTR-300, at 300 kW

IRRADIATION POSITION	MAXIMUM SAMPLE SIZE Diameter x Length (cm x cm)	MAXIMUM THERMAL NEUTRON FLUX ($n\ cm^{-2}\ s^{-1}$)
a. Fast transfer facility		
Large rabbit	3 x 10	2.3×10^{12}
Small rabbit	2 x 5	2.5×10^{12}
b. Central vertical stringer	6 x 15	4.2×10^{12}
c. Thermal column	10 x 150	2.7×10^{12}

The principle activation products of biological samples for a few days after irradiation are Na-24 ($t_{1/2}$=15h) and Br-82 ($t_{1/2}$=35h). Where the required isotopes have sufficiently long half-lives, they can be measured after an appropriate decay time, 5-10 days for Na-24 and 20-30 days for Br-82, which also reduces radiation exposure. For other isotopes, interfering activities must be chemically removed. Such separations have usually been carried out post irradiation, but with increasing concern for radiation exposure the current application principally used pre-irradiation separations which require account to be taken of recoveries.

4. Time of irradiation and the conditions: The maximum amount of radioactivity of a given radionuclide obtained on irradiation (the saturation activity) is almost achieved by an irradiation time of five half-lives of the product. This level is useful when the trace elements sought are at the limit of detection. Where sufficient activity is produced for determination of the required element(s), irradiation time are often comparable to the half-life of the product, or less.

5. Activity measurement: For measurement of radioactivity the limit of detection (minimum detectable count) depends on the background at the specified energy and the net number of counts required to obtain acceptable

statistics. Therefore counter efficiency and background must be considered when selecting a detection system.

Whilst NaI(Tl) detectors provide good efficiency, their energy resolution is determined by statistical fluctuations in the production of photo electrons, and is typically 6% with 10-12% scintillation efficiency for γ-rays of 661 keV. Resolution can be improved by using detectors which produce more carriers per incident radiation event. This can be achieved by solid state detectors, where detection is based on electron-hole pair collection from semiconductors such as Ge and Si (Knoll, 1989, pp 337).

Until the availability of high purity germanium (HPGe) detectors, impurities in germanium were neutralized by drifting in lithium, to produce Ge(Li) detectors. Both types require operation at low temperature, HPGe may be allowed to warm up when not in use, but Ge(Li) detectors required permanent cooling to avoid Li mobility. Energy resolution and detection efficiency are nearly identical for HPGe and Ge(Li) detectors of the same size (0.2-0.3% resolution with 1-2% efficiency).

Recording of output pulses from a detector is performed by a multichannel analyzer (MCA) which comprises of two basic components: an analog-to-digital converter (ADC) and a memory. An analog signal is converted to an equivalent digital number. The output of the ADC is stored in a computer-type memory, which has as many addressable locations as the maximum number of channels into which the recorded spectrum is required to be subdivided (Knoll, 1989, pp 660). Modern radioactivity measurement systems are usually combined with a computer to control the system, which can also be used for calculation of results. A schematic diagram of the detection system used is provided in **Figure-3**.

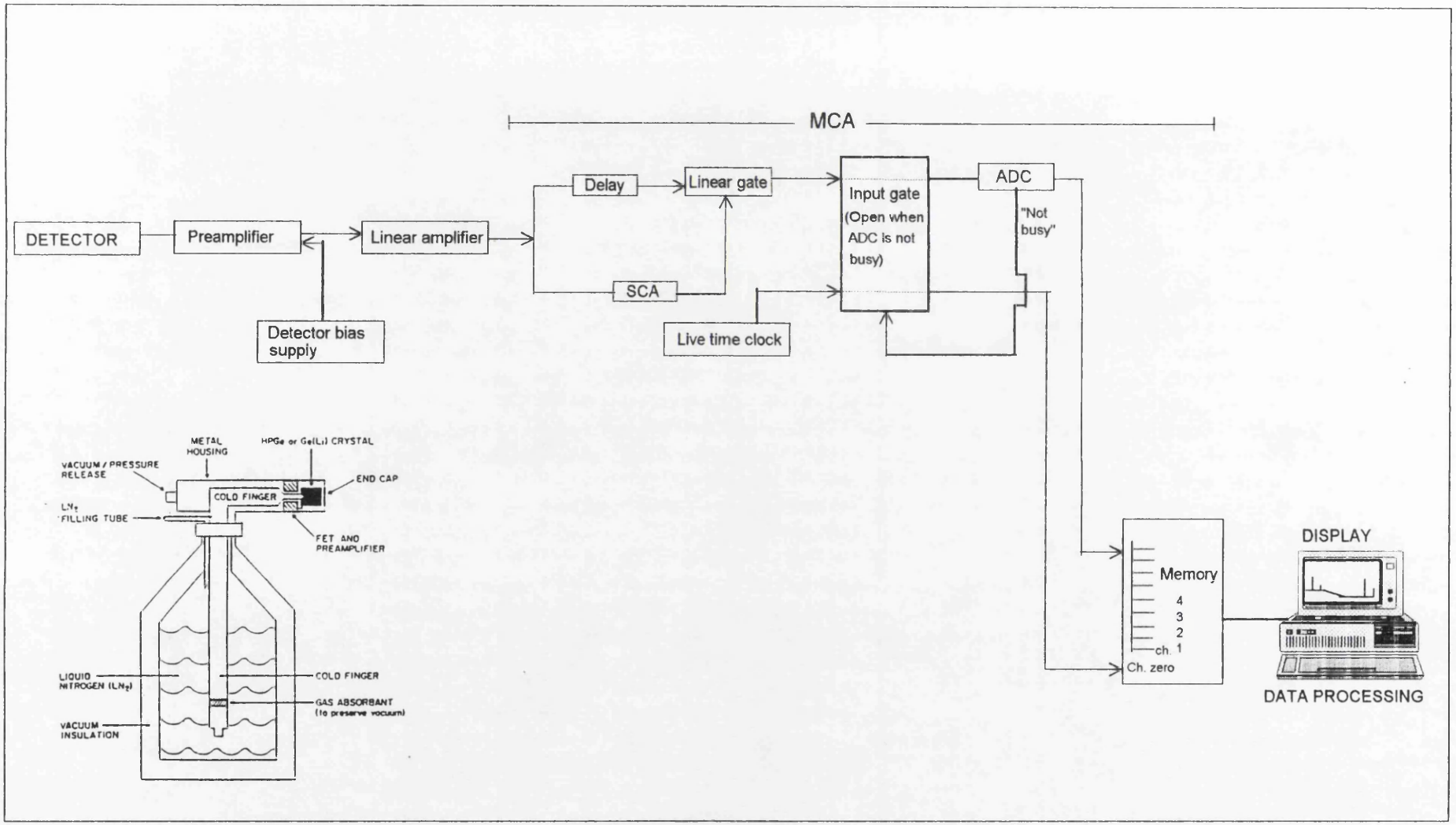


Figure-3: Ge detector and counting system

6. Data processing: The MCA program enables a spectral file to be saved but the file does not contain any readable information. Software designed to analyze the spectra generated by the MCA converts the file to a standard (spectral) format, containing the number of counts in each channel. The program then performs a "semi quantitative" analysis of the data, to provide net photo-peak areas with energy and channel number. The output at this stage contains photo-peak energies and count rates, with additional background and statistical information, but peaks to be further processed require specific identification. Subsequent processing of the data is carried out by an NAA program which ultimately provides quantitative results from inputs of samples and standards, with error propagation and systematic corrections as required.

7. Limit of detection, precision and accuracy :Limit of detection and precision of the measurement of radioactivity depend on the following parameters:

a. Induced activity: The induced activity is the principle parameter affecting the limit of detection and precision, and is a function of the nuclear characteristics of the irradiated isotope.

b. Conditions of radiation measurement: Counting parameters which affect limit of detection and precision are energy resolution and efficiency of the detector for the measured γ-ray, detector background, and minimum resolveable counting rate above background.

c. Sample constituents: The characteristics of other constituents in samples can affect limit of detection where the result in an increased background (continuum) under the photopeaks of interest.

d. Chemical procedure: Since irradiation of the sample can activate other constituents in it, purification of the required elements by chemical treatment (pre- or post-irradiation) improves the limit of detection. The chemical yield of the procedure applied, and decay during the time from the end of irradiation to counting also affect the detection limit and precision of the measurement.

I.5.2 INDUCTIVELY COUPLED PLASMA SOURCE MASS SPECTROMETRY (ICP-MS)

ICP-MS was developed by adaptation of two pre-existing technologies; inductively coupled plasma sources as used in atomic emission spectrometry and mass spectrometry, with an effective interface. This development meets the major requirements for elemental analysis by mass spectrometry: The source used should dissociate the sample as completely as possible, produce a high yield of singly charged ions but the minimum output of polyatomic fragments and multiply charged ions (Denoyer, 1991, Jarvis *et. al.*, 1992 pp 2).

The basic principles of ICP-MS are

1. Samples are introduced into the central channel of an argon plasma at 5000-8000 °K as a finely dispersed mist which is rapidly desolvated and vaporised.
2. During transit through the plasma core dissociation and ionisation occurs.
3. Ions are extracted from the central channel of the plasma through a vacuum pumped interface containing a cooled metal sampler cone, and separated from the bulk of the argon.
4. Extracted ions are transmitted into the mass spectrometer through a cooled metal skimmer cone.
5. In the mass spectrometer, the ions are separated on the basis of their mass to charge ratio.
6. The separated ions are subsequently detected by an electron multiplier (Sargent & Webb, 1993). A diagram of the PlasmaQuad ICP-MS (Fisons Instruments), which was used in this investigation used is provided in **Figure-4**.
7. The instrument is calibrated with standards of known concentrations, and account of variations in operating condition taken by including an internal standard in samples.

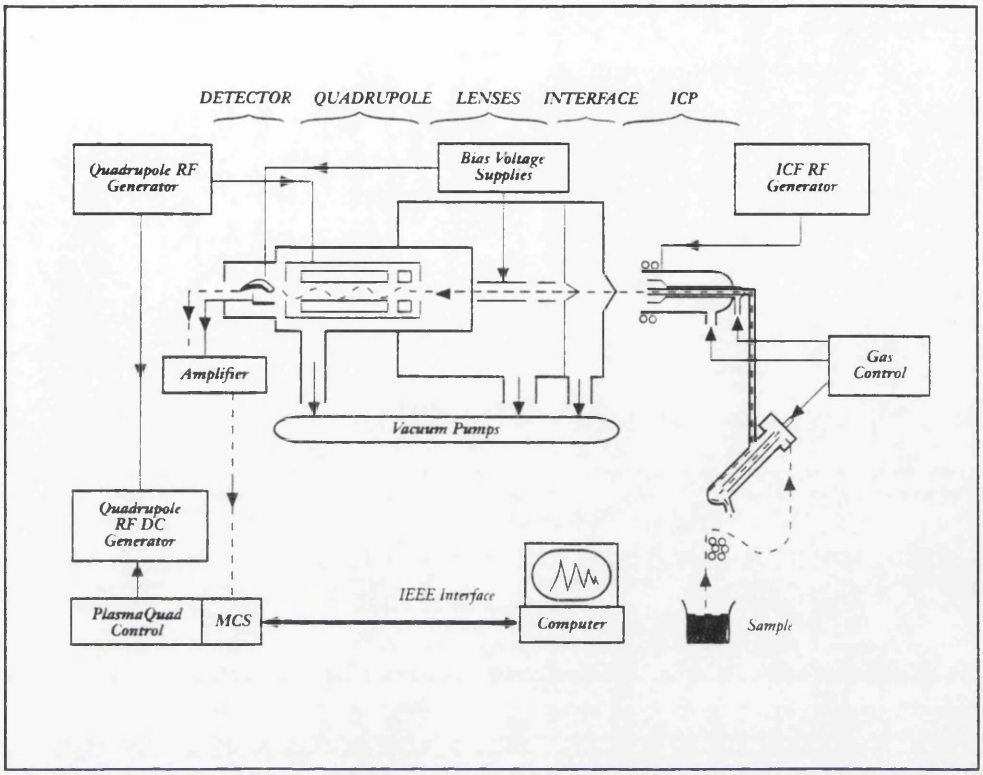


Figure-4: Schematic diagram of the PlasmaQuad ICP-MS

The principle advantages of ICP-MS technique are its capability of providing both elemental and isotopic measurements with high sensitivity. Detection limits in aqueous solution are generally below 0.1 ng mL^{-1}. Instruments provide a wide dynamic range with a resolution of more than 300 , which enables separation of most interferences. Multielemental analysis is possible with a high throughput.

ICP-MS is widely regarded as relatively interference free but as with all analytical techniques there are several significant sources of error. Isobaric interferences (overlapping peaks) can mask the isotope of interest. These interferences may arise from isotopes of other elements within the sample matrix, elemental combination with plasma gas, formation of polyatomic oxides or hydroxides (mostly M/Z<81) and, doubly charged ions. Mass discrimination and variation of sensitivity across the mass scale can cause error in measurement of isotope ratios.

Matrix effects may be overcome by matrix matching, standard addition, chemical separation, or the use of isotopic dilution analysis. Physical affects such as viscosity and surface tension affect the uptake rate and aerosol production, as does high salt content which also results in drift and damaging deposits on the nebuliser, torch, and interface. Dissolved solids give rise the to deposition on the interface cones leading to drift in response and eventually causing blockage. For this reason the sensitivity of ICP-MS is limited, with a maximum concentration of dissolved solids recommended (Ross *et. al.*, 1993).

ICP-MS was principally used for measurement of isotopic concentration of ^{57}Fe, since the neutron activation product is also stable (^{58}Fe). For determination of ^{58}Fe and ^{70}Zn, NAA was preferred because of the overlap of $^{58}Ni^{+}$ with $^{58}Fe^{+}$ (Ni is material of metal cones) and, $^{35}Cl^{35}Cl^{+}$ with $^{70}Zn^{+}$ in ICP-MS.

NAA and ICP-MS are compared in **Table-13**, from Dybczynski (1980), Janghorbani *et al.* (1985), Turnlund (1989), Ward *et al.* (1990), Savory and Wills (1991), Ross *et al* (1993), Sargent and Webb (1993), and Filby and Olsen (1994).

Table-13: Comparison of the principle advantages and disadvantages of NAA and ICP-MS

NAA

ADVANTAGES

1.Low limit of detection for many elements (sub ng to µg)
2.Instrumental, pre- and/or post chemical treatment variations available
3.Minimal sample preparation for a wide variety of matrices
4.Few interferences for elements of interest, corrections possible where necessary
5.Good accuracy and precision demonstrated for many elements
6.Standards may be of different matrix to sample
7.Enables the analysis of many isotopes suitable as tracers of essential minerals, with no isobaric interferences *e.g.* ^{58}Fe, ^{48}Ca

DISADVANTAGES

1.Several important element can not be routinely determined *e.g.* Pb, Be
2.Handling of radioactivity required
3.Time may depend on requirement for decay of interferences
4.Samples must comply with requirements for safe irradiation
5.Access to an operating nuclear reactor is required
6.Not all isotopes can be measured because of the lack of a suitable daughter radionuclide *e.g.* ^{57}Fe, ^{67}Zn
7.Precision is limited by counting statistics

ICP-MS

ADVANTAGES

1.Excellent detection limits for many elements (sub ng to µg)
2.Multi element capability, some elements determined which are not routine by NAA. *e.g.* Li, Sn, Pb
3.Large dynamic range (up to 10 orders of magnitude)
4.Rapid acquisition of mass spectra leads to a high sample throughput (up to 20 samples per day)
5.Relatively simple spectra
6.On-line analysis of molecular sub fractions separated by *e.g.* HPLC
7.ETV or laser ablation can be used for solid samples
8.Application of isotope dilution mass spectrometry is possible for several suitable tracers of essential minerals *e.g.* ^{42}Ca, ^{25}Mg, ^{57}Fe

DISADVANTAGES

1.Spectral interferences from isobaric overlap *e.g.* ^{113}In-^{113}Cd, ^{58}Fe-^{58}Ni
2.Spectral interferences from background species; usually polyatomic formed from the plasma or from the solvent used in the preparation, most of these have M/Z<81 *e.g.* ArO^+-$^{56}Fe^+$, $ArOH^+$-$^{57}Fe^+$, Ar^+-$^{40}Ca^+$ and $^{40}K^+$, $^{35}ClO^+$-$^{51}V^+$, $^{37}ClO^+$-$^{53}Cr^+$
3. Chemical separation is often required
4.Organic or aqueous solution required for nebulization, with low dissolved solids tolerance to avoid blockage in equipment. The same problem arises when samples containing high levels of protein or fat are nebulized
5. Sensitivity depends on elemental ionization potential
6.Mass discrimination effects complicate measurement of accurate isotope ratios

I.5.3 ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS)

NAA and ICP-MS enable determination of the total amount of tracer isotopes in diet and faeces, but measurement of absorption requires account to be taken of the endogenous contribution to the output. This can be obtained by determination of the element by AAS and taking account of the natural isotopic abundance.

The characteristic emission from atoms of an element can be absorbed by other atoms of that element. The basic principle of AAS is the use of an element as light a source and measurement of the absorbed fraction of the emission by the element existing in an atomic vapour of a sample.

Analysis by AAS requires the element to be determined to be present in an atomic vapour. This can be generated by a flame or electrically heated furnace (usually graphite). The spectrophotometer used in the current investigation was based on flame atomization (FAAS). The atomization process consists of two basic steps: generation of a fine aerosol of the sample solution with a nebulizer (*e.g.* pneumatic nebulizer) and, dissociation of the analyte into gaseous ground state atoms by a flame, based on the combination of a fuel gas (*e.g.* acetylene) with an oxidant (*e.g.* nitrous oxide) providing 2000-3000 °C.

I.6 OBJECTIVES

Determination of net luminal disappearance from an input containing isotopic tracer by faecal monitoring requires quantitative faecal recovery of the excreted fraction of the tracer. Collection of faeces may be incomplete because of losses on toilet paper, in general manipulation, and through accidental rejection. Account of incomplete faecal collection can be made by the use of an inert, non-absorbable, marker as a yield indicator.

One of the crucial issues to establish when using faecal monitoring is the time required for faecal collection, to minimise addition of endogenously excreted isotopes for elements with a high intestinal endogenous excretion. By using an inert marker, the difficult task of complete stool collection can be simplified, with optimization of faecal collection period for limited or more desirably single sample collection. This approach is only valid if no absorption of markers occurs during passage through the intestinal tract, and the rates of excretion of marker and isotopic tracer are similar.

Hutcheson *et al.* (1979) have described the use of rare earths as markers in determination of the utilization of macronutrients. The investigation was based on the chemical balance approach. Subjects were provided with a continuous input, with energy, protein, fat, and calcium marked with different rare earths. After a steady state was reached, the absorption of the macronutrients in faecal samples was determined by single sampling. The authors postulated that the method described would allow the use of multiple nutrient markers for monitoring any of several nutrients, and enable determination of the absorption of nutrients without direct monitoring of food intake or extended faecal collection.

The current investigation is concerned with the applicability of rare earth markers in determination of the net luminal disappearance of essential trace

elements by the use of enriched stable isotopic tracers with the following objectives:

Clinical objectives

1. To confirm non absorbability or high faecal recovery of orally administered rare earths.

To accomplish this, a preliminary recovery investigation involving 2 subjects is performed by oral administration of 5 selected rare earths in a range of doses and recoveries of the rare earths are determined by INAA. Having experienced on design and possible procedural difficulties, the investigation for the same rare earths is than extended to a greater number of subjects with an improved study design and chemical separation procedure. Particular attention is also given to background levels of the rare earths in faecal samples provided before administration.

2. To evaluate the faecal recovery pattern of rare earths and their excretion kinetics to establish the time required for recoveries of major fraction of an administered dose.

The recoveries obtained from above investigations are evaluated to obtain recovery profiles of the rare earths for the fractions recovered with individual and consecutively accumulated faecal samples. Attention is also given to parameters related with the intestinal kinetics of rare earths.

3. To demonstrate the validity of the combined use of stable isotopic tracers and rare earth markers for determination of the net luminal disappearance of iron and zinc.

Having confirmed the ability to use rare earths as markers, it is intended to establish a method for measurement of luminal disappearance by limited faecal collection for multi-element and/or multi-isotope studies where the mode

of intakes can be different. For this investigation, attention has been given to all faecal samples obtained during the study period (to provide a maximum collection yield), and changes of the levels of rare earths in urine samples (for further confirmation of non-absorbability). Particular attention is given to the comparability of the faecal excretion kinetics of rare earths and the excreted fraction of isotopic tracers. The possibility of determination of luminal disappearance from limited (specified) samples is investigated.

Cook *et al.* (1979 and 1991) have suggested the use of absorption from a reference solution containing ferrous iron and ascorbic acid to account for inter- and intra-subject variation of absorption from a meal. The use of a worldwide-consumed wheat based Farina meal containing ferric iron has also been proposed as a reference standard to investigate if subject to subject variations in absorption relate to differences in iron status of subjects or to the meal (Forbes *et al.*, 1989). For current investigation, the luminal disappearance of iron from the two standard intakes is determined with the use of a rare earth marker and a stable isotopic tracer for each. Measurement of the luminal disappearance of zinc from the meal is also included in anticipation of wider applicability of the marker recovery approach.

2. Analytical objectives

1. To select 5 rare earths, which have the lowest limits of detection for the rare earths by NAA, and to establish the required doses for specified conditions for investigation to confirm of the absorbability of rare earths. Having determined faecal recoveries of the rare earths by INAA, a procedure for pre-irradiation column separation of rare earths will be established, to reduce radiation exposure, to enable early counting, and to improve limits of detection.

2. To select isotopic tracers and to establish the required doses for investigation of determination of luminal disappearance of iron and zinc with combined use of rare earths as markers. The least abundant isotopes require

addition of the smallest amount of enriched isotopic tracer. Isotopic tracers and rare earths are principally determined by NAA.

A possible approach providing above concerns is to provide iron labelled with ^{57}Fe with the standard drink, and iron and zinc labelled with ^{58}Fe and ^{70}Zn with the meal. For determination of faecal recoveries, a different rare earth for each mode of intakes is used. Rare earths and ^{58}Fe, ^{70}Zn, and ^{57}Fe tracers contents of faecal samples are determined by NAA and ICP-MS (the introduction of a second analytical method arises since ^{57}Fe produces a stable activation product). Total iron and zinc contents of faecal samples are determined by AAS, to account for the isotopic contribution of iron and zinc of natural composition to enrichment in faecal pool. ICP-MS is also employed to determine baseline levels of the rare earths in faecal samples, and in urine samples provided before and after the administrations.

3. To establish a pre-irradiation ion exchange separation of iron and zinc, to achieve the lowest limits of detection by NAA and to avoid isobaric interferences in ICP-MS. By using pre-irradiation separation for determination of ^{58}Fe, time required for decay and radiation exposure are also minimised. For determination of ^{70}Zn, a post-irradiation separation procedure is also considered, to minimise interference caused by the activation product of Mn which has a half-life comparable to that of ^{71m}Zn.

4. Luminal disappearance will be established from results obtained from single samples, and the data considered in terms of cumulative outputs, to identify the minimal collection required for acceptable measurement.

II RARE EARTH RECOVERY INVESTIGATIONS

In this chapter, investigations carried to confirm non-absorbability of rare earths are described. The first part includes theoretical criteria for the choice of rare earths and their required doses for a faecal recovery investigation, a practical assessment of measurement of the selected rare earths by INAA, and a preliminary recovery investigation performed for two subjects.

Rare earth recovery studies with a chemical separation procedure, and a faecal recovery investigation on 6 subjects are described, background levels attained in the recovery investigations are compared with natural levels of rare earths, and the faecal appearance profiles are evaluated in the second part.

II.1 FEASIBILITY STUDIES FOR THE USE OF RARE EARTHS AS MARKERS

II.1.1 CHOICE OF RARE EARTHS AND DETERMINATION OF OPTIMUM DOSE

1. Choice of rare earths as markers

Theoretical limits of determination of rare earths were calculated for the isotopes selected with reference to their cross section, natural abundance, and the intensities of emitted γ-energies of their activation product providing lowest limits of determination. Nuclear characteristics of the selected isotopes are provided in **Table-14**.

For calculation of limits of determination of the isotopes of rare earths, It was considered that the elements were irradiated for 6 h in the CVS (at the flux of 4.2×10^{12} n cm^{-2} s^{-1}). Count rates were calculated for counting at 10 cm above a 130 cc Ge detector, taking account of the absolute intensity of the γ-rays measured and detector efficiency at the specified energy. Limits of

Table-14: Nuclear characteristics and their activation products of stable rare earth isotopes which provide the lowest limit of detection

NUCLIDE				ACTIVATION PRODUCT			
El	A^1	Abund,%	$\sigma_n(b)^2$	A	Half life	γ-energy,keV	Intensity
La	139	99.9	9.2	140	40.3 h	1596	95
Ce	142	11.1	0.95	143	33 h	293	42
Pr	141	100	8	142	19.2 h	1576	3.7
Nd	146	17.2	1.3	147	10.9 d	91	28
Sm	152	26.6	204	153	46.8 h	103	28
Eu	151	47.9	5800	152	13 y	1408	21
Gd	158	24.8	2.5	159	18.6 h	363	10
Tb	159	100	23	160	72.1d	879	30
Dy	164	28.2	1000	165	2.3 h	95	3.6
Ho	165	100	63	165	26.8 h	81	6.2
Er	170	14.9	5.7	171	7.5 h	308	64
Tm	169	100	103	170	128.6 d	84	3.2
Yb	174	31.6	19	175	4.19 d	396	6.5
Lu	175	31.8	16.4	176m	3.7 h	88	8.7

1. Atomic mass of the selected isotopes of rare earths 2. Thermal neutron cross sections for the isotopes, barn ($b=\sigma_n x10^{-24}$ cm^{-2})

determination were taken as the amount which could be determined with 1% precision (10,000 counts) in a 1 hour count. The calculated determination limits of rare earths are provided in **Table-15**.

As can be seen from **Table-15**, the calculated limits of determination for all rare earths for counting immediately after irradiation show that the lowest limit of determination is for Dy whilst Nd is the highest. The use of Dy, Er, and Lu is not practical when compared other rare earths of longer half-life where analysis by INAA is required, because of their short half-life. Since a period of decay is required to reduce radiation exposure and allow for decay of interfering activities, the determination limits of the remaining rare earths were considered in selection of markers. From comparison of the limits of determination, and bearing in mind interference effects, particularly at low energy it is obvious that Sm, La, Yb, Tb, and

Eu are the best available, since the characteristic γ-energies for Ho and Tm occur in the low energy region of the spectra which are influenced by Compton continua and X-rays.

Table-15: Determination limits of rare earths determined immediately after a 6 h, and after 2 and 4 days decay

	Limits of determination, µg		
Rare Earth	Immediate	2 d decay	4 day decay
La	0.16	0.36	0.82
Ce	5.46	15.0	50.0
Pr	1.82	10.4	59.7
Nd	26.3	29.8	82.2
Sm	0.01	0.02	0.05
Eu	4.74	4.74	4.74
Gd	2.56	15.3	-[1]
Tb	2.66	2.66	2.76
Dy	0.002	-	-
Ho	0.02	0.09	0.29
Er	0.14	-	-
Tm	3.07	2.33	2.35
Yb	0.76	1.06	1.47
Lu	0.02	-	-

1.Activity not detectable in view of short half-life

2.Determination of optimum dose

To determine a small fraction of input dose in faeces, a low limit of determination is required. Limits of determination for the proposed procedure, and the consequent amounts of the rare earths required for inputs were calculated on the following theoretical basis:

a.Samples are irradiated for 6 h in the CVS (4.2×10^{12} n cm^{-2} s^{-1}),

b.A precision of ±1% is required for 1 h count (which is equivalent to 3 cps),

c.An average detection efficiency of 1% was assumed (which can be obtained by placing the samples in close proximity to the detector),
d.The minimum detectable amount was required to be 0.1% of the total input in 10% of a faecal sample.

Further assumptions made for dose calculations of Sm and Tb were;

a.Since the 103 keV γ-radiation of Sm-153 is in a lower energy region of the spectrum, where the photo-peak is associated with high background counts, five times the calculated dose was considered sufficient to compensate for this increased background. The ratio of the background counts for Sm-153 at 103 keV to La-140 at 1596 keV was practically found to be 30.

b.For the initially specified precision requirements, a relatively high input was required for Tb. By acceptance of ±2% precision, a dose of 7.5 mg was considered to be adequate.

Calculated limits of determination and the doses of rare earths required to meet these conditions are provided in **Table-16**. The doses used by Luckey *et al.* (1977) are also provided for comparison.

Table-16: Determination limits and proposed doses of the rare earths

	Determination limit	Dose, mg	
ELEMENT	µg	Proposed	Luckey *et. al*
La	0.12	1	60
Sm	0.02	1	39
Eu	0.9	10	15.5
Tb	3.6	7.5	50
Yb	0.5	5	30

II.1.2 DETERMINATION OF RARE EARTHS BY INAA

The use of INAA for determination of the rare earths in studies of nutrition has been described by Gray and Vogt (1974) with detection limits for specific analytical conditions. The objective of this investigation is to consider the suitability of facilities available at SURRC (lower neutron flux, but more efficient detectors) for such studies. Determination of the selected rare earths (La, Sm, Eu, Tb, and Yb) was investigated by analysis of faecal samples to which known amounts of the rare earths had been added.

1. Procedure

Sample preparation: Approximately 20 g samples of freeze-dried faeces were ashed at 400-500 °C for 24 h. Five fractions of 0.2 g were weighed and transferred into labelled aluminium sachets. Two of the fractions were processed for determination of background levels above which output would have to be measured. Three samples were spiked with solutions of the rare earth prepared from 1000 μg mL^{-1} spec pure AAS standards (Johnson Matthey, Materials Technology/U.K.). Three reference standards for the same elements were also prepared by pipetting the solution onto 0.2 g of cellulose in aluminium sachets.

Irradiation and counting: The samples and standards were irradiated for 6 hours in the CVS, and allowed to decay for 3 days. Samples and standards were then transferred to polythene ampoules for counting on a 25 cc Ge(Li) detector for 1 hour each, 10 cm above the detector, to measure La-140 ($t_{1/2}$=40.3 h) and Sm-153 ($t_{1/2}$=46.8 h) activities. Samples were counted again a week after irradiation for measurement of Yb-175 ($t_{1/2}$=4.2 d), Tb-160 ($t_{1/2}$=72.1 d), and Eu-152 ($t_{1/2}$=13 y) for 5000 s each at 5 cm above the detector. One sample and standard together with the baseline samples were counted for 15 h each on contact with the detector after decay of a month for detection of Eu-152. The concentrations of the samples and standards were calculated with reference to one of the standards.

b.Results and conclusion:

The amounts of the rare earths determined in standards and samples are provided in **Table-17**.

Table-17: Amounts of the rare earths in standards and in faecal samples, µg±SD (error of single measurement)

	Reproducibility of rare earth standards				Natural levels of rare earths	
RE	Added	Found	Added	Found	Dupl. I	Dupl. II
La	8.0	8.6±0.1	2.0	2.4±0.02	0.7±0.2	0.9±0.1
Sm	20	17.6±0.1	5.0	4.9±0.02	ND[1]	ND
Eu	0.4	ND	0.1	ND	0.02±0.003	0.02±0.04
Tb	20	19.3±0.2	5.0	5.2±0.2	0.05±0.06	0.06±0.01
Yb	10	9.9±0.02	2.5	2.8±0.1	0.2±0.04	0.2±0.04

	Amounts determined in rare earth added faecal samples						
RE	Added	Found	Added	Found	Added	Found	G&V[2]
La	8.0	8.6±0.3	4.0	5.0±0.1	2.0	2.9±0.3	19.6
Sm	20	16.9±0.05	10	10.6±0.05	5.0	4.2±0.04	40.2
Eu	0.4	ND	0.2	ND	0.1	0.1±0.005	1.1
Tb	20	17.0±0.6	10	11.6±0.4	5.0	4.1±0.1	43.0
Yb	10	9.9±0.3	5.0	5.5±0.3	2.5	2.9±0.1	4.7

1. Not detected
2. Amounts measured in faeces by INAA (Gray and Vogt, 1974)

1. Reasonable agreement between the results expected and the amounts found for standards is evidence that the specific activities are reproducible. Low results for some of the samples can be explained by difficulties of quantitative transfer of the samples from the aluminium irradiation sachets to polythene counting vials.

2. Rare earths were determined in faecal samples to which no rare earths were added, to provide an indication of background levels. The precision obtained for the measurement of baseline samples could be improved by increased irradiation time and chemical isolation of the rare earths from other

interfering elements.

3. The quantities of rare earths added were generally 1/2, 1/4 and 1/8 of those determined by Gray and Vogt (1974). The irradiation and counting facilities used [6 h irradiation in a flux of 4.2×10^{12} n cm^{-2} s^{-1} and counting on a 25 cc Ge(Li)] were approximatly equivalent to the conditions used by Gray and Vogt [20 min irradiation in a flux of 5×10^{13} n cm^{-2} s^{-1} and counting on a 45 cc Ge(Li)] . However, lower amounts of the elements were determined in 0.2 g of ashed samples (equivalent to approximately 2g of dry weight), than for those determined in 1 g of dry samples by Gray and Vogt.

The lowest quantities determined were 2 µg for La and 0.1 µg for Eu. These are comparable to 2% or 0.1% recoveries of a 1 mg dose in a 0.2 g fraction of ashed faecal sample, with the precision indicated. The poor precision obtained for Eu, and the requirement of a long decay period before counting indicates that when using instrumental NAA a higher amount of Eu is required for reasonable precision and to enable earlier counting.

This preliminary study demonstrates that the concentrations chosen for the rare earths can be determined nondestructively. The use of a pure Ge detector with high resolution and greater efficiency (proportional with the volume of detector) would enable measurement of lower concentrations with improved precision.

II.1.3 RECOVERY OF ADMINISTERED RARE EARTHS BY INAA

A practical assessment of recoveries of the rare earths following consumption by two subjects was undertaken to confirm that they could be quantitatively recovered, with analysis by INAA.

II.1.3.1 PROCEDURE FOR DOSE PREPARATION, ADMINISTRATION, AND SAMPLE COLLECTION

1. Ethical approval and protocol:

Ethical approval was obtained from the Joint Ethical Committee of the Grampian Health Board and The University of Aberdeen (since this study was carried out in collaboration with the Department of Medicine, University of Aberdeen), and the participants gave their informed consent. A copy of the information and consent form is provided in **Appendix-1**.

2. Preparation of dose solution:

Specpure oxides (Johnson Matthey, Material Technology/U.K.) containing 4.89 mg of La, 4.83 mg of Sm, 50.0 mg of Eu, 36.9 mg of Tb and 24.9 mg of Yb were weighed and transferred to a 50 mL beaker. The oxides were then converted to chlorides by dropwise addition of analytical grade 6 M HCl and dried by slight heating. The residue was transferred to a 50 mL flask with ~20 mL distilled water. A few drops of 10 M HCl were also added to ensure total dissolution. The volume was then made up. 10 mL fractions of the final solution (containing 0.98 mg of La, 0.97 mg of Sm, 10.00 mg of Eu, 7.38 mg of Tb and 4.98 mg of Yb) were transferred to universal tubes as dosing quantities. The solutions were then further diluted with 20 mL of water to minimize the acidity and possible unpleasant taste.

3. Administration and sample collection:

The subjects consumed the dose solution with their lunch since future application was to be the determination of luminal disappearance from dietary inputs. Two water rinses of the container were also taken to ensure total consumption, and the time of administration was recorded as a reference time for following records of bowel movements. All stool samples, including toilet papers were collected for 6 days by using a toilet ring and biohazardous polythene bags shown in **Figure-5** (as proposed by Hinton *et. al.*, 1969). Individually collected samples were weighed and stored in a deep-freeze at -20 °C for further processing.

Figure-5: Faecal sample collection system used in preliminary rare earth recovery investigation

3.Sample preparation:

Stool samples were autoclaved, transferred to aluminium trays, and freeze-dried (at The Rowett Research Institute, Aberdeen). The samples were ashed

in a furnace at gradually increasing temperatures of 200-450 °C for 16-24 h. Losses from such ashing have been shown to be insignificant (Pietra *et. al.*, 1982, Heydorn, 1984, pp 60-61). On completion of ashing (when sample colour became light green), their ashed weights were recorded. The samples were ground to provide homogeneity, which was confirmed by analysis of duplicate sub-samples. Duplicate fractions of samples were weighed into 1.5 mL polypropylene irradiation containers (0.05-0.2 g as appropriate for the expected rare earths contents) and stored in polythene containers for further use.

II.1.3.2 ANALYTICAL PROCEDURE, RESULTS AND DISCUSSION

1.Irradiation and counting:

Standards of rare earths were prepared from specpure AAS standard solutions (1000 µg mL^{-1} rare earths), by pipetting 10 µg of Sm, and 20 µg of La, Eu, Tb and Yb on to cellulose powder contained in irradiation vials. A fraction of 100 µL of the supplement dose was also prepared in a vial containing cellulose powder. The standards were then dried under an infra-red lamp. The irradiation vials containing samples and standards were heat-sealed, flux monitors (iron wire) were attached, and then wrapped in aluminium foil. Batches of the samples and standards were then irradiated for 3-6 h in the CVS as appropriate for the expected rare earth contents.

Samples were allowed to decay for 4-7 days after irradiation to reduce radiation exposure and to enable reasonable counting geometry. The samples and standards were then unwrapped and counted at 15 cm geometry for 1000-3000 s to obtain counts with good precision (generally <±1%). Flux monitors were also counted at contact geometry for 600 s. The rare earth contents of the samples and the dose solution were then found by matching their specific activities with those for standards. A schematic diagram of overall procedure and a sample spectrum are provided in **Figure-6** and **7**.

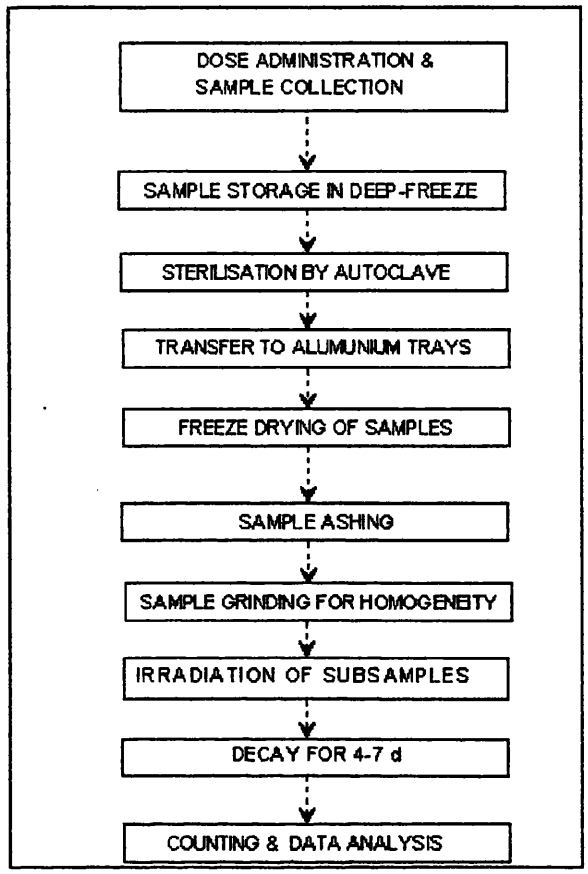


Figure-6: Procedure for rare earth recovery determination by INAA

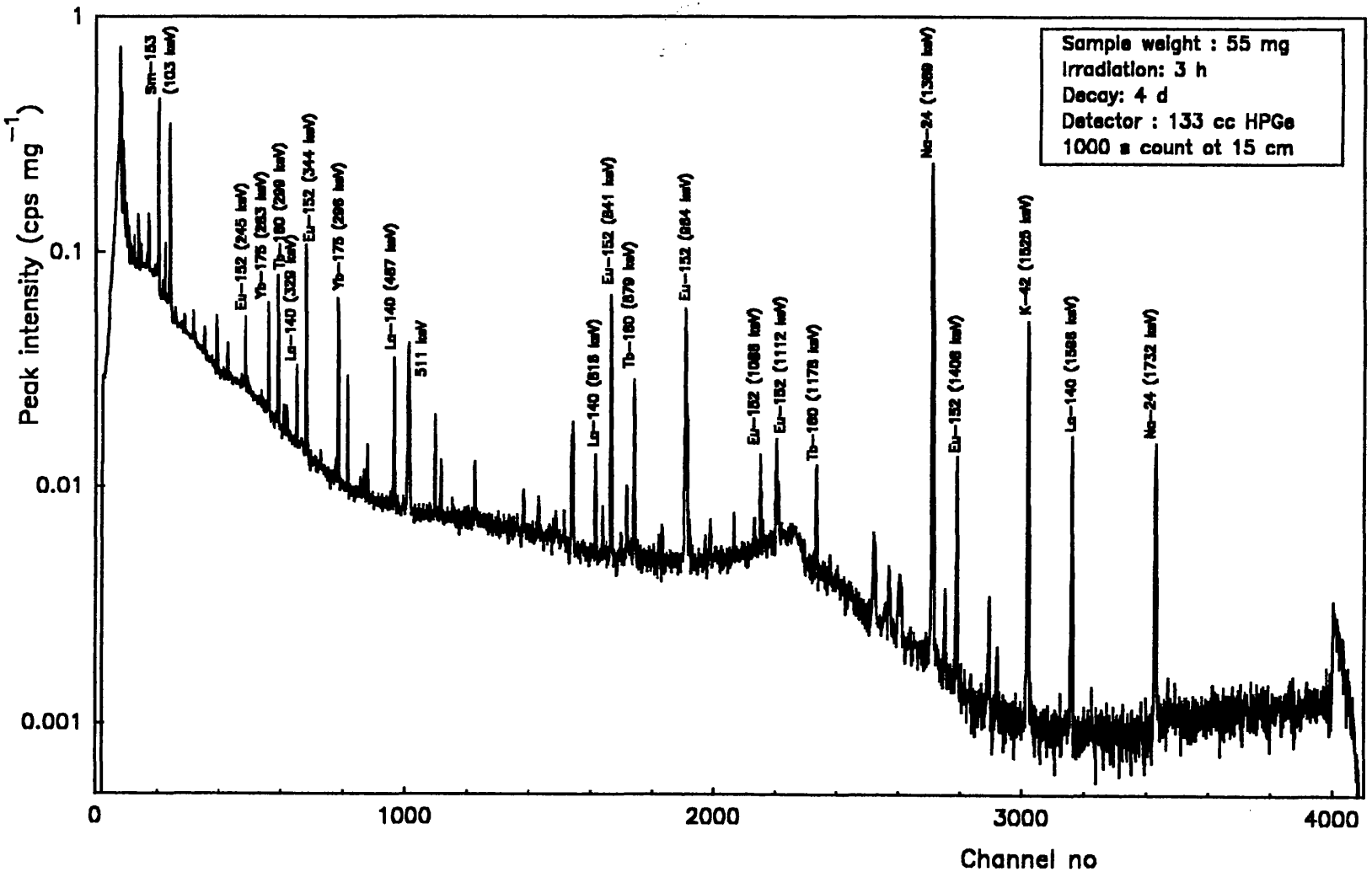


Figure-7: γ- ray spectrum of ashed faecal sample provided 18 h after consumption of rare earths

2. Results and quality control:

Weights of faecal samples and the mean concentrations determined in ashed samples are provided in **Appendix-2**. Sm concentrations determined in duplicate samples (for subject A2) are provided in **Table-18**. Agreement between the results for duplicates show that sample homogenization was satisfactory.

The results for the rare earth contents of the dosing solution for six irradiation batches were calculated by comparing their specific activities with those for the AAS standards irradiated in the same batches. The difference between the results was found to be insignificant (paired t-test, $p>0.05$). The mean of six results and the expected concentrations are also provided in **Table-18**.

Table-18: Concentrations of Sm found in duplicate ashed faecal samples and rare earth concentrations in dosing solution

Sample	Sm conc. in ashed samples µg g^{-1}±SD[1]			Mean RE conc. in dosing solution µg mL^{-1}±SDM		
No	Dupl. I	Dupl. II	Mean	RE	Found	Expected
1	47.6±0.7	48.3±0.7	47.9±0.5	La	95.8±7	97.8
2	63.5±0.9	62.7±0.8	63.1±0.6	Sm	98.5±6	96.6
3	29.2±0.8	30.0±0.8	29.6±0.6	Eu	1029±92	1000
4	4.9±0.2	4.7±0.2	4.8±0.2	Tb	790.5±77	738
5	0.68±0.04	0.64±0.06	0.66±0.03	Yb	498.3±36	498
6	0.11±0.02	0.14±0.05	0.13±0.02			

1.Error of measurement

Quantitative recovery for all rare earths was obtained for subject A2 (99.0±4.9%, mean and 1SD for 5 rare earths, range of 95.1-107.4%) but the mean recovery for subject A1 was 91.8±3.3%, with a range of 88.4-95.6%. This difference is attributed to losses when preparing samples for freeze-drying, and expected to be overcome by oven drying. Recoveries of the rare earths determined in 6 day collection of faeces are provided in **Table-19**.

Table-19: Recoveries of the rare earths in 6 day collection of faeces

	RE recoveries for subjects, %±3SD[1]	
RE	Subject A1	Subject A2
La	93.6±4.7	107.4±7.5
Sm	95.6±1.9	95.1±3.6
Eu	88.4±7.6	99.2±7.8
Tb	93.0±5.8	96.9±6.3
Yb	88.3±3.2	96.2±4.7
Mean±SDM	91.8±3.3	99.0±4.9

1.Measurement error at 99% confidence limit

La recovery for subject A2 was found to be higher than those of the 4 other rare earths. This could indicate that the contribution of the natural level of La in faecal samples to the recovery result is significant. Details of the recoveries are discussed in sections II.2.4-II.2.6 with particular attention to natural levels of La in faecal samples provided before administration.

In conclusion, no problems were encountered in procedures followed for preparation of the dosing solution or dose administration. Samples were stored in a deep-freeze at -20 °C until collection period was complete, and sterilized by autoclave. Sample transfer to aluminium trays after sterilization for freeze drying, was found to be cumbersome, and could cause losses (for subsequent studies, oven-drying was applied to avoid this). The requirement for homogenization before taking a fraction of an individual sample could possibly cause inter sample contamination and requires care.

The results of this preliminary investigation demonstrated that losses during sample preparation could be significant. Recoveries of 4 of rare earths agreed closely, but a variation for La suggests a possible contribution from natural output, which would require further investigation.

II.2 INVESTIGATION OF OUTPUT PROFILE AND RECOVERY OF ADMINISTERED RARE EARTHS, BY PRE-IRRADIATION SEPARATION NAA

II.2.1 ESTABLISHMENT OF PRE-IRRADIATION SEPARATION FOR DETERMINATION OF RARE EARTHS AND ITS APPLICATION TO DETERMINE FAECAL RECOVERIES

Determination of the rare earths by INAA showed that a period of decay after irradiation was required to reduce radiation exposure. This increases the time required for analysis and also effects limits of determination since half-lives of some rare earths activation products (La-140, $t_{1/2}$=40.3 h, Sm-153, $t_{1/2}$=46.8 h, Pr-142, $t_{1/2}$=19.2 h) are comparable with those of major interfering radionuclides (Na-24, $t_{1/2}$=15 h, K-42, $t_{1/2}$=12 h).

Such interferences can be removed before or after irradiation, but the latter does not reduce radiation exposure. A pre-irradiation separation method was established, with the addition of Pr as a yield tracer to take account of recoveries. Elimination of the major interfering elements (alkali and alkaline earths group) in the procedure was demonstrated with Na-24 tracer. The following investigations were carried out to optimise conditions for the pre-irradiation separation of selected rare earths from faeces, based on ion-exchange procedures previously applied to geological samples (Crock & Lichte, 1982, Crock *et al*, 1984 and Terekado, 1989).

II.2.1.1 CONDITIONS FOR Na-24 REMOVAL

Procedure

Duplicates of 200 mg of ashed faecal samples were labelled with 1 mL of Na-24 tracer, and digested in aqua-regia by heating. The solutions were heated to near dryness and diluted to 50 mL with 1M HNO_3. They were then passed through cation exchange columns (100-200 mesh Bio-Rad AG 50W-X8 resin

in Φ=1cm, H=10 cm bed volume, previously equilibrated with 1M HNO_3) at a rate of 1-2 mL min^{-1}. The columns were eluted with 5x25 mL fractions of 2M HNO_3. All effluent and the resin contents of columns were collected in counting bottles and made up 50 mL. The solutions and 50 mL standards were counted for 1000-2000s on contact geometry and recoveries were calculated with reference to the activity of 0.1 mL standard.

Results

The counts of eluent showed that most of the Na is eluted with the initial 1M HNO_3 and 50 mL of 2M HNO_3. The amount of Na-24 detected in the fourth and subsequent fraction of 2M HNO_3 was found to be insignificant. The activity retained on columns were <0.01% of added activity. The fractions of Na-24 eluted are indicated in **Table-20**, where it can be seen that the total recoveries were nearly quantitative, 99.7 and 97.1% for the two columns.

Table-20: Na-24 recovery from doped faecal sample by column separation

	Na-24 recovery, %±1SD	
Eluent	Column I	Column II
1M HNO_3 (sample loading solution)		
50 mL	80.7±0.5	77.0±0.5
2M HNO_3		
I. 25 mL	17.5±0.1	18.2±0.3
II. 25 mL	1.4±0.01	1.7±0.01
III.25 mL	0.1±0.002	0.2±0.003
IV. 25 mL	0.01±0.001	0.02±0.001
V. 25 mL	<0.01±0.003	<0.01±0.003
Total recovery %±SD	**99.7±0.5**	**97.1±0.6**

II.2.1.2 RECOVERY OF RARE EARTHS IN ION EXCHANGE SEPARATION

Having demonstrated effective elimination of Na, the recovery of rare earths from a cation exchange resin was investigated with radioactive rare earth tracers.

Procedure

Fractions of 50 µL of spec-pure AAS standards for La, Sm, Eu, Tb and Yb (1000 µg mL^{-1}) were pipetted into counting vials and dried under an IR-lamp. They were then irradiated for 6 h in the CVS system. The standards were allowed to decay for 4 days to reduce radiation exposure, then transferred into a 50 mL graduated flask and made up with 1M HNO_3. 10 mL fractions of standard solution were loaded onto three columns containing cation exchange resin, previously equilibrated with 1M HNO_3. The Na elimination procedure was then performed as described above.

The rare earth contents of the 3 columns were eluted by 50 mL of 6M and 50 mL of 8M HNO_3 gradually (3x10 and 1x20 mL of 6M, and 1x20 and 1x30 mL of 8M HNO_3 for 2 columns and 50 mL of 6 M and 50 mL of 8M HNO_3 for 1 column). The contents of all eluents, resin contents of the columns and the standard (10 mL of stock solution) were counted after appropriate dilution. Recoveries were calculated with reference to standard activities.

Results

Recoveries of the rare earths with the eluents are represented by results for La in **Table-21** for the 3 columns. It can be seen from **Table-22**, providing total recoveries of the rare earths that they are not removed from the columns by 1M and 2 M HNO_3 and that they are completely removed by 6M and 8M HNO_3. The residue on the columns were below the limit of determination.

Table-21: Sequential recovery of La from cation exchange columns

	Recovery of La, %±SD			
Eluent	Column I	Column II	Eluent	Column III
6 N HNO_3			**6 N HNO_3**	
I.10 mL	6.2±0.1	10.2±0.2	50 mL	84.6±0.8
II.10 mL	31.1±0.6	31.2±0.7		
III.10 mL	28.4±0.6	25.0±0.5		
IV.20 mL	24.1±0.3	25.7±0.4		
8 N HNO_3			**8 N HNO_3**	
I.20 mL	6.8±0.1	7.6±0.3	50 mL	12.1±0.1
II.30 mL	2.6±0.2	2.0±0.2		
TOTAL, %±SD	99.2±1.0	101.7±0.8		96.7±0.8

Table-22: Recovery of rare earths by ion exchange separation (summation of all eluents)

			Recovery, %±SD			
RE	1M HNO_3	2M HNO_3	Column I	Column II	Column III	Resin
La	<0.04	<0.04	99.2±1.0	101.7±0.8	96.7±0.8	<3.4
Sm	<0.02	<0.02	98.1±0.4	100.4±0.4	96.1±0.3	<0.8
Eu	<1.63	<1.58	103.1±2.5	106.7±2.6	102.0±1.7	<1.7
Tb	<1.15	<1.11	102.6±2.1	94.9±2.2	103.9±1.1	<1.1
Yb	<0.35	<0.34	100.4±1.0	108.8±1.2	95.8±0.5	<2.1
Mean±SD			100.7±2.1	102.5±5.5	98.9±3.8	

II.2.1.3 RECOVERY OF RARE EARTHS FROM SPIKED FAECAL SAMPLES

In order to demonstrate that efficient separation can be attained with faecal samples, where other constituents could effect the separation, the procedure was applied to faecal samples to which radioactive rare earth tracers were added.

Procedure

After preparation of a 50 mL of rare earth tracer solution, as described above, 10 mL fractions of the solution were added to 200 mg duplicates of ashed faecal samples. The samples were dissolved in aqua-regia with addition of 5 mL of 30% H_2O_2 (if necessary) and evaporated to near dryness. The residues were then re-dissolved in 50 mL of 1M HNO_3. The solutions were passed through columns, prepared as described above, and major interfering elements eluted with 125 mL of 2M HNO_3. The rare earth contents of the columns were recovered with 50 mL of 6M and 50 mL of 8M HNO_3. All eluents, loading solution and the rare earths standard (10 mL of stock solution) were collected in counting bottles and counted on a 130 cc pure Ge detector after appropriate dilution. The recoveries were calculated with reference to standard activities.

Results

The results are provided in **Table-23** showing that the losses with the initial dilute eluent acid can be significant, where cation contents of sample is exceed ion-exchange capacity of column. The recoveries obtained for Column I, lower than 100%, indicates that there is a need for a tracer to determine the yield of separation. Obtainable quantitative recoveries from a solution, containing the rare earths (as described in the previous procedure) and from ashed faecal sample, spiked with the rare earths suggests that there is no matrice effect to the separation procedure.

Table-23 :Rare earth recovery from spiked ashed faecal samples (sum of 6M and 8M HNO_3 elution)

	Recovery, %±SD	
RE	Column I	Column II
La	96.9±0.9	101.5±0.7
Sm	95.3±0.2	101.4±0.2
Eu	99.1±2.4	105.6±2.5
Tb	96.1±1.5	101.3±1.3
Yb	96.8±0.9	100.4±0.8

II.2.1.4 RECOVERY MEASUREMENTS WITH Pr AS YIELD TRACER

The results of the column separation procedure for 5 rare earths, as described above demonstrates that good recoveries can be obtained for a representative set of rare earths. Because ^{141}Pr is 100% abundant and its neutron activation product (Pr-142) has a relatively short half-life (19.1 h) and an interference-free characteristic γ-ray at 1576 keV (4% intensity), Pr was used as a yield tracer for the pre-irradiation separation described above. To demonstrate the applicability of the use of Pr yield tracer, recoveries derived from separation were compared with those derived from nondestructive analysis.

Procedure

100µL of spec-pure Pr solution (1000 µg mL^{-1} AAS standard) was pipetted into duplicates of an ashed faecal sample (obtined from the preliminary faecal recovery investigation containing ~40% of the supplement dose) and processed by following the acid digestion and separation procedure (**Figure-8**). The rare earth standards (prepared from 1000 µg mL^{-1} spec-pure AAS standards as 20 µL of Eu and Tb, 10 µL of La, Sm and Yb, and 100 µL of Pr) and 100 µL of dosing solution (fraction of supplement dose) were pipetted into polypropylene vials and heat-dried under an IR-lamp.

Fe-wires were then attached to the irradiation vials and they were irradiated for 6h in the CVS. After 2 days decay, the samples and standards were counted on a 130 cc Ge detector at the same geometry for 2000 s each. The flux monitors were also counted on contact geometry for 600 s. The yield of the procedure was found by comparison of the Pr activities produced in samples and standard. The rare earth contents of the samples and the dosing solution were determined with reference to the standards used, and the recoveries were calculated by comparison of the contents of samples and dosing solution.

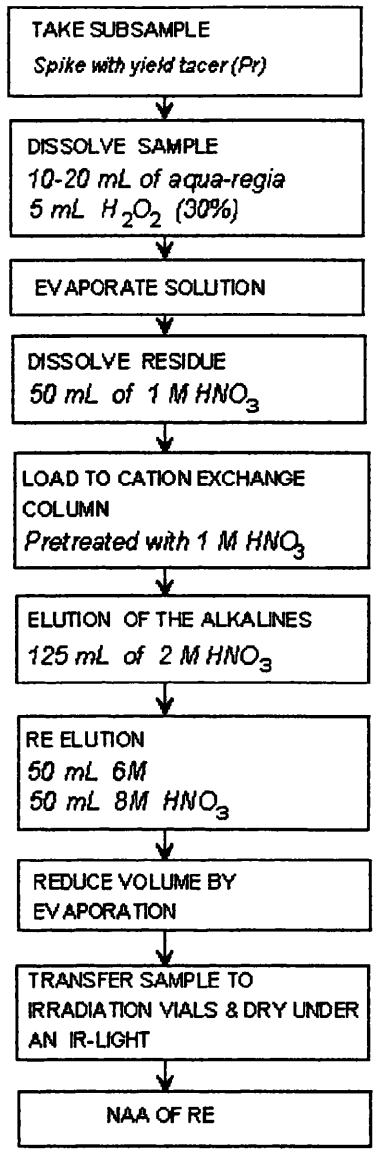


Figure-8: Schematic diagram of rare earths chemical separation procedure

Results

For validation of the separation procedure with a yield indicator, the recovery results of the rare earths with a faecal sample (from preliminary application on two subjects) obtained by INAA are compared with those obtained after chemical separation procedure (CNAA) by using Pr as chemical yield indicator in **Table-24**.

Table-24: Comparison of rare earth recoveries determined by chemical separation with Pr yield tracer and INAA

	Recoveries,%±SD of ingested dose					
	Column separation (CNAA)			INAA		
RE	Column I	Column II	Mean±SDM	Dupl. I	Dupl.II	Mean±SDM
La	40.1±1.0	40.5±1.1	40.3±0.7	43.2±1.0	41.4±1.0	42.3±1.0
Sm	40.2±0.8	39.8±0.8	40.0±0.6	38.1±0.9	37.4±0.8	37.8±0.9
Eu	38.9±2.6	40.6±2.8	39.7±1.9	40.7±1.9	40.2±2.7	40.6±1.9
Tb	42.7±2.1	40.5±2.2	41.6±1.5	39.4±1.7	40.0±2.4	39.6±1.7
Yb	40.9±1.2	40.4±1.6	40.7±1.0	39.1±1.1	38.1±1.5	38.7±1.1
Column yield, %±SD						
	87.1±0.8	93.6±0.7				

Good agreement between the results obtained from two different techniques ($p>0.05$ by paired t-test where mean of the recoveries of rare earths were 40.5±0.7 and 39.8±1.7 for CNAA and INAA respectively) demonstrate that the use of Pr as a yield tracer is an applicable technique for the chemical separation procedure. Agreement between the recovery results by CNAA and INAA in 95% confidence limit is also illustrated in **Figure-9**.

Conclusion

1.The acid digestion procedure followed for ashed faecal samples was found satisfactory. Samples were heated to dissolve after addition of 15 mL of aqua-regia for a 200 mg sample. For complete digestion, on some occasions addition of an extra 5 mL of aqua-regia and 5 mL of 30% H_2O_2 was required.

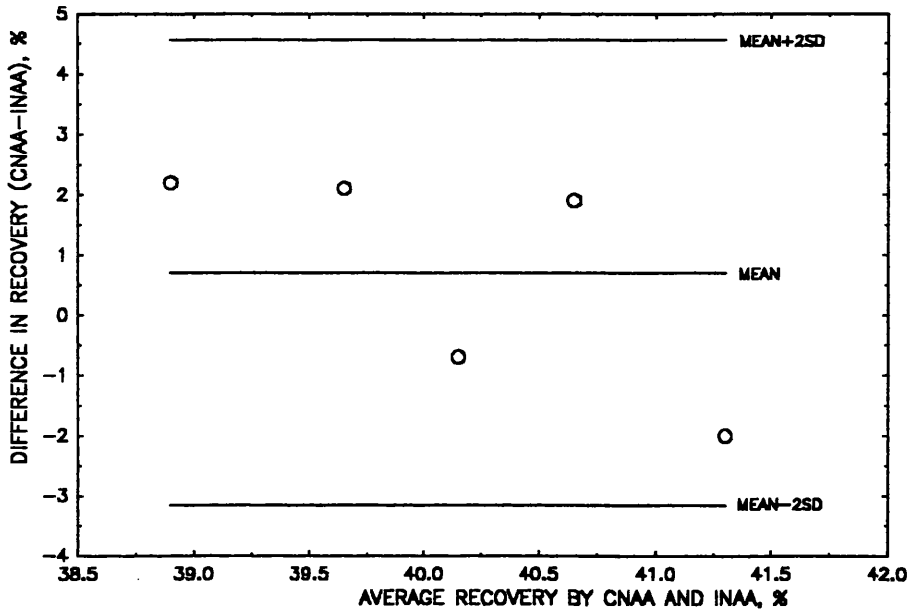


Figure-9: Difference between recoveries (average of 5 rare earths) obtained by CNAA and INAA

2. Effective elimination of the alkali and alkaline earths (Na, K, Mg, Ca etc.) was demonstrated by separation of Na-24 tracer from the cation exchange columns. The elimination of major interfering elements can be seen in **Figure-10**. It can be seen that K-42 is eliminated from the processed sample and the reduction of Na-24 to a level comparable with that of K-40 in the natural background is attributed to trace quantities of Na in the sample containers.

The pre-irradiation separation procedure described provides less radiation exposure and/or permits counting at a shorter time after irradiation. The dose rates from unshielded samples after 2 days decay were 50 μSv h^{-1} (at 20 cm) for column separated samples whilst the dose rates were more than 200 μSv h^{-1} for unprocessed samples 4 days after irradiation. Practical exposure was significantly less than these levels, since irradiated samples were stored in shielded containers. No significant exposure was observed on finger monitors, which were worn in addition to a film badge and personal radiation monitor.
3. Recovery results of rare earth separation procedure confirmed that recovery of rare earths with 50 mL of 6M HNO_3 was 90% and 50 mL of 8M HNO_3 was

required to recover remaining 10%.

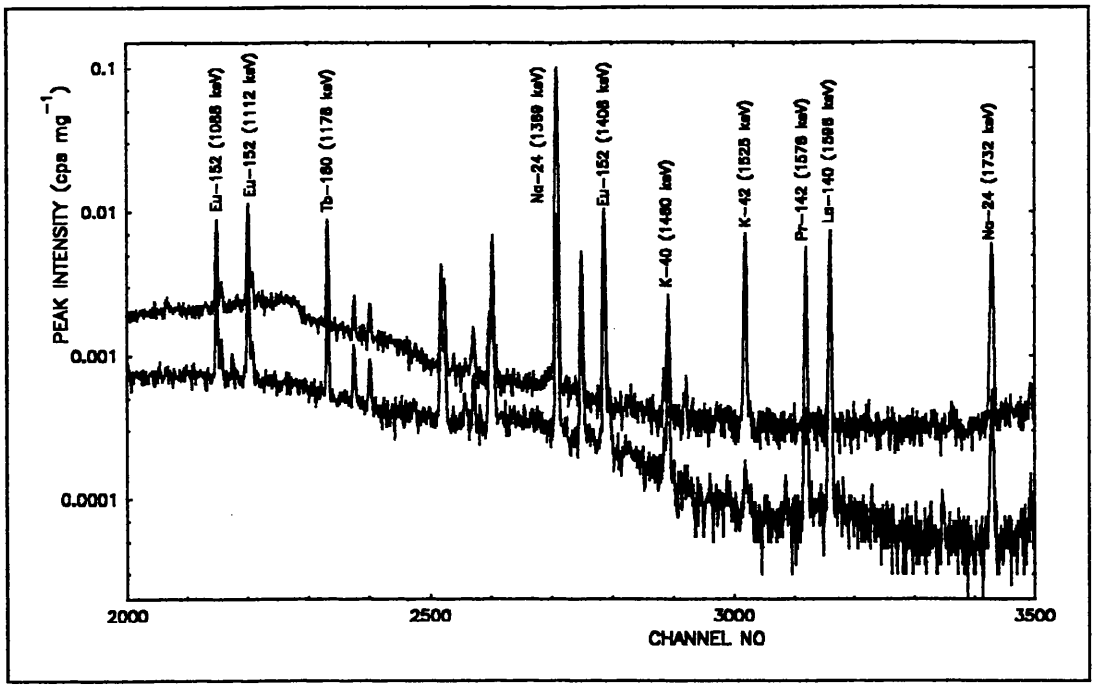


Figure-10: Spectra for the same sample obtained before (upper spectrum) and after chemical separation

II.2.2 RARE EARTH RECOVERY WITH PRE-IRRADIATION SEPARATION

The investigation for faecal recovery of the rare earths, with inclusion of the separation procedure, was extended to six subjects for further confirmation of their non-absorbability, with particular attention to their natural levels in faeces. The fractions of supplement dose recovered in individual faecal samples, and in faecal pools consisting of their consecutive accumulation were evaluated to obtain faecal recovery profiles for each subject. The principle aim was to identify the fraction of output required to obtain sufficient recovery for measurement of luminal disappearance from mineral nutrients labelled with stable isotopic tracers.

II.2.2.1 PROCEDURE FOR DOSE PREPARATION, ADMINISTRATION, AND SAMPLE COLLECTION

1. Ethical approval and protocol:

The investigation was approved by The Joint Ethical Committee of Grampian Health Board and The University of Aberdeen.

The subjects, 5 males and 1 female in good health, pursuing normal sedentary activities were provided with an explanatory form containing the theoretical background, requirements of the investigation, and instructions for the use of the sample collection system. The physical characteristics of 6 subjects who participated in two investigations [2 subjects (AS1-2) in the preliminary recovery investigation and 6 subjects (BS1-6) in the extended, including the same participants] are provided in **Table-25**. The sample collection system and instruction for its use was described to each of the subjects and they were requested to collect all stool samples and toilet papers used, starting from the day before administration (one sample as baseline) followed by a period of a week. Their informed consent was obtained before participation. A copy of the information and consent form is provided in **Appendix-1**.

Table-25: Physical characteristics of the subjects participated in rare earth recovery investigations

Subject	Sex	Age, y	Height, m	Weight, kg
AS1 (BS1)	M	56	1.74	79
AS2 (BS2)	M	37	1.68	65
BS3	M	54	1.85	95
BS4	M	42	1.83	78
BS5	F	28	1.57	55
BS6	M	32	1.77	60
Mean±SDM		42±11	1.74±0.1	72±15

2. Preparation of dose solution

Specpure oxides of the rare earths consisting of 9.75 mg of La, 9.79 mg of Sm, 100.10 mg of Eu, 77.24 mg of Tb and 50.55 mg of Yb were converted to chloride form by HCl treatment and contained in 100 mL of solution as described previously. Six fractions of the final solution of 10 mL for each (containing 0.98 mg of La, 0.98 mg of Sm, 10.00 mg of Eu, 7.72 mg of Tb and 5.05 mg of Yb) were transferred to universal tubes and diluted with additional 20 mL of water to reduce the acidity for consumption.

3. Administration and sample collection

Subjects drank the solution containing a dose of rare earths with their lunch and took two further water rinses of the container to ensure total consumption. They recorded the time of preceding and following bowel movements on record cards provided with the volunteer information sheet as requested. Each of the stool samples was collected in a "biohazard" (autoclave resistible) sample bag, contained in a plastic bowl. Samples were brought to laboratory, weights recorded and stored in a deep-freeze at -20 °C.

II.2.2.2 ANALYTICAL PROCEDURE

The overall procedure including analytical processes is provided in **Figure-11**.

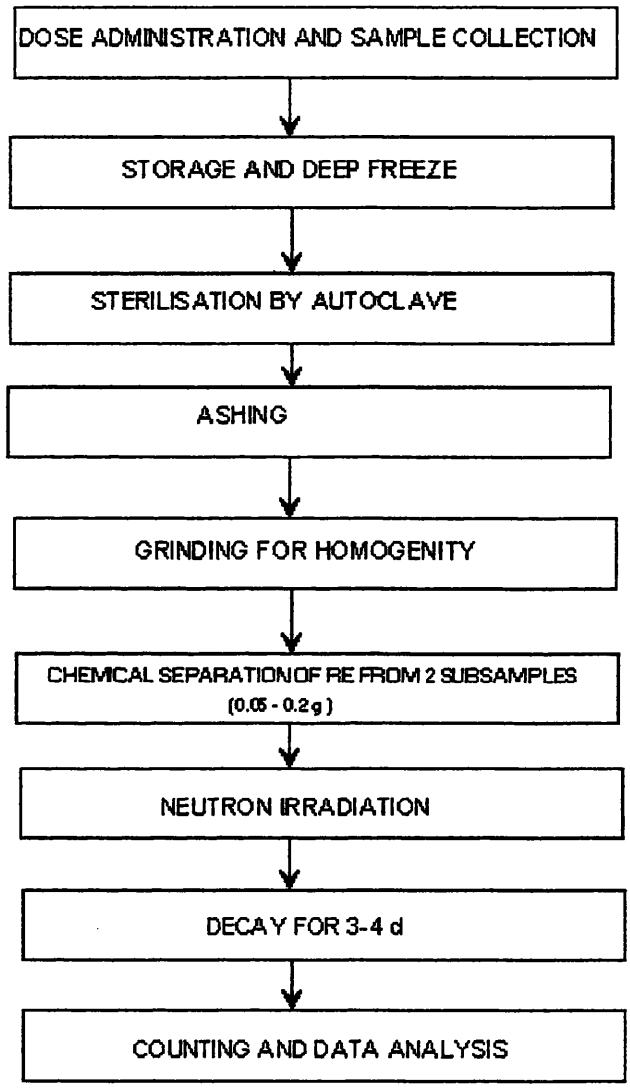


Figure-11: Procedure for the investigation of rare earth recovery

Sample and standard preparation

After completion of sample collection, the samples for each of the subjects were transferred to a "biohazard" bag and they were then autoclaved. The requirement of sample transfer to aluminium trays for freeze-drying (which caused sample losses in the preliminary application) was eliminated by oven-drying. The samples were dried in an oven at 50-70 °C and their weights

recorded. They were then ashed at gradually increased temperatures of 200-450 °C for 16-24 h and their ashed weights also recorded. The samples were ground to ensure homogeneity and stored in polythene containers for further use.

Duplicate fractions of samples were weighed (0.05-0.2 g as appropriate for the expected rare earth contents) and spikes of 100 µg of Pr were added. Samples were dissolved, their rare earth contents were separated by cation exchange and transferred to irradiation vials as previously described. Standard vials were prepared to contain;

a) 20 µg of La and Tb, and 10 µg of Sm
b) 20 µg of Eu and 10 µg of Yb
c) 100 µg of Pr standard (for chemical yield measurement)
d) 100 µg of supplement dose as dosing standard.

All standards were then dried under an IR-light, iron flux monitors attached to each, and wrapped in aluminum foils for irradiation.

Irradiation and counting

Batches of samples (provided by the same subject) and standards were irradiated in the CVS system for 3-6 h as appropriate for the expected rare earth contents. After 3 days decay, the samples and standards were counted at an optimum geometry for 1000-2000 s. The standards and dose solution were also counted at a constant geometry (15 cm above the detector) for all irradiation batches for quality control. Flux monitors were counted at contact geometry for 600s.

The yields of the chemical procedure were found by comparing the specific activities of Pr for the samples with those of the standards. The rare earth contents of the samples (after chemical yield correction) and the supplement dose were found by comparing the specific activities of the rare earths for samples and for the dose with those for the standards. The recoveries of the administered dose were then calculated as the fraction of supplement dose, taking into account the ashed weights of the samples and the concentrations found (as means of duplicate measurements). Weight of faecal samples and the mean concentrations determined in ashed samples are provided in **Appendix-3**.

II.2.2.3 COMMENTS ON PROCEDURE

Sample collection and preparation for analysis:

No problems were reported by any of the subjects relating to dose consumption and/or sample collection throughout the study. None of the subjects had any illness whilst participating in the study. All subjects completed the study period without overlooking any samples, but it was noted that some samples did not include toilet paper although subjects were specifically requested to include this.

Analytical procedure and quality control:

Yields of 80-100% were obtained for the separation procedure. The use of a pre-irradiation separation reduced the dose rates from irradiated samples. For a 1000 s count for measurement of the Pr activity in samples and standards a standard error of <1% was obtained, resulting in a negligible contribution to the precision of rare earth concentrations determined. Agreement between the concentrations for duplicates, with chemical yield correction, confirmed that sample homogenization was satisfactory and it justified yield measurements for chemical separation by Pr tracer. **Table-26** provides a set of Sm concentrations found for duplicates samples after chemical yield correction for subject B2 as an example. As can be seen from the table that differences between concentrations, with reference to their means, before yield correction is significantly higher than that of after yield correction.

Table-26: Sm concentrations in ashed faeces for duplicate samples, determined by chemical separation with yield correction, for subject B2

Chemical yield,%		Sm concentration in ash, μg g^{-1}±SD[1]			Difference, %	
Col. I	Col. II	Column I	Column II	Mean±SDM[2]	Before	After
91	98	0.26±0.01	0.28±0.01	0.27±0.01	28	7
86	96	112.3±1.7	114.0±1.8	113.1±1.2	13	1
87	95	83.1±1.3	84.4±1.3	83.8±0.9	10	2
83	93	27.8±0.4	28.2±0.4	28.0±0.3	13	1
90	91	5.6±0.1	5.8±0.1	5.7±0.1	5	3
86	88	0.47±0.02	0.49±0.02	0.48±0.01	6	4
84	85	0.23±0.01	0.25±0.01	0.24±0.01	9	8

1)Error of measurement, 2) SD for mean

The rare earth content of dosing solution was determined for 10 irradiation batches with reference to specific activities of the simultaneously irradiated AAS standards. The mean concentrations in the dosing solution obtained for all irradiation batches are compared with the expected values in **Table-27**. Insignificant difference between the specific activity or the rare earth concentrations determined for the dosing solution and expected values are evidence that the results of measurement are reliable ($p>0.05$, paired t-test).

Table-27: Comparison of the mean concentrations of rare earths determined and those expected of dosing solution

	Mean concentrations (n=10), µg mL^{-1}	
RE	Found±SDM	Expected
La	88.2±11	97.5
Sm	94.8±10	97.9
Eu	1031.1±122	1001
Tb	762.2±81	772.4
Yb	482.1±49	505.5

Conclusion

1. The procedure used for sample collection and preparation for analysis minimized sample losses during the process. However, sterilization by autoclave and sample drying in polythene bags remained inconvenient.

2. The pre-irradiation separation of the elements of interest provided better precision and reduced radiation exposure, so that the time required for radioactive decay was minimized.

3. Agreement between results obtained for duplicates subject to the chemical procedure demonstrated that the processes for homogenization and the measurement were satisfactory.

Discussion of the recovery results and their kinetic interpretations is provided in section II.2.4 and II.2.5. Investigation of the natural (background) levels of rare earths found in faeces and their effect on calculation of recoveries are described in the following section.

II.2.3 NATURAL (BACKGROUND) LEVELS OF RARE EARTHS IN FAECAL SAMPLES

The natural levels of rare earths in faeces were determined in samples provided before administration of the dose solution, to confirm that this level was regained by the end of collections. Concentrations were determined principally by ICP-MS. The rare earths used in the recovery investigation were also determined by INAA to confirm results obtained by ICP-MS.

II.2.3.1 ANALYTICAL PROCEDURE

Procedure for INAA

Duplicates fractions of 0.2 g of ashed samples, blank (cellulose powder), and standard for the rare earths of interest (10 µg of each) were prepared and irradiated in the CVS for 6 hours, as described previously. The samples were allowed to decay for a week to reduce the contribution of major interfering nuclides to the rare earths counts.

The samples were counted for 4000 s for determination of La, Sm and Yb at 10 cm geometry. After 4 weeks decay, counting was repeated for 5-10h at contact geometry for determination of Tb and Eu. The contents of the samples were determined with reference to the specific activities of the standards which were counted under the same conditions.

Procedure for ICP-MS

0.1 g of ashed samples (equivalent to 5 g of wet weiight) were dissolved in 10 mL of 16 M aristar HNO_3 by heating and solutions were evaporated to near dryness. The digestion procedure was then repeated with addition of 5 mL of the HNO_3, 5 mL of 30% H_2O_2 was also added dropwise for complete digestion of organic material and the solutions were evaporated to dryness. The residues were dissolved in 2% v/v HNO_3, transferred to 100 mL graduated flasks, and made up to provide a sample concentrations of 0.1%, w/v. A blank

solution was also run through the sample digestion procedure.

Multi-element standards containing all rare earths in 0.2, 1 and 10 ng mL^{-1} concentrations were prepared from specpure standards (1000 µg mL^{-1}). The standards, samples and the blank solutions were spiked with ^{115}In as internal standards to monitor signal fluctuations. The detection system was then calibrated for each of rare earths by introduction of the standard, the regression coefficient for the calibration was found 0.9999 for the isotopes chosen for the rare earths analysis provided in **Table-28**.

Table-28: Stable isotopes used for determination of rare earths by ICP-MS

Isotope	Natural abundance, %	Isotope	Natural abundance, %
^{139}La	99.99	^{159}Tb	100
^{140}Ce	88.5	^{162}Dy	25.5
^{141}Pr	100	^{165}Ho	100
^{146}Nd	17.2	^{166}Er	33.4
^{147}Sm	15.0	^{169}Tm	100
^{151}Eu	47.8	^{174}Yb	31.8
^{157}Gd	15.7	^{175}Lu	97.4

The rare earth contents of the blank and samples were determined by triplicate measurements. No significant internal standard drift was found in any of the measurements. The rare earth concentrations for the ashed samples were then calculated by application of the sample dilution factor, after subtraction of blank levels. The characteristics of the ICP-MS instrument used and the analysis parameters are provided in **Table-29**.

Table-29: Operational parameters of the **Plasma Quad PQ1**

Plasma		
R.f. power	Forward	1.35 kW
	Reflected	<10 W
Gas controls	Auxiliary	0.55 L min^{-1}
	Coolant	14 L min^{-1}
	Nebulizer	0.73 L min^{-1}
Nebulizer	Meinhart: concentric type	
Spray chamber	Scott-type double bypass, water cooled (ambient)	
Ion Sampling		
Sampling cone	Nickel with 1.0 mm orifice	
Skimmer cone	Nickel with 0.75 mm orifice	
Sampling distance	10 mm from load coil	
Optimization		
	The lenses were adjusted to maximize the ^{115}In signal	
Vacuum		
Expansion stage	2.4 mbar	
Intermediate	$<1x10^{-4}$ mbar	
Analyzer	$4x10^{-6}$ mbar	
Data acquisition (Peak scanning)		
Sweeps	1600	
Dwell time	80 µs	
Channels	512	
Dead time	20 ns	

II.2.3.2 RESULTS

The concentrations of rare earths in the blank solution (containing chemicals used in sample preparation for ICP-MS) and irradiation vials (sample containers for irradiation) were found to be $<0.01x10^{-3}$ µg mL^{-1} and $<0.1x10^{-3}$ µg g^{-1} respectively, and were insignificant compared with concentrations detected in the ashed faecal samples. The concentrations of all rare earths determined by ICP-MS and of those obtained in the recovery investigation (determined by INAA) are provided in **Table-30**.

Conclusion

It can be seen from **Table-30** that good agreement was found between the results obtained by ICP-MS and by INAA. Chondrite normalized (Evensen *et al.*, 1978) rare earth concentrations determined by ICP-MS, as a function of atomic numbers, are depicted for subject BS4 with the measurement precision and for the average of 4 subjects with the standard deviation of the mean in **Figure-12**.

Table-30: Background levels of rare earths in ashed faeces (μg g^{-1}±SD) determined by ICP-MS and INAA

	B1	B2	B4	B5	MEAN±SDM
RE	ICP-MS (INAA)	ICP-MS (INAA)	ICP-MS (INAA)	ICP-MS (INAA)	ICP-MS
La	1.06±0.005 (1.11±0.05)	2.16±0.02 (1.97±0.02)	1.08±0.04 (0.88±0.02)	1.42±0.002 (1.38±0.07)	1.43±0.51
Ce	1.37±0.003	3.18±0.006	1.54±0.01	1.61±0.022	1.92±0.84
Pr	0.21±0.004	0.49±0.005	0.30±0.01	0.29±0.01	0.32±0.12
Nd	0.76±0.03	1.55±0.08	0.86±0.04	1.07±0.007	1.06±0.35
Sm	0.15±0.005 (0.20±0.01)	0.22±0.02 (0.15±0.01)	0.17±0.03 (0.13±0.01)	0.22±0.01 (0.17±0.01)	0.19±0.03
Eu	0.059±0.002 (0.063±0.02)	0.23±0.02 (0.21±0.05)	0.094±0.005 (0.081±0.02)	0.18±0.004 (0.16±0.02)	0.14±0.08
Gd	0.17±0.003	0.27±0.006	0.17±0.02	0.24±0.03	0.21±0.05
Tb	0.027±0.003 (0.035±0.01)	0.17±0.002 (0.17±0.05)	0.055±0.004 (0.031±0.02)	0.11±0.003 (0.088±0.02)	0.09±0.06
Dy	0.14±0.003	0.21±0.01	0.14±0.005	0.20±0.009	0.18±0.04
Ho	0.028±0.002	0.039±0.002	0.032±0.003	0.042±0.003	0.035±0.007
Er	0.087±0.004	0.12±0.007	0.093±0.008	0.12±0.009	0.10±0.02
Tm	0.013±0.001	0.017±0.002	0.016±0.002	0.015±0.004	0.015±0.002
Yb	0.10±0.005 (0.075±0.01)	0.17±0.01 (0.082±0.04)	0.15±0.01 (0.094±0.02)	0.15±0.01 (0.088±0.03)	0.14±0.03
Lu	0.020±0.002	0.016±0.001	0.015±0.003	0.018±0.004	0.017±0.002

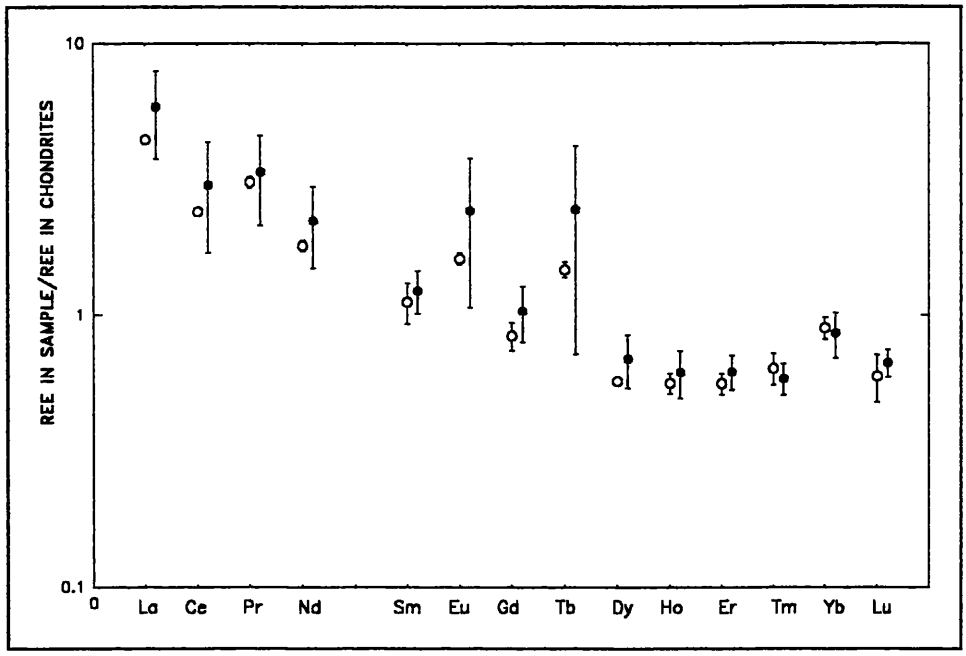


Figure-12: Chondrite normalised rare earth pattern in baseline faecal samples (O, results for subject B4 with measurement precision and ●, average of 4 subjects with standard deviation of the mean)

The production of smooth trends can be taken as further indication of the reliability of the results. As can be seen from the figure, the normalised concentrations descends from 10 to 1 (from La to Lu). These values would be from 1 to 0.1 if the concentrations were given on the basis of dry weight of the faecal samples (the ratio of ashed/dry weight was always close to 0.1) which agrees with the results of Laul and Gosselin (1989) for vegetable standards. This suggests that the amounts found in baseline faecal samples probably originate from vegetables.

II.2.4 EFFECT OF NATURAL RARE EARTH LEVELS ON RECOVERY RESULTS

Contribution of the natural levels of rare earths to recovery measurements

Rare earth concentrations obtained in the final collections for 5 subjects are compared with the pre-dose concentrations in **Table-31**. Recovery was incomplete for subject B6 when the investigation ended. In the final sample from this subject, the average recovery was 1%.

Table-31: Concentrations of rare earths (μg g^{-1} in ashed sample) in samples provided at the end of the investigation

	RE concentration, μg g^{-1}±3SD				
Subject (time, h)[1]	La	Sm	Eu	Tb	Yb
B1 (143)	2.16±0.12	0.28±0.01	1.88±0.24	2.11±0.10	1.38±0.05
B2 (142)	2.30±0.21	0.24±0.02	2.48±0.76	1.91±0.90	1.15±0.36
B3 (163)	1.30±0.06	0.18±0.01	0.55±0.06	0.42±0.04	0.39±0.02
B4 (113)	0.76±0.05	0.12±0.001	0.32±0.21	0.32±0.21	0.25±0.15
B5 (163)	1.25±0.08	0.10±0.02	0.39±0.18	0.30±0.15	0.20±0.10
Mean±SDM	1.56±0.65	0.18±0.08	1.12±0.99	1.00±0.93	0.68±0.55
	Natural levels in pre-dose samples				
Mean±SDM	1.43±0.51	0.19±0.03	0.14±0.08	0.09±0.06	0.14±0.03

1 Time post-dosing

It can be seen that when samples are provided 110-160 h (5-7 days) after consumption of the dose solution the concentrations of the elements of Eu, Tb and Yb were found to be higher than their pre-dose levels, but it should be emphasized that these represent less than 0.1% of the input. The contribution from a recovery of 0.1% of an input 1 mg of La and Sm to background level in the final collection can not be discriminated because the background abundances of these elements are significant. For elements where a higher levels of input was used (10 mg for Eu, 7.5 mg of Tb and 5 mg for Yb), the contribution of the background concentrations to the levels determined in the samples provided at the end of the investigation were found to be not

significant.

The background level of La was subtracted from output levels in calculating La recoveries, assuming the level remained constant through the collection period. Sm recoveries were not similarly adjusted, since its background level was less than the error of measurement. The effect of taking account of the La background for each of individual collection can be seen in **Table-32**, where recoveries of La are compared with the mean of recoveries of other 4 rare earths for subject B4, as an example. It is evident that the contribution of the low abundant (heavy) rare earths to recovery results is negligible but that for La recoveries account must be taken of the background level. The La background is more significant when correcting lower recoveries.

Table-32: Effect of the background contribution to La recovery (subject BS4)

Sample no	Time post-dosing, h	Recovery, %±3SD		
		La before correction	La corrected	Mean of 4 RE±SDM
1	19	26.25±1.15	25.25±1.18	25.16±0.3
2	43	61.15±3.47	59.36±3.50	59.79±0.7
3	67	9.04±0.54	7.94±0.62	7.68±0.3
4	90	2,36±0.16	1.36±0.32	1.40±0.07
5	116	0.70±0.03	-	0.10±0.01
6	141	0.72±0.05	-	0.05±0.04
Total		100.22±3.70	93.91±3.76	94.19±0.82

Conclusion

The significant difference between the concentrations determined in pre-dose and in final samples (6-7 days after administration) for Eu, Tb and Yb can be explained by the fact that a longer period of collection is required to reach natural levels for the higher inputs of these elements. The recoveries of these elements in final samples were 0.1% or less.

Intersubject variations in recoveries for the final samples may be due to

individual idiosyncrasies and/or the composition of diets consumed, but the regular descending recovery with time post-dosing indicates that the remaining insignificant quantities would be excreted in the following samples. This regularity also indicates that absorption of the elements are unlikely, otherwise good agreement between the recoveries of La after background effect correction and the mean of the other 4 rare earths would not be found. No significant difference was found when corrected La recovery results were compared with the average of those for the other 4 rare earths in **Table-33** ($p>0.05$, paired t-test) for all investigations (INAA and CNAA). This insignificant difference indicates that natural levels of the rare earths were very low (except La) and remained constant through the study period, and might be evidence that the losses occurred during sample collection but not due to absorption where recoveries were found not to be quantitative.

Table-33: Effect of the background contribution on recoveries of La for 8 subjects

	Recovery, %±3SD		
Subject	La before correction	La corrected	Average of 4 RE±3SDM
A1	93.6±4.7	88.6±4.7	90.0±6.7
A2	107.5±8.0	102.7±8.0	96.9±5.0
B1	100.7±3.5	98.1±3.6	96.3±2.4
B2	103.8±4.2	99.9±4.4	100.3±3.4
B3	97.0±2.1	90.5±2.2	91.3±3.2
B4	100.2±3.7	93.9±3.8	94.2±2.0
B5	89.4±2.6	86.1±2.7	88.6±2.8
B6	100.9±2.0	97.6±2.7	93.9±3.9
Mean±SDM	99.1±5.7	94.7±5.9	93.9±3.9

II.2.5 RESULTS OF RARE EARTH RECOVERY INVESTIGATIONS

Results

Faecal rare earth recoveries (% of ingested dose) obtained in the initial investigation, where samples from 2 subjects (A1-2) were analysed by INAA are provided in **Table-34.a** and **b** with recovery profiles in **Figure-13.a** and **b**. Recoveries and recovery profiles for the second study, where subsamples from 6 subjects (B1-6) were analysed after a pre-irradiation separation are provided in **Table-34.c-h** and **Figure-13.c-h** respectively. Experimental data is provided in **Appendix-2** and **3**. All recoveries for La are given after background correction.

The tables contain the time elapsed after administration of the dose, recoveries for each of the rare earths in individual stools, means of recoveries for 5 rare earths for each sample, and cumulative recoveries obtained by addition of sequential outputs. Standard deviations for total recoveries take account of measurement errors for each element (99% confidence limit), whilst the deviation for total of the mean recoveries takes account of standard deviations for the mean of recoveries for each of the samples.

As can be seen from the tables, standard deviations at total recovery for individual collections vary between the elements of interest, since the detection sensitivity depends on the nuclear characteristics of the isotopes, and the efficiency of detector for the characteristic gamma emission measured. Precision of measurement of the elements was found to decrease in the order Sm:Yb:La:Tb:Eu, i.e. the best precision was obtained for Sm and Yb.

The recoveries of rare earths as function of time post-dosing for individual and cumulative collections with standard deviations are provided in the figures. As can be seen, the recoveries were extrapolated to the time when the dose was consumed, to make the recovery profiles more visible.

Table-34.a: Faecal recoveries of rare earths for subject A1

SUBJECT A1								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	18	23.45	24.19	23.41	24.78	23.67	23.90	23.90
2	41	48.28	51.22	47.94	50.88	49.76	49.62	73.52
3	48	13.66	15.32	15.15	13.80	12.86	14.16	87.68
4	67	1.21	1.23	1.28	1.46	1.12	1.26	88.94
5	100	-	0.37	0.57	0.45	0.39	0.45	89.39
6	124	-	0.04	0.08	0.15	0.11	0.10	89.49
Total,% ±3SD		86.60 ±4.65	92.37 ±1.81	88.43 ±7.65	91.52 ±5.68	87.91 ±3.15	89.49 ±1.91	89.49 ±1.91

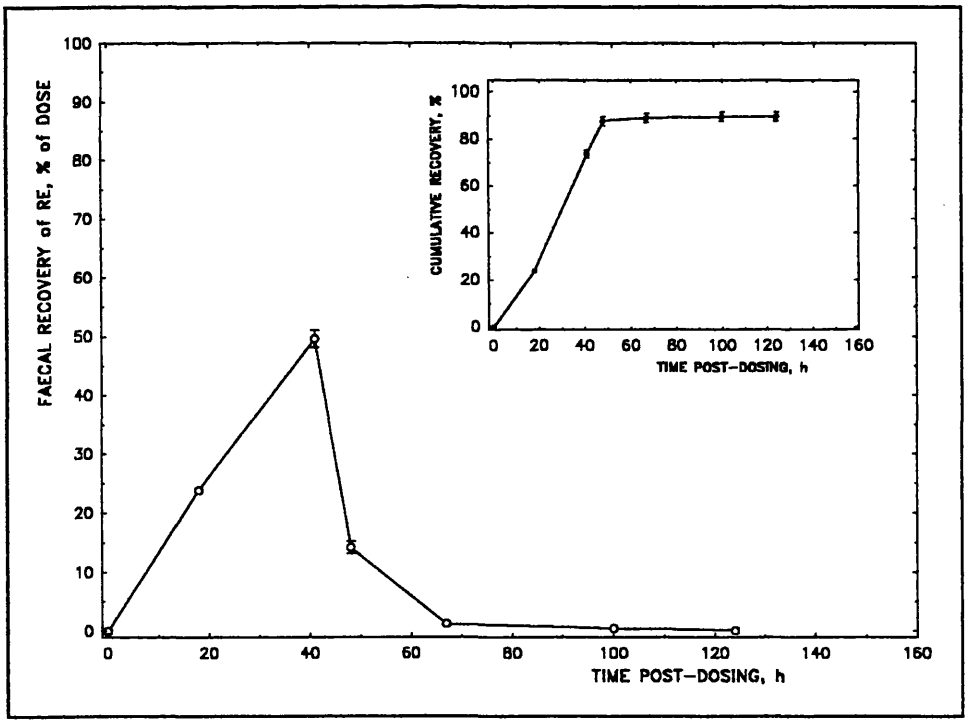


Figure-13.a: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject A1

Table-34.b: Faecal recoveries of rare earths for subject A2

SUBJECT A2								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	18	5.91	4.44	4.06	4.13	4.21	4.55	4.55
2	35	41.73	37.80	40.60	39.60	38.70	39.68	44.23
3	60	35.21	33.90	34.70	34.20	34.30	34.44	78.67
4	78	16.38	15.60	16.10	15.60	15.60	15.85	94.52
5	110	3.44	3.29	3.49	3.34	3.34	3.38	97.90
6	152	-	0.12	0.21	0.13	0.13	0.15	98.05
Total,% ±3SD		102.7 ±8.0	95.15 ±2.6	99.16 ±8.2	96.90 ±6.4	96.38 ±4.2	98.05 ±1.83	98.05 ±1.83

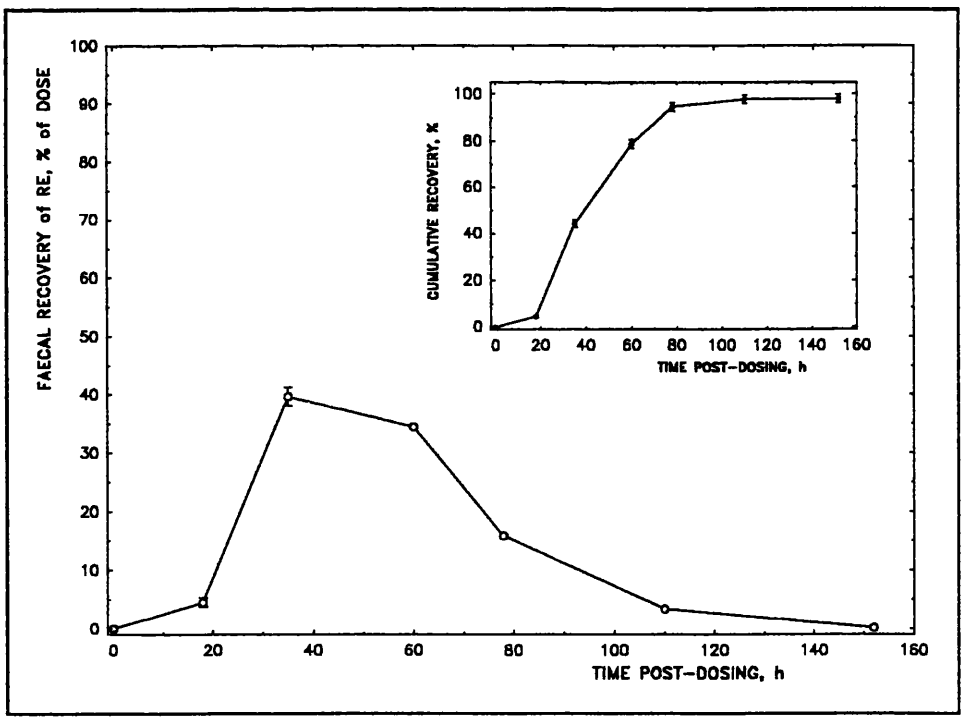


Figure-13.b: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject A2

Table-34.c: Faecal recoveries of rare earths for subject B1

SUBJECT B1								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	20	43.50	44.12	43.76	43.14	43.14	43.53	43.53
2	44	49.66	50.43	49.88	51.20	50.09	50.25	93.78
3	67	3.58	1.74	1.38	2.26	2.01	2.19	95.97
4	92	0.55	0.28	0.22	0.29	0.29	0.31	96.28
5	120	0.33	0.11	0.06	0.07	0.10	0.13	96.41
6	143	0.44	0.17	0.11	0.16	0.16	0.21	96.62
Total,% ±3SD		98.06 ±3.56	96.85 ±3.23	95.41 ±4.69	97.12 ±3.86	95.79 ±3.69	96.62 ±1.13	96.62 ±1.13

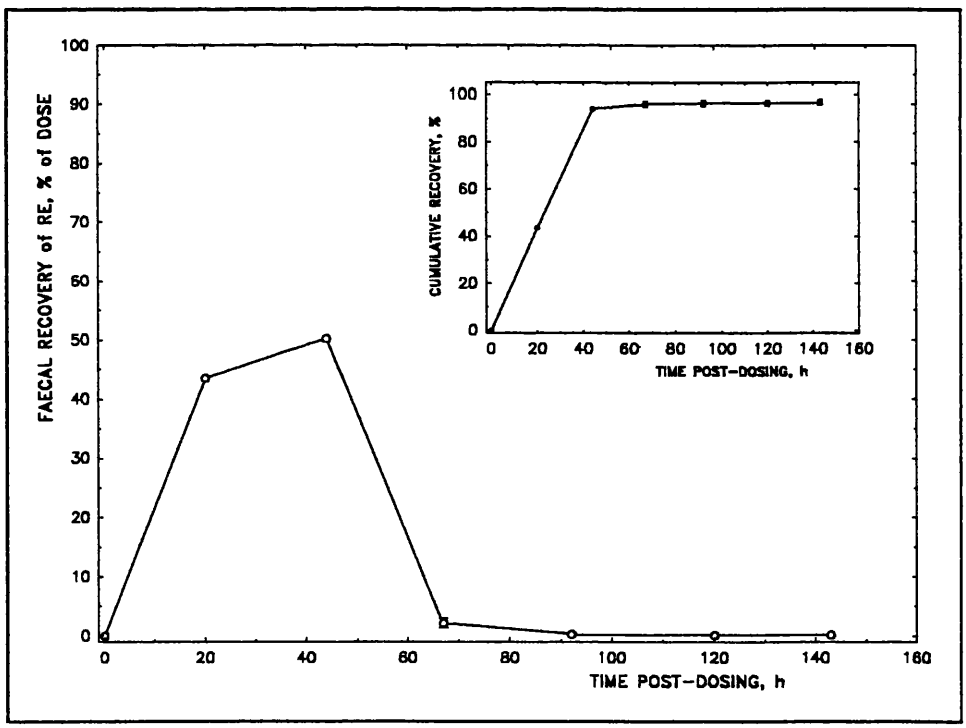


Figure-13.c: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B1

Table-34.d: Faecal recoveries of rare earths for subject B2

SUBJECT B2								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	11	0.21	0.04	0.01	0.01	0.01	0.06	0.06
2	30	63.29	65.64	67.53	65.48	64.00	65.19	65.25
3	55	31.17	29.52	27.88	29.76	29.24	29.51	94.76
4	77	3.38	3.24	3.18	3.27	3.21	3.26	98.02
5	98	0.98	2.27	1.90	2.09	1.96	1.84	99.86
6	118	0.44	0.10	0.07	0.07	0.09	0.15	100.0
7	142	0.43	0.12	0.12	0.12	0.11	0.18	100.2
Total %±3SD		99.90 ±4.42	100.9 ±2.44	101.0 ±3.06	101.0 ±3.73	98.62 ±2.77	100.2 ±1.13	100.2 ±1.13

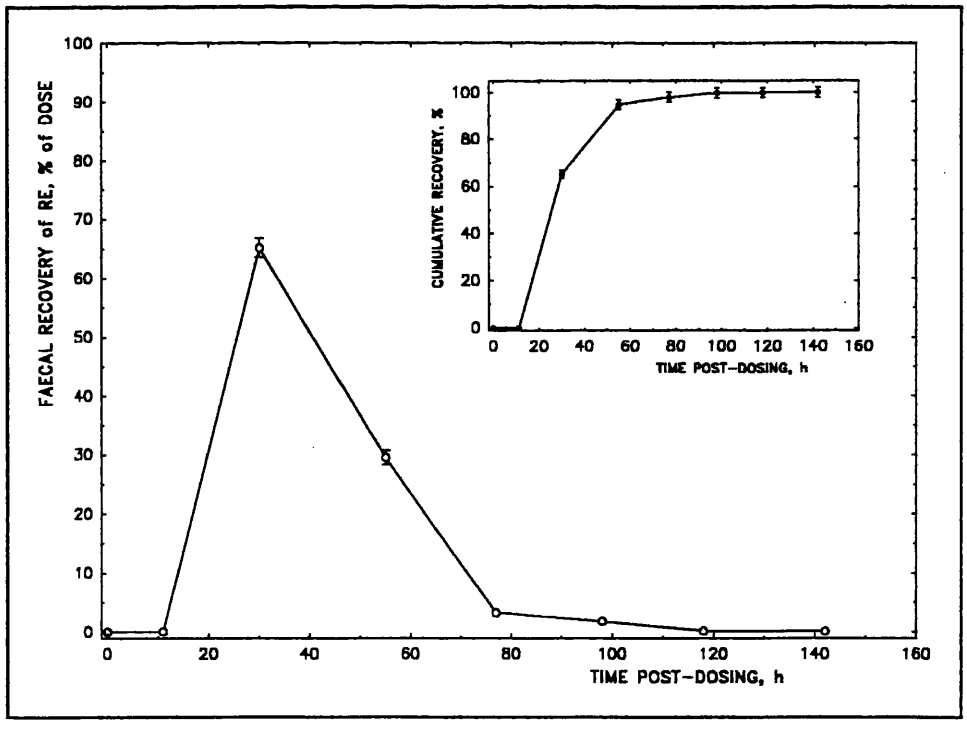


Figure-13.d: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B2

Table-34.e: Faecal recoveries of rare earths for subject B3

SUBJECT B3								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	18	30.31	30.19	30.22	29.56	30.58	30.17	30.17
2	42	46.33	44.71	47.48	46.65	45.01	46.04	76.21
3	66	11.31	12.61	12.34	12.09	11.89	12.05	88.26
4	90	2.13	2.04	1.97	2.33	2.33	2.16	90.42
5	117	0.40	0.54	0.52	0.55	0.55	0.51	90.93
6	141	-	0.13	0.11	0.12	0.10	0.11	91.04
7	163	-	0.17	0.05	0.05	0.07	0.08	91.12
Total,% ±3SD		90.48 ±2.15	92.69 ±0.64	92.69 ±4.06	91.35 ±2.41	90.53 ±2.21	91.12 ±1.33	91.12 ±1.33

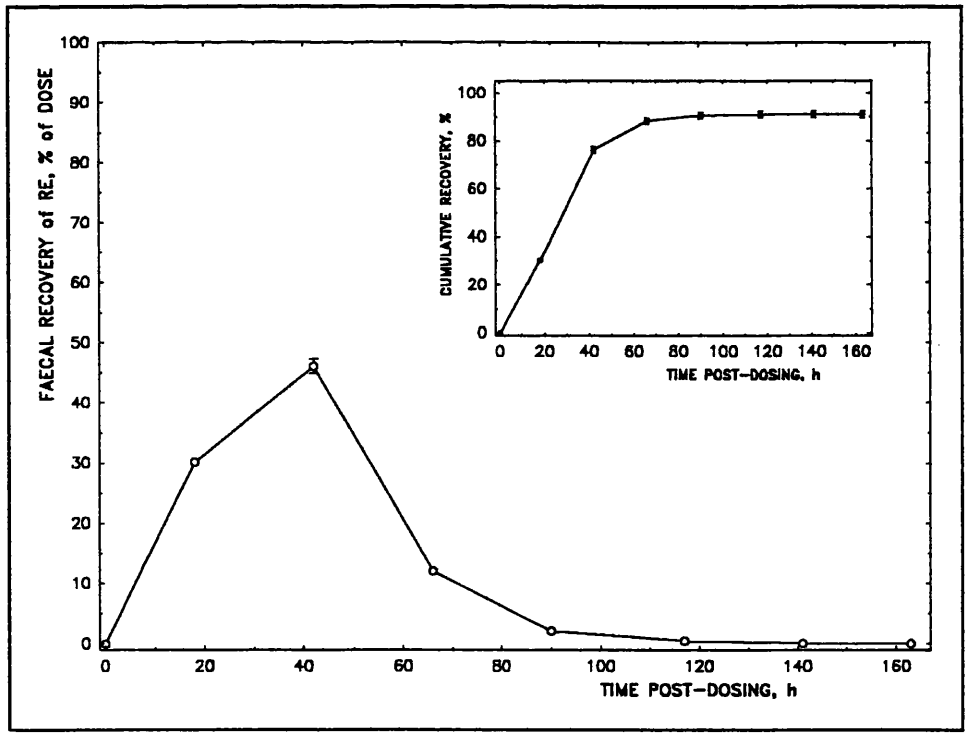


Figure-13.e: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B3

Table-34.f: Faecal recoveries of rare earths for subject B4

SUBJECT B4								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	19	25.25	25.37	25.25	24.73	25.29	25.18	25.18
2	42	59.36	58.79	60.21	60.47	59.69	59.70	84.88
3	67	7.94	7.41	7.52	7.87	7.94	7.74	92.62
4	90	1.36	1.38	1.32	1.44	1.47	1.39	94.01
5	116	-	0.09	0.09	0.10	0.10	0.10	94.11
6	140	-	0.11	0.03	0.03	0.05	0.05	94.16
Total,% ±3SD		93.91 ±3.76	93.15 ±1.13	94.42 ±4.93	94.64 ±4.03	94.51 ±3.03	94.16 ±0.60	94.16 ±0.60

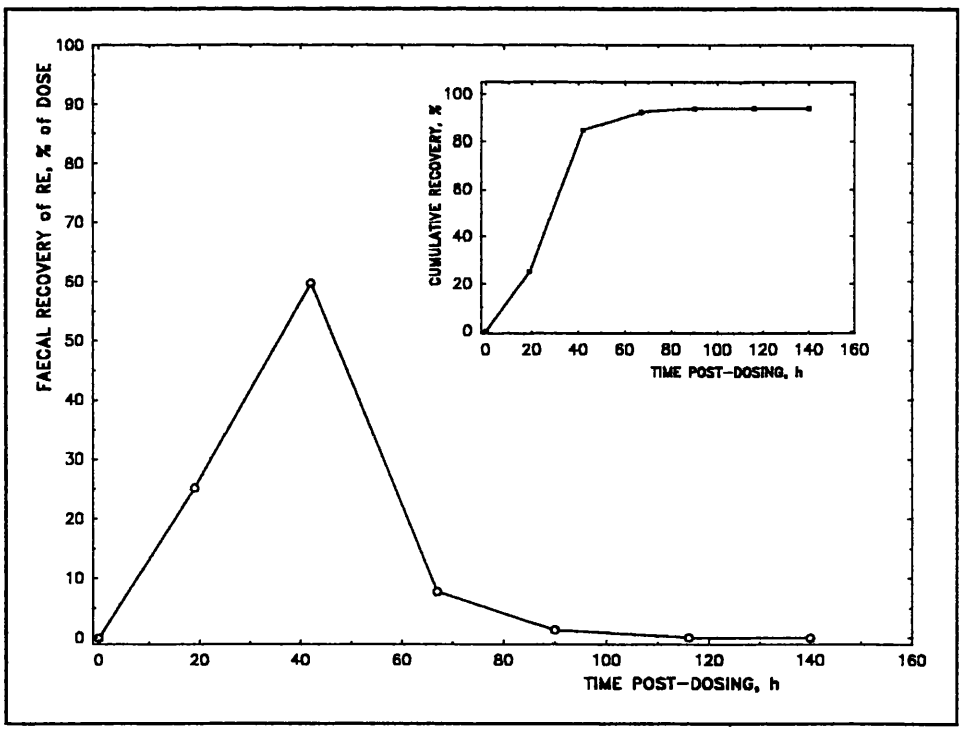


Figure-13.f: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B4

Table-34.g: Faecal recoveries of rare earths for subject B5

SUBJECT B5								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	14	2.83	3.02	3.08	3.40	3.00	3.07	3.07
2	39	74.70	77.70	75.94	74.91	75.32	75.71	78.78
3	62	7.87	8.26	8.99	8.90	8.68	8.56	87.34
4	86	0.45	0.51	0.57	0.48	0.50	0.50	87.84
5	98	0.26	0.22	0.27	0.23	0.24	0.24	88.08
6	113	-	0.02	0.01	0.01	0.01	0.01	88.09
7	135	-	0.01	0.004	0.004	0.004	0.003	88.09
Total,% ±3SD		86.11 ±2.66	89.74 ±0.83	88.86 ±4.16	87.93 ±3.04	87.75 ±0.92	88.09 ±1.31	88.09 ±1.31

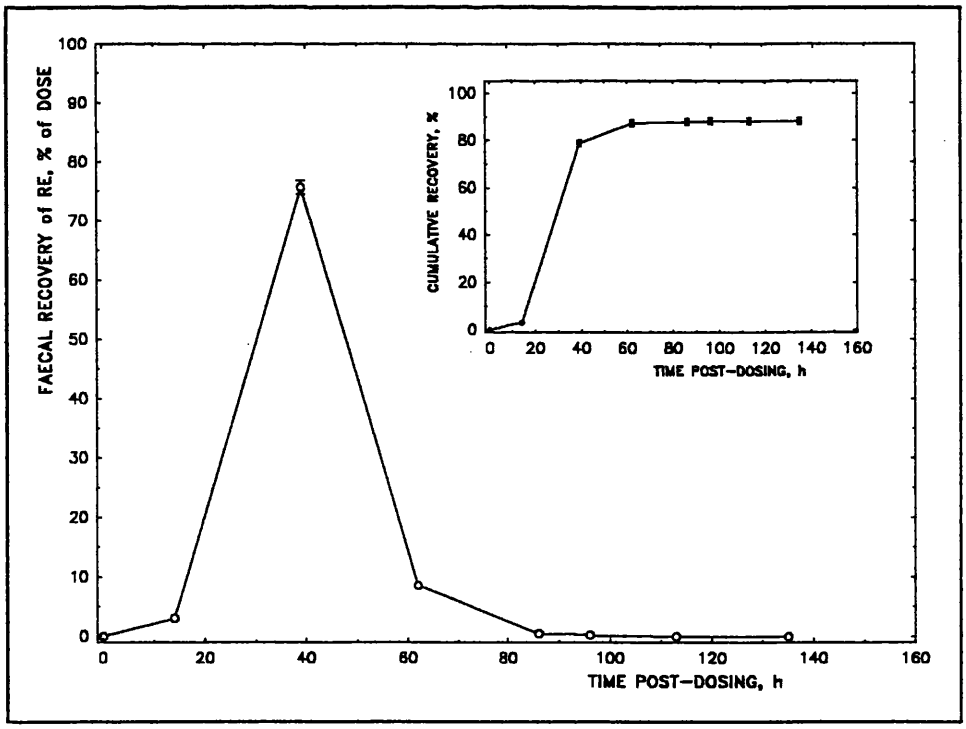


Figure-13.g: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B5

Table- 34.h: Faecal recoveries of rare earths for subject B6

SUBJECT B6								
Faecal sample No	Time post-dosing (h)	% of INGESTED RE DOSE IN STOOL						
		La	Sm	Eu	Tb	Yb	Mean	CUMULATIVE
1	7	0.10	0.02	0.01	0.01	0.01	0.03	0.03
2	28	41.73	39.53	40.93	41.16	41.16	40.91	40.94
3	54	29.49	27.81	28.47	28.77	27.96	28.50	69.44
4	73	19.95	20.39	20.67	19.77	20.11	20.18	89.62
5	99	2.76	2.03	1.80	2.44	1.89	2.18	91.80
6	124	2.34	1.38	1.31	1.63	1.51	1.63	93.43
7	144	1.20	0.85	0.78	1.08	1.25	1.03	94.46
Total,% ±3SD		97.59 ±2.03	92.01 ±0.66	93.97 ±3.67	94.86 ±2.31	93.89 ±2.19	94.46 ±1.28	94.46 ±1.28

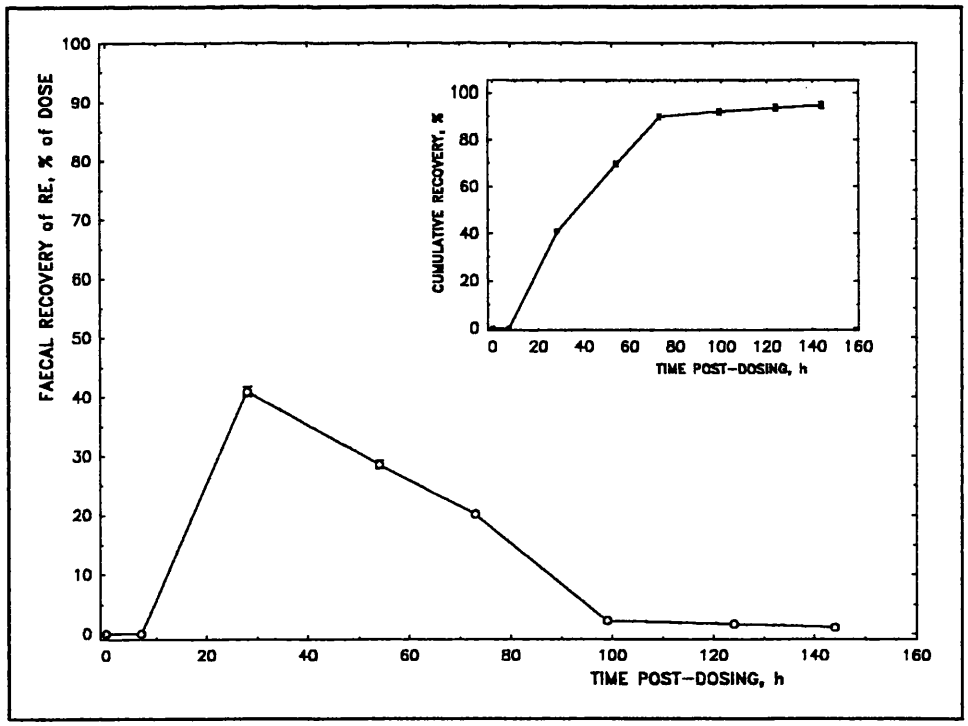


Figure-13.h: Faecal profile of recovery (mean of 5 rare earths) for individual samples and composite of sequential outputs for subject B6

II.2.6 DISCUSSION OF RARE EARTH RECOVERIES

In this investigation, the same 5 rare earths as used by Luckey *et al.* (1977) were used, but in lower quantities and with a single input, to ascertain of quantitative recovery of the doses could be achieved following administration as chloride solution. These studies were carried out to confirm the feasibility of the use of single inputs of markers in studies of trace element absorption with stable isotopic tracers.

II.2.6.1 DOSE AND PROCEDURE

Luckey *et al.* (1977) investigated the faecal recovery kinetics of rare earths on 3 subjects using single inputs of 5 rare earth oxides contained in capsules. This was repeated on the same subjects to check reproducibility and/or intra subject kinetic variations. Samples were collected the day before consumption and for the following week. Subsamples of a 0.7g of dried samples were analyzed by INAA as described by Gray and Vogt (1974).

Six subjects were involved in this study; 5 rare earth chloride solutions were used, and the amounts administered were calculated to be at a level which would enable detection of 0.1% of the dose in a fraction of output by using a high resolution 133 cc HPGe detector. The precision of measurement, particularly significant at lower concentrations, was improved by pre-concentration (ashing and pre-irradiation chemical separation). This improvement enabled investigation of rare earth recovery kinetics with 60, 5, 1.5, 7 and 6 times lower than those of used by Luckey *et al.* (1977) for La, Sm, Eu, Tb and Yb respectively. The procedure also enabled use of larger amounts of sample, which also allowed detection of lower concentrations (less than 0.1% of recoveries were measured with ±5% precision with a 99% confidence limits where 0.2 g of ash sample were used equivalent to 2 g of dry sample).

II.2.6.2 FAECAL RECOVERIES OF THE RARE EARTHS

Faecal recoveries obtained from this and those obtained by Luckey *et al.* (1977) are provided in **Table-35**. The recoveries of the rare earths consumed (mean of 8 applications, average of 5 rare earths for all subjects with 1SDM) were found to be 94.1±4.2% in a range of 88.1-100.2%. For 5 of the 8 recovery studies the results were approximately quantitative, 96.7±2.5% (mean of 5 applications with 1SDM, average of 5 rare earths) in a range of 94.2-100.2%, while the other three were 89.6±1.5% (mean with 1SDM, average of 5 rare earths) in a range of 88.1-91.1% because of losses with accidental rejection (reported by subject with 88.1% recovery), and with toilet papers which were not included in the sample collection.

The variability of recoveries after correction for the La background effect (specified in II.2.3) was found to be less than ±6%, and differences were found to be insignificant for all applications ($p>0.05$, paired t-test).

Luckey *et al.* (1977) obtained variations of inter-subject and inter-element recoveries, 5 of 7 applications and 3 of the 5 elements were significantly different from the mean of recoveries for the applications and for the elements. The results obtained from this study are more consistent (insignificant inter-subject and inter-element variation, $p>0.05$). It is apparent that the use of pre-concentration and chemical separation together with the improved detection limit provides more precise results. Comparison of the inter-element variations found in subjects A1-2 and B1-6 demonstrates the benefit of chemical separation.

Table-35: Faecal recoveries of rare earths

A. THIS INVESTIGATION							B. LUCKEY *et al.* (1977)						
	% of ingested RE dose in faeces							% of ingested RE dose in faeces					
Subject	La	Sm	Eu	Tb	Yb	Mean±SDM	Subject	La	Sm	Eu	Tb	Yb	Mean±SDM
A1	86.6	92.4	88.4	91.5	87.9	89.5±2.5	BV1	94.4	86.4	101.4	88.5	101.2	94.4±7.0
A2	102.7	95.1	99.1	96.9	96.4	98.0±3.0	BV2	86.8	92.2	99.8	91.9	104.2	95.0±6.9
							DH1	110.2	98.9	116.1	100.6	116.8	108.5±8.4
B1	98.1	96.8	95.4	97.1	95.8	96.7±1.1	DH2	88.3	77.5	83.5	82.0	82.3	82.7±3.9
B2	99.9	100.9	100.7	100.9	98.6	100.2±1.0	TL1	85.9	76.1	90.6	80.1	90.5	84.6±6.4
B3	90.5	92.7	92.7	91.4	90.5	91.1±1.1	TL2	85.8	81.9	91.5	84.4	93.3	87.4±4.8
B4	93.9	93.2	94.4	94.6	94.5	94.1±0.6	TB	88.9	79.7	86.7	92.0	89.9	87.4±4.7
B5	86.1	89.7	88.9	87.9	87.8	88.1±1.3							
B6	97.6	92.0	94.0	94.9	93.9	94.5±2.0							
							Mean	91.5	84.7	95.7	88.5	96.9	91.4±8.8
Mean	94.4	94.1	94.2	94.4	93.2	94.1±4.2	**±SDM**	±8.8	±8.4	±11.1	±7.1	±11.4	
±SDM	±6.2	±3.5	±4.3	±4.1	±4.0								

Reproducibility of recoveries was also examined for the investigation carried out by Luckey *et. al.* (1977) (applied to 3 subjects) and for this investigation (applied to 2 subjects). Good agreement is found for 2 of the 3 subjects investigated by Luckey (94.7±0.4% with 1SDM for subject BV and 86.0±2.0%, 1SDM for subject TL) whilst the reproducibility for subject DH significantly poor (95.6±18.2% with 1SDM). The reproducibilities for this study were found to be 93.1±5.1% and 99.5±2.1% (with 1SDM) for Subject-1 and 2 respectively. The variation of intra-subject recoveries can be explained by losses during sample collection (due to losses beyond control) in both cases. However the reproducibility of nearly quantitative recovery results, particularly for Subject-2 (99.5±2.1% with 1SDM) confirms the conclusion made for incomplete recoveries and indicates that the losses due to significant absorption is less probable.

II.2.6.3 FAECAL APPEARANCE PROFILE

Faecal recoveries of the rare earths for all 8 applications demonstrate that rare earth markers enable determination of the yield of sample collection. The profiles of faecal output as a function of time after consumption were considered, to identify the optimum time for limited sample collection when applied to determination of luminal disappearance with stable isotopic tracers. Parameters related to intestinal kinetics of the rare earths were also derived from the recoveries for possible applicability to measuring intestinal passage times of pharmaceuticals and to calculate exposure of dose where radioactive tracer(s) or marker(s) are used.

Recoveries of the rare earths with the first samples following consumption ranged between 0.03 and 43.5% (16.3±16.5% in 16±5 h with 1SDM). The major fraction of the doses were recovered in 2 consecutive samples (69.7±18.7% in 38±6 h with 1SDM). Recoveries were nearly quantitative with in summation of 3 faecal outputs (86.8±8.9% in 60±7 h with 1SDM) for all subjects, providing no significant difference ($p>0.05$; paired t-test) from the

following consecutive accumulations (92.4±3.7% in a period of 82±9 h with 1SDM). Recoveries obtained with 5 and 6 sample collection were nearly quantitative (93.6±4.2% in 115±16 h and 93.9±4.2 in 135±13 h respectively). Fractions of the total rare earth recoveries for 2-4 consecutive sample and time post-dosing are provided in **Table-36**. Cumulative recoveries (as % fraction of total recoveries) as a function of time post-dosing for 1-6 sample accumulation are depicted in **Figure-14** with standard deviations for both recoveries and time post dosing.

Table-36: Faecal recoveries of rare earthsin composites of 2-4 sequential outputs

	2 Samples		3 Samples		4 Samples	
SUBJECT	Time, h	Recovery,%*	Time, h	Recovery,%	Time, h	Recovery,%
AS1	41	82.1	48	98.0	67	99.3
AS2	35	45.1	60	80.1	78	96.4
BS1	44	96.6	67	98.9	92	99.2
BS2	30	65.3	55	94.8	77	98.0
BS3	42	83.6	66	96.9	90	99.3
BS4	42	90.2	67	98.4	90	99.9
BS5	39	89.5	62	99.1	86	99.7
BS6	28	42.8	54	72.7	73	93.9
MEAN±SDM	38±6	74.4±20.9	60±7	92.3±10.1	82±9	98.2±2.1

* Recoveries for the composites represent percentage of the total recoveries provided in **Table-34.a-h** for each subject

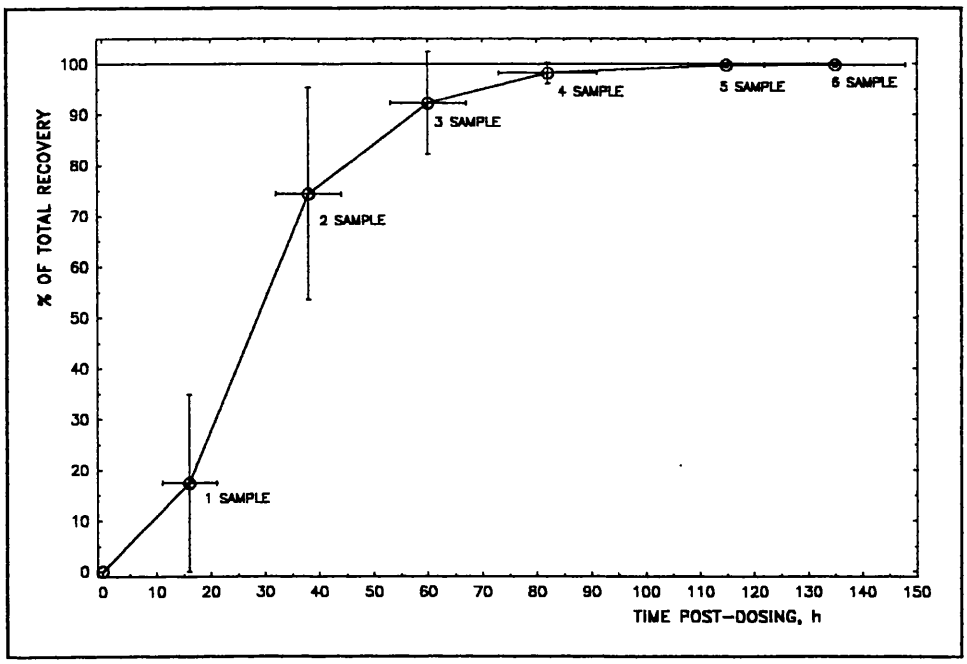


Figure-14: Cumulative faecal rare earth recoveries as a function of time post dosing (mean of 8 applications with ±1SDM)

Since the transit time of digesta can be estimated from fractional retention as a function of time (Kotb and Luckey, 1972, and Luckey, T.D., 1979), the retention patterns of the rare earths were assessed to provide further information on their intestinal kinetics. Retention patterns for each of the subjects were evaluated to determine the **first appearance time** (duration required for digesta to enter excretion compartment) by extrapolating to 100% retained, the **gastro intestinal half life** of the rare earths, and **quantitative excretion time**. The faecal excretion parameters derived from recoveries in 8 applications are provided in **Table-37** with standard deviations obtained in the linearity equation;

$$LogR\% = (a \pm SD_a)t + (b \pm SD_b)$$

and with regression coefficients. The retention pattern for subject B2 is provided in **Figure-15**.

Table-37: Parameters for intestinal kinetics of the rare earths

Subject	Time of first appearance,h	G.I. half- life,h	Time for quantitative excretion,h	r^2 (linearity)
AS1	16.4±4.2	23.3±6.0	61.9±16.0	0.920
AS2	26.0±4.3	36.0±6.0	92.7±15.5	0.947
BS1	11.2±2.5	18.5±4.1	60.0±13.3	0.968
BS2	10.8±0.9	22.4±2.0	87.8±7.7	0.990
BS3	12.1±0.5	23.2±0.9	86.1±3.5	0.998
BS4	15.5±1.0	23.7±1.6	70.0±4.7	0.996
BS5	11.7±1.5	20.1±2.7	67.3±9.6	0.976
BS6	11.1±1.5	28.1±3.6	122.5±15.7	0.956
Mean±SDM	14.3±5.2	24.4±5.5	81.0±20.9	

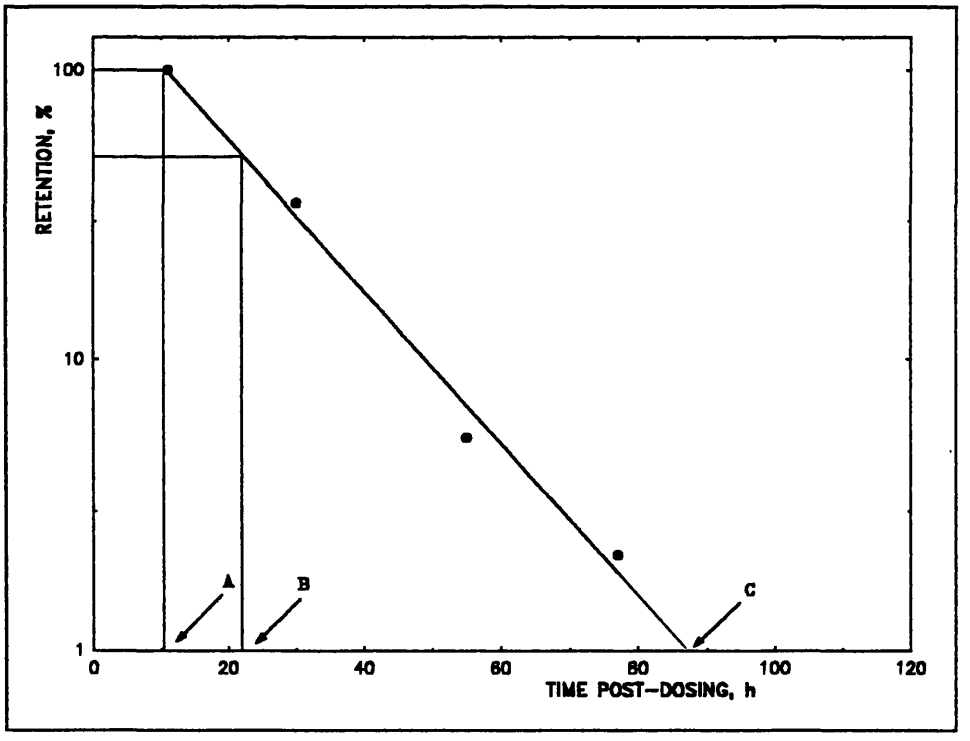


Figure-15: Recovery kinetics for the mean of 5 rare earths for subject B2 (A:time of first appearance time, B:gastro intestinal half-life, and C: time for quantitative excretion)

It can be seen from **Table-37** that both the first appearance time and gastro-intestinal half-life for subject A2 were found to be significantly difference from those found for the other subjects ($p>0.05$). This significance for subject A2 can be explained by the subject have slowest digesta flow rate comparing with the others.

The recoveries obtained from this investigation were also interpreted in terms of the rate of flow of the rare earths through the alimentary tract, as suggested by Luckey *et. al.* (1977) and Luckey (1979). Patterns of recovery rates obtained from recoveries of the rare earths for the same subjects (1 and 2) are provided in **Figure-16.a** and **b**. As can be seen from the figures, the patterns were reproducible for Subject1-1 (A1 and B1 in **Figure-16.a**) whilst those for Subject-2 (A2 and B2 in **Figure-16.b**) were different. These observed differences might be explained by individual idiosyncrasies or differences in composition of foods (e.g. fibre contents) consumed.

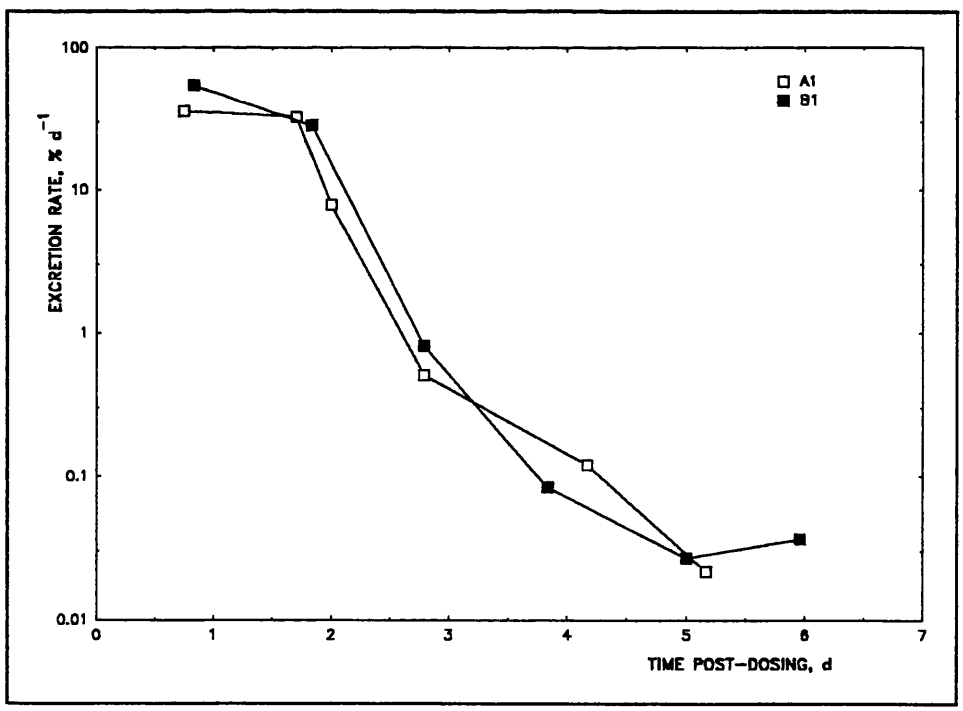


Figure-16.a: Faecal excretion rates of administered rare earths for Subject-1

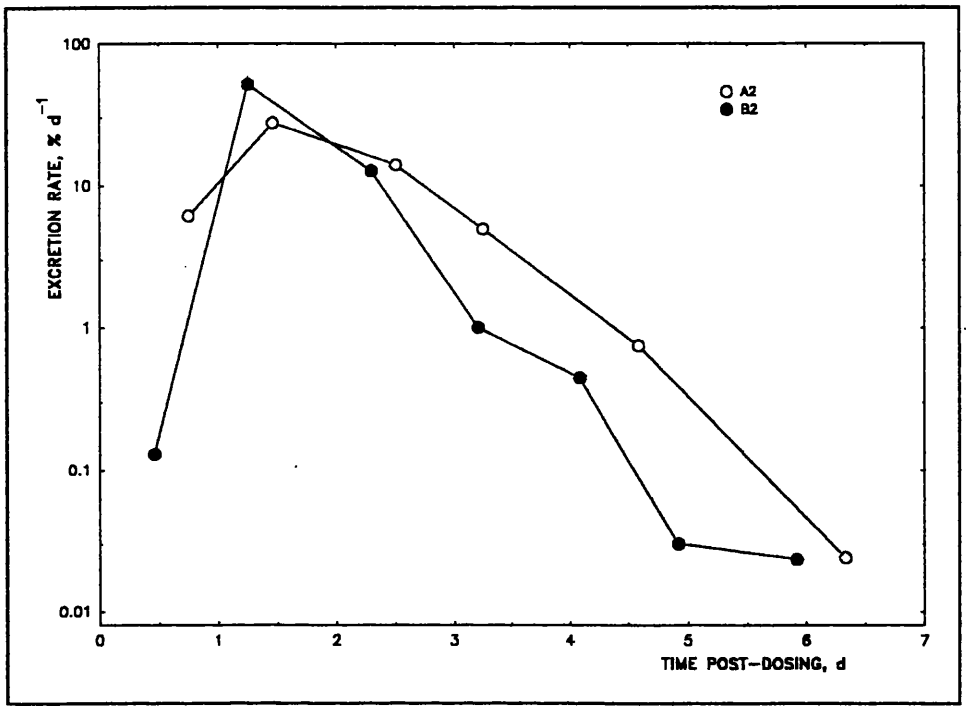


Figure-16.b: Faecal excretion rates of administered rare earths for Subject-2

Luckey (1979) found that significant quantities of digesta enter the excretion compartment in about 10 h (first appearance time) and the half-life of digesta is about 25 h. Luckey *et. al.* (1977) and Luckey (1979) showed that digesta flow rate into the excretion compartment vary between subjects and that this could not be related to physical characteristics, diet, sex, age, or life style but could be related to intestinal physiology. The results of this investigation for the kinetics of rare earth markers agree with those obtained by Luckey *et. al.* (1977).

II.2.6.4 CONCLUSION

The results of this investigation performed on 6 subjects with 8 applications show that the rare earths are nearly quantitatively recovered following consumption of a solution of rare earths chlorides with a meal. The determination of a significant effect of La baseline levels on recovery results

and agreement between marker elements recovered with each sample, even for low recoveries, might be a further evidence that losses are due to incomplete collection (losses particularly with toilet papers) where recoveries are less than 100%.

The mean of total recoveries (as an average of 5 elements) was found to be 94.1±4.2% with SDM (in a range of 88.1-100.9%) with better reproducibility than those found by Luckey *et al.* (1977) for the same rare earths for 7 applications (91.4±8.8% with SDM in a range of 82.7-108.5%). Good agreement is obtained when the results of this investigation are compared with those of Hayes *et al.* (1964) (96±4% with SDM in a range of 91-104% for 22 applications) where the recoveries determined by the use radioactive La-140.

Patterns of recoveries of the rare earths with individual samples have a similar configuration for each of the applications. In all of the subjects, the first appearance was detected 24 h after consumption. The patterns showed a maximum recovery in 40 h and nearly quantitative excretion in 72 h. The amounts appearing by 120 h were approximately 0.1%. A major fraction of the dose, as percentage of total recoveries, was recovered with the first 2 samples (74±21% with SDM in a range of 43-97% for 8 applications). The mean recovery with the first 3 samples was nearly quantitative (92±10% in a range of 73-99%) providing no significant difference from 100%.

III. MEASUREMENT OF LUMINAL DISAPPEARANCE USING STABLE ISOTOPIC TRACERS AND RARE EARTH MARKERS

Having investigated the faecal recovery and recovery rates of potential rare earth markers, with establishment of suitable analytical procedures and demonstration of near quantitative recovery, their application in determination of the luminal disappearance (intestinal uptake) of trace elements by using enriched stable isotopic tracers was investigated. Faecal recovery profiles of rare earth marker and of the excreted fractions of isotopic tracers were compared to investigate the applicability of the rare earth marker approach in trace element absorption studies

To demonstrate the applicability of rare earths for determination of the luminal disappearance, the luminal disappearance of iron from 2 modes of sequentially administered intakes, a standard solution and a wheat flour based meal (Farina), each being labelled with a stable isotope of iron and a rare earth was measured. The inclusion of an isotopic tracer of zinc with the Farina meal enabled consideration of measurement of uptakes of two elements. Attention was given to faecal recovery profiles of the rare earths markers and the excreted fractions of the isotopic tracers.

III.1 STUDY DESIGN AND PRE- AND POST-IRRADIATION SEPARATION FOR DETERMINATION OF ISOTOPIC TRACERS

III.1.1 CHOICE OF ENRICHED STABLE ISOTOPES AND DETERMINATION OF REQUIRED INPUTS

For measurement of the luminal disappearance of iron and zinc , selection of appropriate stable isotopes and the required inputs are determined by the factors:

1.Range of luminal disappearance

Luminal disappearance varies widely, depending on status of subjects, mode of intake, oxidation state of the elements, and constituents of diet. The following examples illustrate these variations for iron and zinc,

Luminal disappearance of ferrous iron, from a solution containing 1 mg of Fe was found to be 37.6% (SD 16.8) with a range of 3.6-60.7% for 15 male subjects (Marx, 1979). In 96 normal men, Magnusson *et al.* (1981) found the mean absorption to be 21.6% (SD 11.3), with a significant negative correlation between absorption and serum ferritin level. Uptake by 2 male 1 female subjects from a meal containing 3.2 mg of ferric iron was 14.5±9.4% (range of 5-23.9%), Jasani *et al.* (1971), whilst the uptakes by 8 female subjects from a cereal-based diet containing 14 mg d^{-1} iron was 9.0% (Turnlund *et al.*, 1990).

The luminal disappearance of zinc however has shown less variability compared. Uptake by 2 male and 2 female subjects, from a solution containing 2 mg Zn was 61±3.0% in a range of 57-65% (Wastney *et al.* 1991), whilst the uptake by 9 male subjects, from a breakfast diet containing 2 mg of zinc was 38.9% (SD 9.8) in a range of 27.5-54.2%, Couzy *et al.* (1993).

2. Natural abundance of the enriching isotope

The natural abundance of the enriching isotope is a major factor influencing the choice of dose. The total dose of an element containing enriched isotopic tracer should be similar to the normal daily intake, and should not exceed the physiologically required dose, unless so required for specific investigation. By considering this, the amounts of iron and zinc for a single input administered with breakfast are considered to be approximately 3 mg since the average contents of food obtained for daily consumption in the U.K. are 10.9 mg for iron and 9-12 mg for zinc (Department of Health, 1991). The intake for measurement of iron absorption from a standard solution has been recommended to be 3 mg by WHO (Magnusson, *et al.*, 1981).

It is evident that enrichment of the least abundant isotope of the element of interest results in use of the lowest amount of isotopic tracer for labelling the dose. This choice should lead to better accuracy compared with that obtained from the same quantity of a more abundant isotopic tracer. A disadvantage of using enriched isotopic tracers of the lowest abundant isotope is the high cost.

3. Degree of isotopic enrichment

The degree of isotopic enrichment required to enable measurement of luminal disappearance from a dose depends on the precision of the analytical technique used.

The required doses of pure isotopes for iron and zinc can be calculated with the following assumptions, with regard to the above factors.

a. The luminal disappearance of the tracer from the dose is 60% for iron and 50% for zinc,

b. A week after administration, measurement of 1% of the excreted dose is achievable in a single stool sample,

c. The analytical technique used is capable of measuring 5% enrichment over natural levels of the isotopes,

d. For adult subjects, the natural levels of the elements in daily faecal output are about 16.8 mg for iron and 13.0 mg for zinc (Mason *et al.*, 1990).

The calculated inputs to meet these criteria and the cost and enrichment of commercially available enriched stable isotopes are provided in **Table-38**.

Table-38: Input of stable isotopes required to enable measurement of 1% of excreted dose (when 60% of iron and 50% of zinc are absorbed)

Isotope (Mass no)	Natural abundance[1] (Atom %)	Required dose (mg of isotope)	Available[2] enrichment (Atom %)	Cost[2] (£ mg^{-1})
Fe				
54	5.8	12.2	95-97	10-20
56	91.7	NA[3]	-	-
57	2.2	4.6	80-97	10-28
58	0.28	0.6	65-93	100-300
Zn				
64	48.6	NA	-	-
66	27.9	NA	-	-
67	4.1	5.3	76-94	12-28
68	18.8	24.4	37-99	2-6
70	0.6	0.8	65-88	80-300

1. Commission atomic weights and isotopic abundances (1991)

2.Aggett and Whitley (1994)

3. Not applicable since the required input exceeds physiological requirements

It can be seen that the use of ^{54}Fe or ^{68}Zn is not practical for a single dose where the daily requirement and intended detection in a single stool sample obtained a week after administration are considered. For these isotopes, lower doses can be used if the enrichment is measured in a total faecal pool, where enrichment over natural levels of the isotopes can be much more than 5%. Such an approach has been followed by Turnlund *et al.* (1990) with inputs of 1 mg ^{54}Fe, by Sian *et al.* (1993) with a range of 3-10 mg of ^{67}Zn and ^{68}Zn and by English *et al.* (1989) with 4 mg of ^{68}Zn.

III.1.1.1 DETERMINATION OF REQUIRED DOSES

By the use of the least abundant isotopes (^{58}Fe, ^{57}Fe and ^{70}Zn) as tracers, the required doses to investigate luminal disappearance were determined by considering the analytical technique used to measure their enrichment. The

following assumptions were made in calculating the required amount of enriched stable isotopes.

1) Fractional luminal disappearance is 10% for iron and 50% for zinc when they are administered with a meal, and 60% for iron sulphate (+2 oxidation state) when it is given with a solution containing ascorbic acid.

2) 1% of a nonretained fraction of the isotopic doses is measurable in an ashed sample obtained 5 day after administration.

3) The tracer isotopes for the elements administered with meal, ^{58}Fe and ^{70}Zn, are determined by NAA. The isotope for iron given with the solution is ^{57}Fe and is determined by ICP-MS.

4) The solution and meal are marked with Sm and Yb, which provide the lowest limit of determination by NAA, of the rare earths considered.

By taking account of nuclear parameters and detection efficiency, it can be calculated that;

a) When approximately 1 µg of ^{58}Fe is irradiated for 6 h in a flux of $3.6x10^{12}$ n cm^{-2} s^{-1} and counted on a 130 cc HPGe detector (~2% efficiency for characteristic γ-ray of Fe-59 at 1099 keV), it provides 10000 counts, producing 1% precision.

b) When approximately 0.5 µg ^{70}Zn is irradiated 1 h in the pneumatic transfer system (in a flux of $2.4x10^{12}$ n cm^{-2} s^{-1}) and counted on the detector (~3% efficiency for characteristic γ-ray of Zn-71m at 386 keV) on contact, it can be determined with 2% precision.

c) 3% over the natural level of ^{57}Fe can be determined by ICP-MS where the precision for measurement of the isotope is 1%.

With the above assumptions, the required doses are 1 mg for each of ^{58}Fe and ^{70}Zn, assuming 1 µg of ^{58}Fe and 0.5 µg ^{70}Zn are measured in 10% of the total ash weight of a single sample. The dose for ^{57}Fe is 2 mg.

d) The required dose for Sm and Yb are 1 and 5 mg respectively where 0.1% of the dose in 10% of ashed faecal samples is determined with 1% precision by CNAA (Section II.1.1). The use of 1 mg of Yb could be sufficient where the samples are counted for a longer period than 1 h on contact geometry with higher detection efficiency.

III.1.1.2 EFFECT OF THE TIME INTERVAL BETWEEN SEQUENTIAL ADMINISTRATIONS

The process used to enrich a specified isotope of low abundance can enrich other similar isotopes with low abundance (*e.g.* 95% ^{57}Fe enriched iron could contain 2% ^{58}Fe for which the natural abundance is 0.28%). Attention should therefore be given to time interval required between the two administrations to minimize the possible effects of isotopic overlap in the outputs.

The isotopic and elemental composition of each of sample collection following both administrations was calculated, with following assumptions;

1) The standard solution is administered first.

2) The recovery of excreted dose is completed within 5 days following consumption. The recovery kinetics of the isotopic tracer is the same as that of the rare earth marker. The fractional distribution of the marker in 5 consecutive collections after dose consumption, based on results obtained for rare earth recoveries are 0.2, 0.6, 0.15, 0.03 and 0.02.

3) The elemental composition of a regular daily output, based on results provided by Mason *et al.* (1990) is constant and contains 16.8 mg of iron and 13 mg of zinc.

4) The elemental and isotopic composition of the mode of intakes are considered to be;

a) A standard drink containing 3 mg of iron, including 2 mg of ^{57}Fe and 0.02 mg of ^{58}Fe (enrichment in ^{57}Fe enriched Fe),

b) A standard meal containing 3 mg of iron, including 1 mg of ^{58}Fe and 0.2 mg of ^{57}Fe (enrichment in ^{58}Fe enriched Fe). The meal also contains 3 mg of zinc including of 1 mg of ^{70}Zn,

c) The drink and the meal contain 1 mg of Sm and 1 mg of Yb respectively.

The distribution of the isotopes and elements for each of the individual samples for ^{57}Fe are provided in **Tables-39** and **40**, for ^{58}Fe in **Tables-41** and **42** for intakes consumed at 1 and 3 day intervals, and in **Table-43** for ^{70}Zn for 3 days interval. The isotopic distribution in the samples are also provided with the figures illustrating the cases for ^{57}Fe, as example (**Figures-17** and **18**). The abbreviations for the tables are as follows;

M is fractional marker recovery

E_s and E_m are elemental contribution from the standard drink and meal respectively

E_t is total element (consisting of E_s , E_m and the natural level of the element in daily faecal output)

I_s and I_m are isotopic contribution from the standard drink and meal

I_b is isotopic baseline containing the natural level of the isotope in daily faecal output and the isotopic contribution from the doses in natural composition

I_t is total isotope in sample

Table-39: Elemental iron and ^{57}Fe composition of faecal samples when the standard drink and meal are given at a time interval of 1 day (drink enriched in ^{57}Fe)

		Fe content, µg			^{57}Fe content, µg			
DAY	M	E_s	E_m	E_t	I_s	I_m	I_b	I_t
0	0	0	0	16800	0	0	369.6	369.6
1	0.2	240	0	17040	160	0	371.4	531.4
2	0.6	720	540	18060	480	36	374.9	890.9
3	0.15	180	1620	18600	120	108	370.9	598.9
4	0.03	36	405	17241	24	27	369.9	420.9
5	0.02	24	81	16905	16	5.4	369.8	391.2
6	0	0	54	16854	0	3.6	369.6	373.2

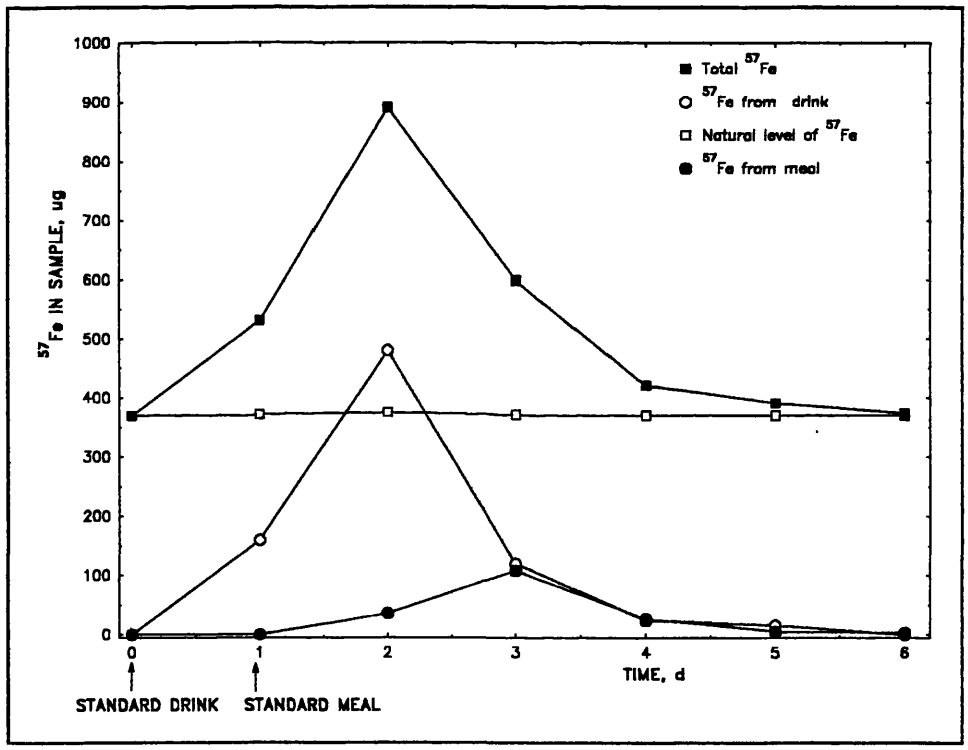


Figure-17: Calculated ^{57}Fe isotopic distribution in faecal samples when the standard drink and meal are administered at a time interval of 1 day

Table-40: Elemental and ^{57}Fe composition of faecal samples when the standard drink and meal are given at a time interval of 3 days (drink enriched in ^{57}Fe)

		Fe content, µg			^{57}Fe content, µg			
DAY	M	E_s	E_m	E_t	I_s	I_m	I_b	I_t
0	0	0	0	16800	0	0	369.6	369.6
1	0.2	240	0	17040	160	0	371.4	531.4
2	0.6	720	0	17520	480	0	374.9	854.9
3	0.15	180	0	16980	120	0	370.9	490.9
4	0.03	36	540	17376	24	36	369.9	429.9
5	0.02	24	1620	18444	16	108	369.8	493.8
6	0	0	405	17205	0	27	369.6	396.6
7	0	0	81	16881	0	5.4	369.6	375.0
8	0	0	54	16854	0	3.6	369.6	373.2

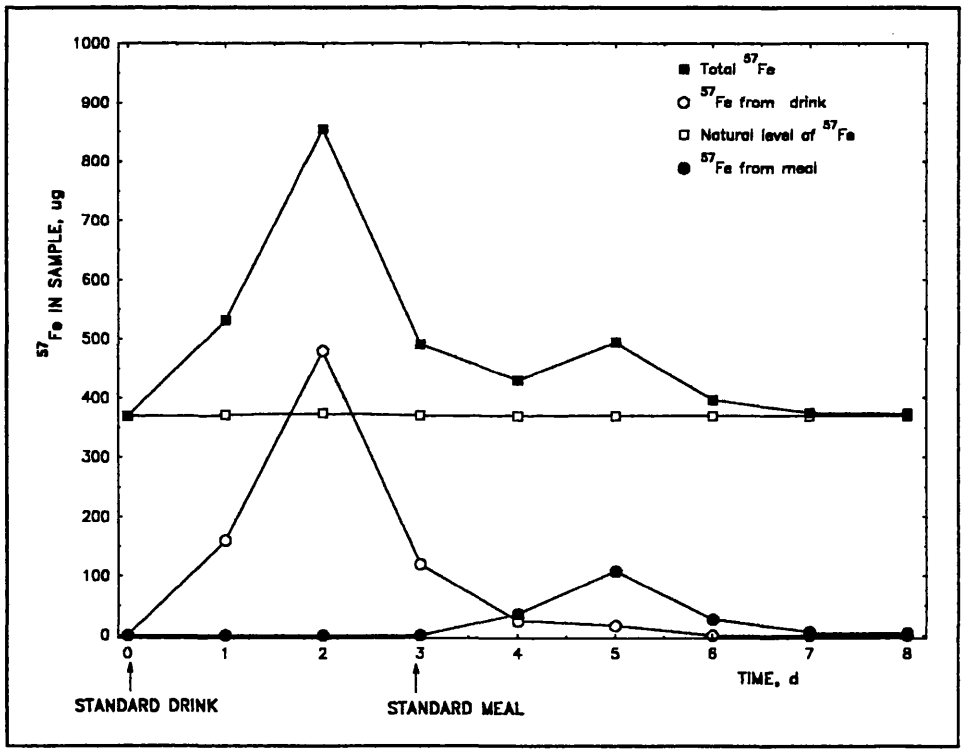


Figure-18: Calculated ^{57}Fe isotopic distribution in faecal samples when the standard drink and meal are administered at a time interval of 3 days

Table-41: Elemental iron ^{58}Fe composition of faecal samples when the standard drink and meal are given at a time interval of 1 day (meal enriched in ^{58}Fe)

		Fe content, µg			^{58}Fe content, µg			
DAY	M	E_m	E_s	E_t	I_m	I_s	I_b	I_t
0	0	0	0	16800	0	0	47	47
1	0	0	240	17040	0	1.6	47.0	48.6
2	0.2	540	720	18060	180	4.8	48.1	232.9
3	0.6	1620	180	18600	540	1.2	50.1	591.3
4	0.15	405	36	17241	135	0.2	47.8	183.0
5	0.03	81	24	16905	27	0.2	47.2	74.4
6	0.02	54	0	16854	18	0	47.1	65.1
7	0	0	0	16800	0	0	47.0	47.0

Table-42: Elemental iron ^{58}Fe composition of faecal samples when the standard drink and meal are given at a time interval of 3 day (meal enriched in ^{58}Fe)

		Fe content, µg			^{58}Fe content, µg			
DAY	M	E_m	E_s	E_t	I_m	I_s	I_b	I_t
0	0	0	0	16800	0	0	47.0	47.0
1	0	0	240	17040	0	1.6	47.0	48.6
2	0	0	720	17520	0	4.8	47.0	51.8
3	0	0	180	16980	0	1.2	47.0	48.2
4	0.2	540	36	17376	180	0.2	48.1	228.3
5	0.6	1620	24	18444	540	0.2	50.1	590.2
6	0.15	405	0	17205	135	0	47.8	182.8
7	0.03	81	0	16881	27	0	47.2	74.2
8	0.02	54	0	16854	18	0	47.1	65.1
9	0	0	0	16800	0	0	47.0	47.0

Table-43: Elemental and ^{70}Zn composition of faecal samples when the standard drink and meal are given at a time interval of 3 days

		Zn content, µg		^{70}Zn content, µg		
DAY	M	E_m	E_t	I_m	I_b	I_t
0	0	0	13000	0	78.0	78.0
1	0	0	13000	0	78.0	78.0
2	0	0	13000	0	78.0	78.0
3	0	0	13000	0	78.0	78.0
4	0.2	300	13300	100	79.2	179.2
5	0.6	900	13900	300	81.6	381.6
6	0.15	225	13225	75	78.9	153.9
7	0.03	45	13045	15	78.2	93.2
8	0.02	30.0	13030	10	78.1	88.1
9	0	0	13000	0	78.0	78.0

The luminal disappearance of tracers for the cases given in the above tables were calculated using **Equation-5.b** provided in section I.5. The calculated fractional luminal disappearances for each of the single samples, and for summation of sequential collections are provided in **Table-44**.

Table-44: Calculated fractional luminal disappearance of ^{57}Fe (consumed with a standard solution) and of ^{58}Fe and ^{70}Zn (consumed with a meal) for single (F_s) and cumulative (F_c) faecal collections, for the drink and the meal given at times interval of 1 and 3 days

	Luminal disappearance of tracer, % of dose									
	^{57}Fe				^{58}Fe%				^{70}Zn, %	
	1 d interval		3 d interval		1 d interval		3 d interval		3 d interval	
Day	**F_s**	**F_c**	**F_s**	**F_c**	**F_s**	**F_c**	**F_s**	**F_c**	**F_s**	**F_c**
0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	60.0	60.0	60.0	60.0	NA	NA	NA	NA	NA	NA
2	57.9	58.5	60.0	60.0	8.6	8.1	NA	NA	NA	NA
3	35.3	54.8	60:0	60.0	9.9	9.4	NA	NA	NA	NA
4	29.2	54.0	18.9	58.7	9.9	9.5	9.9	9.9	50.0	50.0
5	50.7	54.0	-125	55.1	9.7	9.5	10.0	10.0	50.0	50.0
6	NA	53.8	NA	54.1	10.0	9.5	10.0	10.0	50.0	50.0
7	NA	NA	NA	54.1	NA	9.5	10.0	10.0	50.0	50.0
8	NA	NA	NA	54.0	NA	NA	10.0	10.0	50.0	50.0
9	NA	NA	NA	53.8	NA	NA	NA	10.0	NA	50.0
Expected F, %			**60.0**			**10.0**				**50.0**

NA: Not applicable

As can be seen from the calculated luminal uptake of iron, overlap between the the isotopic contents of the two inputs significantly effects calculation of iron uptake, when the inputs are consumed at an interval of 1 day. This effect is approximately 25% when luminal disappearance of iron from the standard drink is measured in a single sample 3 days after consumption of the drink (15% recovery of marker). The luminal disappearance from the meal deviates by 14% from the expected value 1 day after consumption (marker recovery 20%). Administration of intakes with a 3 day interval provides insignificant isotopic overlap between the two measurements for the first 3 samples from which 95% of marker is recovered. Possible deviations from the expected luminal disappearance, as found for ^{57}Fe for the fourth and fifth samples after consumption of the drink, when there is a small effect of the meal input should be taken into account. Such overlap can be corrected by using marker recovery with those particular faecal samples. The calculated levels of isotopic

tracers and Sm (with the standard drink) and Yb (with the standard meal) markers in samples collected when there is such a time interval between administrations is provided in **Figure-19**.

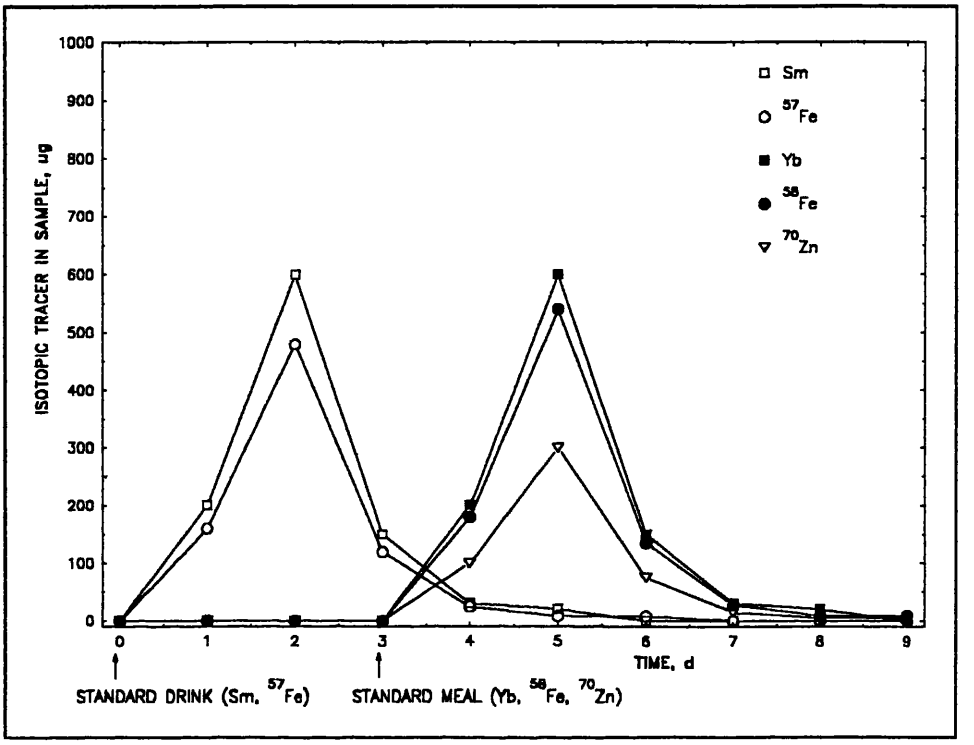


Figure-19: Calculated isotopic tracer and marker distributions in faecal samples when the intakes are administered at a 3 day interval

III.1.2 PRE-IRRADIATION SEPARATION OF IRON AND ZINC

For determination of isotopes in faecal samples by NAA, pre-irradiation chemical separation enables reduction of the Compton background (improving precision) and reduction of radiation exposure. This approach is also advantageous when determining elements which require post-irradiation separation (e.g Zn-71m, $t_{1/2}$=4.1 h) since it reduces the time required to allow for decay of major interferences (Na-24, K-42, Mn-56). Nuclear characteristics of the tracer isotopes, ^{58}Fe and ^{70}Zn, the selected rare earths markers and of potentially interfering isotopes, together with estimated induced activities, when 0.1 g of ashed faecal sample is irradiated are provided in **Table-45**. This shows the benefit of pre- and/or post-irradiation separation when the isotopes of interest are determined in human faecal samples.

Table-45: Properties of selected tracers, markers and potential interferences (Browne *et al.*, 1978)

Isotope	Abundance,%[1]	$\sigma_{n,\gamma}$, b	Radioisotope	$t_{1/2}$	γ-Energy, keV	Induced act[2]. kBq
Tracer isotopes						
^{58}Fe	0.28	1.1	Fe-59	45 d	1099	1.48
^{70}Zn	0.6	0.008	Zn-71	4 h	386	0.33
Rare earth markers[3]						
^{174}Yb	31.8	65	Yb-175	4.2 d	396	259
^{152}Sm	26.7	206	Sm-153	46.7 h	103	1184
Major interferences						
^{23}Na	100	0.1	Na-24	15 h	1369	2220
^{55}Mn	100	13.3	Mn-56	2.6 h	847	2220
^{37}Cl	24.2	0.4	Cl-38	37.2 m	1642	1480
^{41}K	6.73	1.5	K-42	12.4 h	1525	85.1
^{63}Cu	69.2	4.4	Cu-64	12.7 h	1346	0.11
^{48}Ca	0.003	0.7	Ca-47	4.6 d	1297	29.6

1.Commission on atomic weights and isotopic abundances (1991)

2.Activity produced when 0.1 g of ashed faecal sample is irradiated in the flux for 6h

3.Activities produced for 10 µg of Yb and Sm

Chemical separation of the elements also reduces isobaric interferences in their determination by mass spectrometry. In ICP-MS, serious interference problems occur due to the formation of polyatomic ions resulting from combination of analyte constituents and plasma gases. This effect which is particularly serious for isotopes with atomic number between 40-80 (Jarvis *et al.* 1992, pp 129) is also minimized by chemical isolation of the element of interest.

The following investigations were carried out to establish a separation procedure for iron and zinc:

1.Pre-irradiation separation procedure: This procedure reduces radiation exposure and minimizes the time required for decay of major interfering radionuclides (Na-24, K-42, Mn-56) in application of NAA. The procedure also reduces the matrix effect causing isobaric and polyatomic interferences in isotopic quantification by ICP-MS *e.g.* ^{58}Fe and ^{58}Ni.

2.Post-irradiation separation procedure: This procedure is applied to reduce Compton contribution from Cl-38 and Mn-56 in determination of Zn-71m.

Ion exchange separation of iron and zinc is more convenient than pre- and/or post-irradiation procedures described previously by Janghorbani *et al.*, (1980) and Ting *et al.* (1982). They have described separation of iron or zinc from dissolved ashed faecal samples by precipitation with ammonium pyrrolidinedithiocarbamate (APDC) and followed this with post-irradiation procedures of co-precipitation and solvent extraction. The use of radioactive Zn-65 as yield indicator in trace amounts enables determination of the yield in the zinc separation procedure, whilst there is no such practical availability for iron since Fe-59 or Fe-55 requires different detection system where ^{58}Fe or ^{54}Fe is intended to be used as isotopic tracer. This procedure is inconvenient since each extra separation step can cause unknown losses and require additional yield measurements. The application of a separate chemical

separation for each of the elements is also impractical since it requires different procedures and reagents *e.g.* ether extraction for iron, and ion exchange column for zinc purification (Turnlund et al., 1982). Considering the disadvantages of the approaches given above, an ion exchange separation procedure which enabled separation of both elements was investigated.

Investigations of the anion exchange distribution coefficients for all elements in HCl show that none of the alkaline and rare earth elements are adsorbed by the anion exchange resin (Saito, 1984). The anion exchange separation procedure applied in this study has been modified for iron and zinc from the procedure used by Kraus and Moore (1953) for the transition elements, Mn to Zn.

Pre-irradiation separation of iron and zinc:

The anion exchange procedure was investigated by separation of radioactive tracers for Fe and Zn from spiked faecal samples.

Tracers solutions were prepared from Fe which had been irradiated for 20 h in 2.7×10^{12} n $cm^{-2}s^{-1}$ flux (in the thermal column) and from Zn which had been irradiated for 3 h in 4.2×10^{12} n $cm^{-2}s^{-1}$ flux (in the CVS), to contain 60 kBq mL^{-1} of Fe-59 and 20 kBq mL^{-1} of Zn-69m, and 1kBq mL^{-1} of Zn-65.

Duplicates of 0.2 g of ashed faecal samples obtained from the rare earth recovery investigation, and containing rare earth markers were spiked with 200 µL of each of the tracer solutions. The samples were then dissolved in 10 mL aqua regia with dropwise addition of 5 mL of H_2O_2 to promote the oxidation of remaining organic matter, and evaporated to dryness.

1) 100-200 mesh AG 1X-8 anion exchange resin (Bio-Rad Laboratories) was soaked in distilled water overnight. 10 mL of the resin was packed in two columns of 1 cm radius and equilibrated with 20 mL of 9 M HCl,

2) The acid digested samples were loaded on to the columns with 3x2 mL of 9 M HCl,

3) The columns were washed with 20 mL of 4 M HCl to remove alkali metals and Ni, Mn and Co,

4) The iron contents of the columns were eluted with sequential volumes of 0.5 M HCl,

5) The zinc contents of the columns were eluted with sequential volumes of 0.005 M HCl.

6) The resin contents of the columns were transferred to counting bottles to investigate if any tracers remained in the resin.

The flow rate for all eluent were in 1-1.5 mL min^{-1}. All fractions of eluent were collected in polythene counting bottles and made up to 20 mL. All eluent and the tracer standard (200 µL fractions of the standard solutions diluted to 20 mL) were counted on a 130 cc HPGe detector at contact geometry and the contents of the eluent were determined by comparing the activities of eluent with those of standard.

Results and conclusion

Besides the major activity of Fe-59 in the iron standard, Co-60 ($t_{1/2}$=5.2 y) and Mn-54 ($t_{1/2}$=291 d) were also found. These two radionuclides were used to examine the effectiveness of the separation procedure. The only activities identified in the Zn standard were Zn-69m, $t_{1/2}$=14 h and Zn-65, $t_{1/2}$=245 d.

The distribution of iron and zinc Recoveries in fractions of eluent are provided in **Table-46**.

Table-46: Recoveries of iron and zinc in fractions of eluent, determined with Fe-59 and Zn-69m

	Fe recovery, %±SD			Zn recovery, %±SD	
Eluent	Column I	Column II	Eluent	Column I	Column II
6 mL 9 M HCl					
	ND	ND		ND	ND
20 mL 4 M HCl					
	1.80±0.1	1.30±0.1		ND	ND
0.5 M HCl			**0.005 M HCl**		
I. 10 mL	63.8±0.5	68.1±0.5	I. 5 mL	ND	1.90±0.3
II. 10 mL	29.9±0.3	27.1±0.2	II. 5 mL	34.2±0.7	22.8±0.7
III. 5 mL	2.59±0.1	2.10±0.1	III. 10 mL	59.3±1.1	72.9±1.4
IV. 5 mL	0.63±0.03	0.49±0.03	IV. 10 mL	3.10±0.2	1.23±0.1
V. 10 mL	0.29±0.03	0.25±0.03	V. 10 mL	0.65±0.2	1.83±0.5
VI. 10 mL	0.10±0.02	0.10±0.02	VI. 20 mL	1.80±0.5	0.80±0.2
Total rec, %	97.3±0.6	98.1±0.5	**Total rec, %**	99.0±1.4	101.5±1.7
Resin	ND	ND		1.88±0.2	ND

In counting of the eluent, it was found that;

1) The impurities in the iron tracer standard (Mn, Co) were totally removed with 9M and 4 M HCl eluent,

2) Recoveries of iron with 50 mL of 0.5 M HCl were 97.3±0.6% and 98.1±0.5% (SD indicates error of the counts),

3) Whilst recoveries of zinc with 60 mL of 0.005 M HCl were 99.0±1.4% and 101.5±1.7%, an increased elution volume was considered since traces of zinc were detected on one of the columns,

4) The amount of iron eluted with 20 mL of 4M eluent (to remove impurities) could be reduced by using a smaller volume at this molarity.

The separation was repeated with 4 columns, with a smaller volume of 4 M HCl eluent to further optimise volumes (10 mL) and minimise losses, and with a larger volume of eluent for zinc (70 mL).

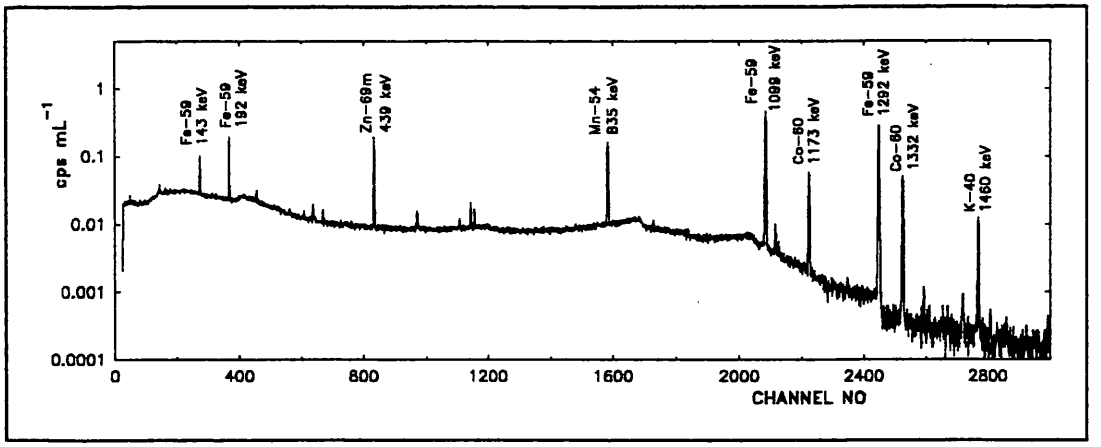
The recoveries of iron and zinc showed that the impurities were effectively removed with the loading solution and with 10 mL of 4M HCl. Recoveries

were quantitative with the volumes of eluent used (**Table-47**). The effectiveness of the procedure also can also be seen from the spectra provided in **Figure-20**, showing removal of the impurities and separation of Fe and Zn.

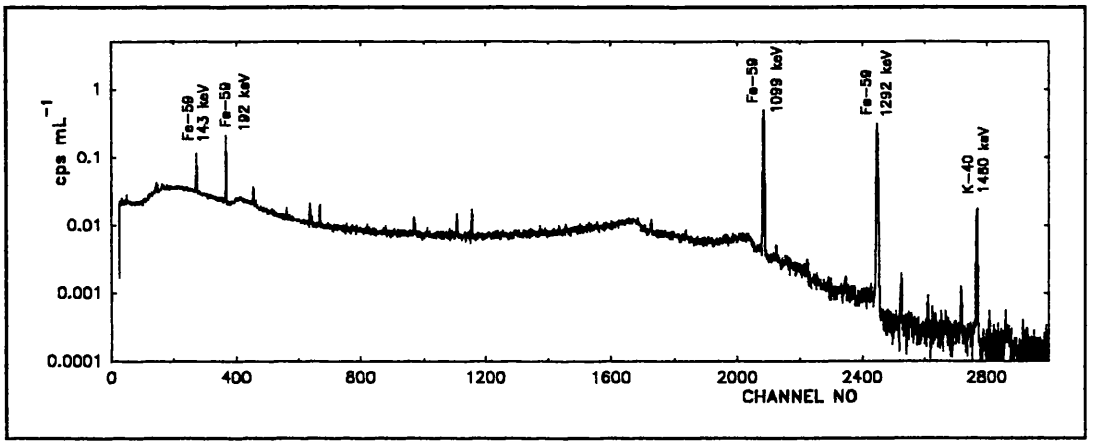
Table-47: Iron and zinc recovery results for 4 columns

Eluent, HCl	Col. I	Col. II	Col.III	Col. IV
Fe recovery, %±1SD				
50 mL 0.5 M	100.9±0.4	101.8±0.4	102.8±0.9	102.1±0.9
70 mL 0.005 M	1.2±0.1	1.1±0.1	0.9±0.06	1.0±0.1
Zn recovery, %±1SD				
50 mL 0.5 M	ND	ND	ND	ND
70 mL 0.005 M	100.6±1.9	99.4±1.8	100.7±2.9	102.8±2.9

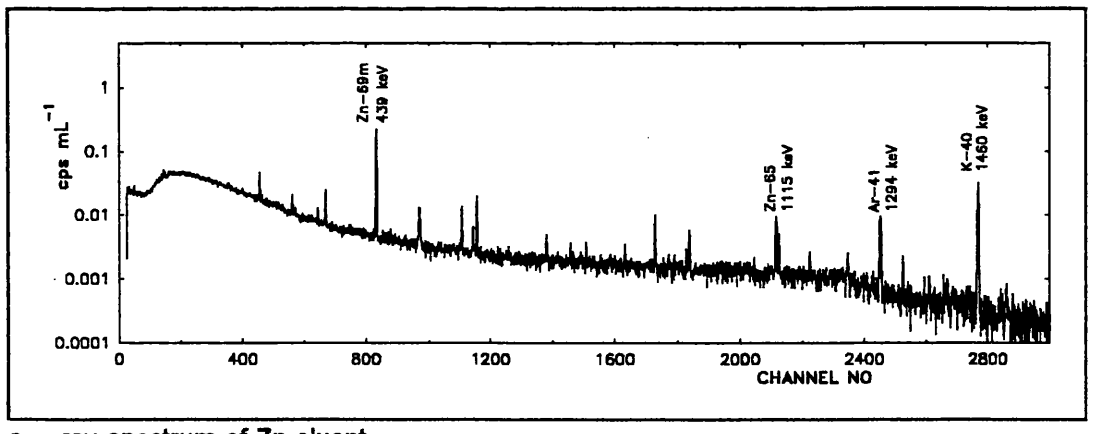
Losses with the eluent used to remove impurities and any residue on the resin were insignificant. The contribution of iron to the zinc eluent was found to be approximately 1%. This contribution can be measured where required by comparing the analysis of iron and zinc eluent of the samples.



a. γ-ray spectrum of loading solution containing Fe, Zn, and the impurities



b. γ-ray spectrum of Fe eluent



c. γ-ray spectrum of Zn eluent

Figure-21: γ-spectra of the tracer solution used for ion exchange separation, and of the Fe and Zn fraction of eluent

III.1.3 POST-IRRADIATION SEPARATION OF ZINC

The anion exchange procedure removes many potential interfering elements, including the alkali metals, but activated eluents were found to contain traces of Mn-56 ($t_{1/2}$=2.6 h comparable to Zn-71 $t_{1/2}$=4 h) and Cl-38 ($t_{1/2}$=37.2 m) from the acid eluent. A post-irradiation clean-up was applied to reduce these activities.

Janghorbani *et al.* (1980) and Ting *et al.* (1982) have described a post-irradiation separation for determination of ^{70}Zn in faecal samples which followed a pre-irradiation separation involving precipitation by APDC. This was based on precipitation of MnO_2 and solvent extraction of Zn.

The efficiency of post-irradiation precipitation of MnO_2 for purification of ion-exchange eluent of Zn was investigated.

Procedure

One fraction of 0.2 g ashed faecal sample (obtained from the previous rare earth recovery investigation) was spiked with 36.569 µg of ^{70}Zn and a similar fraction was processed without addition of the spike. The samples were dissolved, and their zinc contents separated by anion exchange as described above.

The eluents (70 mL) were evaporated until 5 mL of solution remained with addition of 16M HNO_3 to expel chlorine. The samples were then transferred to 10 mL graduated flasks and the volumes made up. Duplicates of 2 mL of the samples were pipetted to irradiation vials (the vials were washed with 0.5 M HNO_3 and demineralised water, and dried), and evaporated under an IR lamp. The samples were then irradiated in the pneumatic system at a flux of 2.4 n cm^{-2} s^{-1} for 1 h. After decay of 30 min, the contents of vials were transferred to 50 mL beakers with a few mL of 16M HNO_3 and the samples

containing enriched ^{70}Zn were each counted for 2000 s to determine Zn-71m at 386 keV and Zn-69m at 438 keV. The samples of natural zinc composition were counted for only a short period since the energy band for Zn-71 was covered with the compton continuum from Mn-56 at 846 keV.

Separation procedure

The samples were then subjected to Mn separation procedure as follows;

1.After addition of 10 mg of Mn^{2+} carrier, the samples were heated to boiling on a hot plate,

2.0.2 g of $NaBrO_3$ was added to oxidize manganese, and the heating continued until completion of precipitation of MnO_2,

3.The precipitates were filtered through double GF/A filter disks,

4.The filtrates and precipitates were transferred to counting bottles and counted for 2000 s.

Results and conclusion

The Mn activities found in the samples are included in **Table-48**, where a major reduction is evident. Zn was not detected on the filter papers indicating insignificant losses with the MnO_2 precipitate. The ratio of the decay-corrected activities of Zn-71m at 386 keV and Zn-69m at 439 keV for samples containing natural level of ^{70}Zn (natural sample) were compared to evaluate the effect of separation procedure. The enrichment factor for the samples spiked with enriched ^{70}Zn (enriched sample) was also calculated from the ratio of activities by the equation of

$$Enr=\frac{\left[\frac{Zn\text{-}71m}{Zn\text{-}69m}\right]_{Enr}-\left[\frac{Zn\text{-}71m}{Zn\text{-}69m}\right]_{Nat}}{\left[\frac{Zn\text{-}71m}{Zn\text{-}69m}\right]_{Nat}}$$

where [Zn-71m/Zn-69m] is the ratio of the activities for the enriched (enr) and

natural (nat) samples. The results are provided in **Table-48.**

Table-48: Determination of enrichment in ash faecal sample with and without post irradiation separation of zinc for comparison

Procedure	**Manganese activities, cps**			
Before Mn separation	380	478		
After Mn separation	0.1	9.7		
	Counting ratio, (Zn-71m/Zn-69m)±SD			**Enrichment factor±SD**
	Natural-I	Natural-II	Mean±SDM	
Before Mn separation	NA	NA	NA	
After Mn separation	0.0099±0.0005	0.0104±0.0005	0.0101±0.0003	
	Enriched-I	Enriched-II		
Before Mn separation	0.0928±0.009	0.0945±0.009	0.0936±0.007	8.267±0.7
After Mn separation	0.0965±0.0002	0.0973±0.0002	0.0969±0.003	8.594±0.4

The accuracy of the results showed that the overall zinc separation procedure is quantitative. Losses of zinc due to adsorption on the manganese precipitate was below limits of determination. From a comparison of the precision of the results, it is obvious that the manganese precipitation procedure is satisfactory (98-100% elimination of Mn-56 counts at 847 keV). However the counting ratio, Zn-71m/Zn-69m, for natural sample was not obtainable before, whilst the ratio obtained after the separation provided 5% precision. Effective manganese elimination can also be seen from **Figure-21** for a sample spiked with enriched ^{70}Zn.

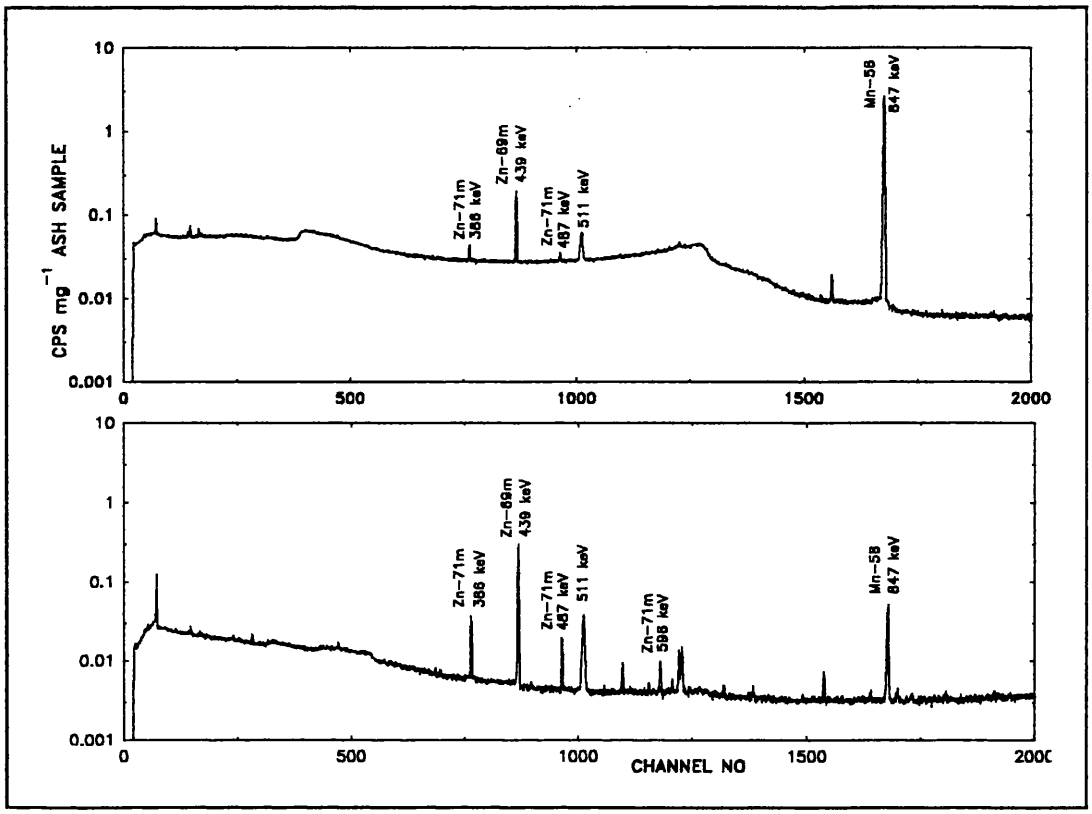


Figure-21: γ-ray spectra of irradiated Zn eluent containing ^{70}Zn enriched spike, counted before and after manganese precipitation [The ratio of the counts at 847 keV (Mn-56) is a factor of 100-see text]

Enrichment was measured with reference to the counts ratio for the sample of natural composition (with ~5% precision). It can be seen that this was found to be 8.267±0.7 before and 8.594±0.4 after chemical separation, compared with 8.646 obtained for the enriched standard solution. Both results provided reasonable accuracy, but better precision was obtained after chemical separation. The measurement precision for the ratio for enriched samples from which Mn had been removed was found to be 5 times better (~2%) than that obtained from which Mn had not been removed.

The γ-ray spectrum of Zn eluent (equivalent to 40 mg of ashed faecal sample) containing natural level of ^{70}Zn, counted after Mn separation is provided in **Figure-22** from which Zn-71m is clearly visible in the presence of Na-24 and Cl-38.

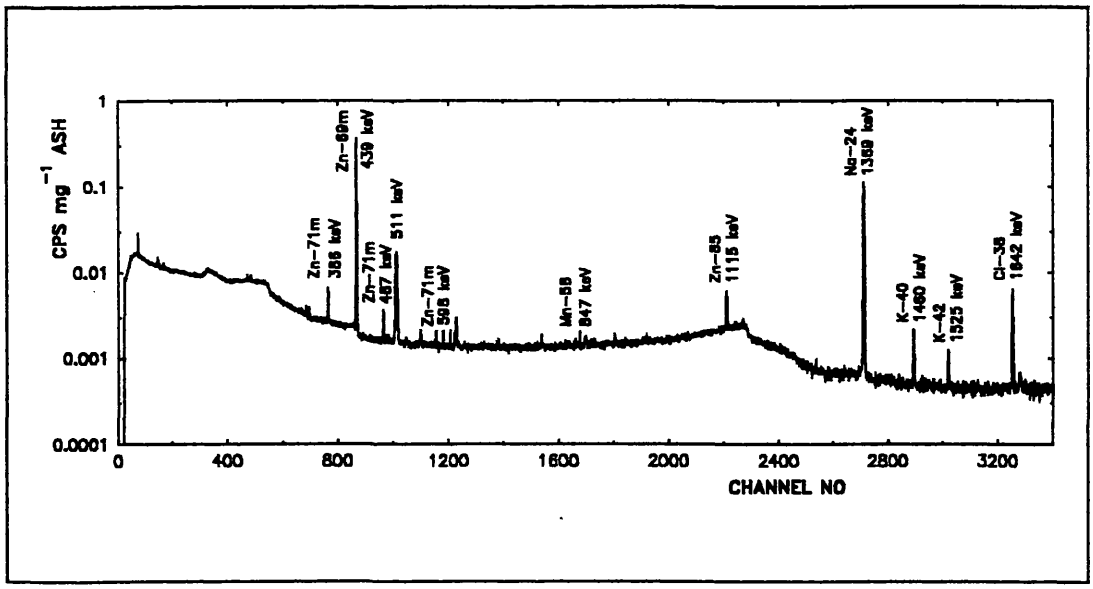


Figure-22: γ-ray spectrum of irradiated Zn eluent (40 mg ash) containing Zn in natural composition, counted after Mn precipitation

III.2 THE USE OF ENRICHED STABLE ISOTOPIC TRACERS AND RARE EARTH MARKERS FOR MEASUREMENT OF LUMINAL DISAPPEARANCE

III.2.1 ETHICAL APPROVAL AND CONSENT OF VOLUNTEERS

The investigation was approved by The Research Ethics Committee of Glasgow Royal Infirmary, Greater Glasgow Health Board. Seven subjects (6 male, 1 female), all in good health and pursuing normal sedentary activities participated in the investigation. In all subjects, haemoglobin and ferritin concentrations and red cell counts were within the normal range (**Table-49**). They were informed of the theoretical background of the investigation and provided written consent. A schematic diagram of the overall procedure is provided in **Figure-23**.

Table-49: Physical and haematological characteristics of the participants in the investigation

	PHYSICAL CHARACTERISTICS				HAEMATOLOGICAL DATA		
Subject	Sex	Age y	Height m	Weight kg	Hb g dL^{-1}	RBC x10^{12} L^{-1}	Ferritin µg L^{-1}
C1	M	56	1.74	79	15.0	4.74	100
C2	M	37	1.68	65	16.8	5.18	131
C3	M	54	1.85	95	14.4	4.46	82
C4	M	42	1.83	78	15.9	5.34	110
C5	M	28	1.75	78	15.4	5.34	47
C6	M	32	1.70	70	13.7	4.72	152
C7	F	28	1.57	55	13.5	4.47	49
Normal range[1]					13-16	4.2-5.9	40[2]

1. Entwistle, 1992

2. Recommended value for subjects who are borderline iron deficient based on measurement of iron absorption from the standard solution (Bezwoda et al., 1979, Magnusson et al., 1981and Cook et al., 1991)

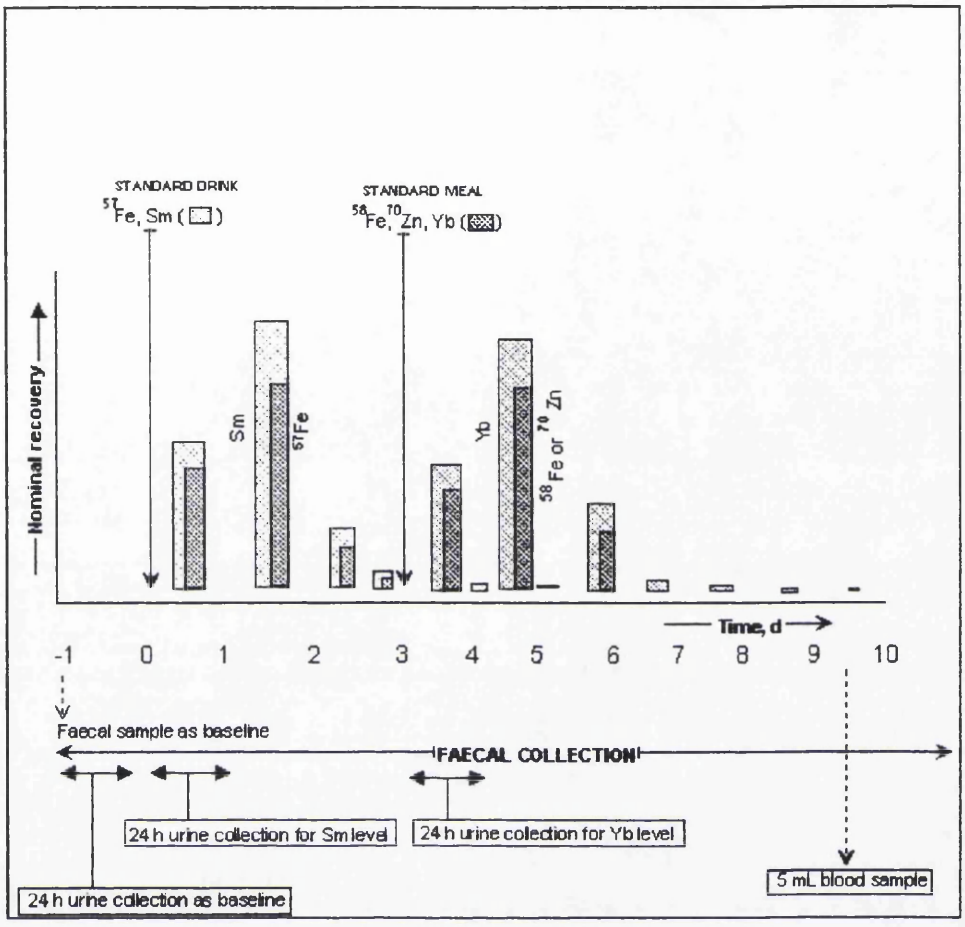


Figure-23: Outline of procedure for measurement of intestinal uptake of 2 sequential inputs of trace elements

III.2.2 PREPARATION OF DOSES, ADMINISTRATION, SAMPLE COLLECTION, SAMPLE PREPARATION

III.2.2.1 PREPARATION OF DOSES

1.Rare earth marker solutions:

Sm solution for standard drink: 58.60 mg of specpure Sm_2O_3 was dissolved in 10 mL of 0.1 M H_2SO_4 and the volume made up to 50 mL to provide 1.0107 mg mL^{-1} of Sm.

Yb solution for standard meal: 59.20 mg of specpure Yb_2O_3 was dissolved in 10 mL of 0.1 M and the volume made up to 50 mL to provide 1.0398 mg mL^{-1}

of Yb.

2. Isotope enriched solutions:

Isotope enriched iron and zinc were obtained in the metallic form (Europa Scientific Ltd/UK). The isotopic composition of the elements in enriched and natural forms are provided in **Table-50**.

Table-50: Isotopic composition of iron and zinc in natural and in enriched form

	ISOTOPIC COMPOSITION, % of weight					
IRON	NATURAL[1]	ENRICHED[2]		ZINC	NATURAL[1]	ENRICHED[2]
		^{57}Fe	^{58}Fe			^{70}Zn
^{54}Fe	5.8	-	<0.01	^{64}Zn	48.6	7.05
^{56}Fe	91.72	3.0	0.03	^{66}Zn	27.9	7.65
^{57}Fe	2.2	95.15	8.07	^{67}Zn	4.1	2.55
^{58}Fe	0.28	1.85	91.90	^{68}Zn	18.8	8.65
				^{70}Zn	0.6	74.1±1.4

1.Commission on atomic weights and isotopic abundances, 1991
2.Composition provided by the manufacturer, based on ICP-MS measurements

^{57}Fe enriched solution for standard drink

15 mg of ^{57}Fe enriched metallic Fe was dissolved in 5 mL of 0.5 M H_2SO_4. After addition of 1 mL of 8.01 mg mL^{-1} natural iron solution (in 0.5 M H_2SO_4) the volume was made up to 50 mL to provide 0.460 mg mL^{-1} Fe containing 0.289 mg mL^{-1} ^{57}Fe (0.286 mg from enriched and 0.003 mg from natural Fe sources), providing 28.6-fold ^{57}Fe enrichment.

Seven 7 mL fractions of the solution were transferred to tubes to provide 3.22 mg of total Fe containing 2.023 mg ^{57}Fe (2.002 mg from enriched source) for each of the subjects, and the residue (approximately 1 mg) retained for use as standard.

^{58}Fe enriched solutions for standard meal

8.2 mg of ^{58}Fe enriched metallic Fe was dissolved in 5 mL of 11M HCl and the solution was then evaporated to dryness. The residue dissolved in 5 mL 0.01 M HCl. The solution was transferred to a universal tube with several rinses of water using a 1mL pipette to provide a total volume of 14 mL. The volume was then made up to 15 mL by addition of 1 mL of 15.003 mg mL^{-1} natural Fe solution (in 0.01 M HCl) to provide 1.547 mg mL^{-1} Fe containing 0.505 mg mL^{-1} of ^{58}Fe (0.502 mg from enriched and 0.003 mg from natural sources), providing 116.6-fold enrichment.

^{70}Zn enriched solutions for standard meal

10.1 mg of ^{70}Zn enriched metallic Zn was dissolved in 3 mL of 0.5 M HCl. The solution was then evaporated to dryness. The residue dissolved in 5 mL 0.01 M HCl. The solution was transferred to a universal tube with several rinses of water using a 1mL pipette to provide a total volume of 14 mL. The volume was then made up to 15 mL by addition of 1 mL of 12.989 mg mL^{-1} natural Zn solution (in 0.01 M HCl), to provide 1.539 mg mL^{-1} Zn containing 0.504 mg mL^{-1} ^{70}Zn (0.499 mg from enriched and 0.005 mg from natural sources), providing 54.6 fold enrichment.

Standard meal (Farina): Wheat flour based Farina meal containing following ingredients was prepared as described by Forbes *et al.* (1989).

Ingredients (one portion)	
Farina flour	40 g uncooked (approximately 250 g cooked)
Milk (whole)	120 mL (4% fat)
Butter	14 g
Sugar (white granulated)	24 g
Ferric chloride	3 mg Fe
Salt	0.5 g
Water	250 mL

Preparation

Heat water to boiling and add salt. Reduce heat to low, stir farina flour in gradually, and cook 1 minute, stirring constantly. Spoon into serving bowls to equal (approximately 250 g) portions. Mixture will be slightly thin; thickens during sitting.

III.2.2.2 ADMINISTRATION AND SAMPLE COLLECTION

The procedure followed for administration of enriched tracers and markers, and collection of faeces and urine is described below:

Day -1: Subjects collected one faecal sample and a 24 h urine output for measurement of background levels of iron, zinc, and rare earths.

Day 0: Having fasted overnight subjects consumed a standard solution, finally diluted to 50 mL with demineralised water, containing 3.22 mg of total iron including 2.023 mg of ^{57}Fe, 1.017 mg of Sm, 20 mg freshly prepared ascorbic acid. After complete consumption of the solution, two rinses of water were also taken to ensure total consumption. The subjects did not take any food or drink for 3 h following the administration.

Urine samples were collected for 24 h following the administration and all faecal output with associated was collected for 10 days.

Day 3: The subjects having fasted overnight consumed a standard meal containing approximately 3 mg of iron as ferric chloride

Immediately after the preparation of the meal 2 mL of Fe solution containing a total of 3.094 mg of Fe including 1.010 mg of ^{58}Fe, 2 mL of Zn solution containing a total of 3.078 mg of Zn including 1.008 mg of ^{70}Zn, and 1 mL of Yb marker solution (1.0398 mg mL^{-1}) were added to each portion. Portions of the meal were then mixed well, sugar and butter added on while they were

hot, and served with milk. After complete consumption of meals, serving bowls were rinsed with milk, and this was also consumed to ensure total consumption. The subjects did not take any food or drink for 3 h following the administration.

Urine samples were collected for 24 h following administration of the meal. Faecal samples were collected for 7 days after the Farina consumption. After completion of the period of sample collection, subjects were taken to hospital for blood sampling (sample is taken by an authorized person).

The participants were provided with an information form explaining the required procedures (**Appendix-4**) and the following sample collection systems.

1. A card to record time of bowel movement.

2. A polypropylene commode stool collection system (SAGE Products Inc, USA) consisting of a bowl with lid and a frame (**Figure-24**), "biohazard" polythene bags, and pieces of twist wires. After the use of the collection unit, the lid is snapped tightly and the frame removed from the unit. The bowl is then contained a numbered polythene bag and the opening bound with a twist wire.

3. Urine collection container: This was a plastic container of 2 L volume containing 25 mL of 2 M HCl as preservative.

Figure-24: Faecal sample collection system used in absorption investigation

III.2.2.3 PREPARATION OF SAMPLES

1.Faecal samples :The collected samples were stored in a deep freeze at -20 °C. Since the plastic sample collection system was not resistant to autoclave temperature (120 °C), they were then sterilized by irradiation, receiving a total dose of 75 000 Sv from a Co-60 γ-source. The samples were oven-dried at 50 °C and ashed at 450 °C for 16-24 h. They were then ground to provide homogeneity and stored in plastic containers for further use.

2.Urine samples: For determination of rare earths in urine samples by ICP-MS, 100 mL fractions of the collected urine were digested with gradual addition of 20 mL of 16M HNO_3 and 10 mL of 30% H_2O_2 until clear colourless solutions were obtained. The solutions were then transferred to 100 mL graduated flask and the volumes were made up. 5 mL of the acid digested

samples were diluted to 20 mL with 2% v/v HNO_3 and transferred to polythene bottles for further use. A blank solution was also run throughout the procedure to take account of rare earth levels in the chemicals.

3.Blood samples: 5 mL of blood sample was taken from each of the subjects (Glasgow Royal Infirmary, Department of Nutrition) and transferred to tubes containing potassium-EDTA as an anticoagulant. Haematologic characteristics were determined by Department of Haematology, Glasgow University. Serum ferritin concentrations were determined by a radioimmunoassay.

III.2.3 ANALYTICAL PROCEDURE

Markers (Sm and Yb) and tracer isotopes (administered with the Farina meal, ^{58}Fe and ^{70}Zn) contents of faecal samples were determined by CNAA. Total elemental Fe and Zn contents of faecal samples were determined by AAS. For determination of rare earths in faecal and urine samples, and ^{57}Fe in faecal samples, ICP-MS was employed. An outline of procedure applied to faecal samples is provided in **Figure-25**.

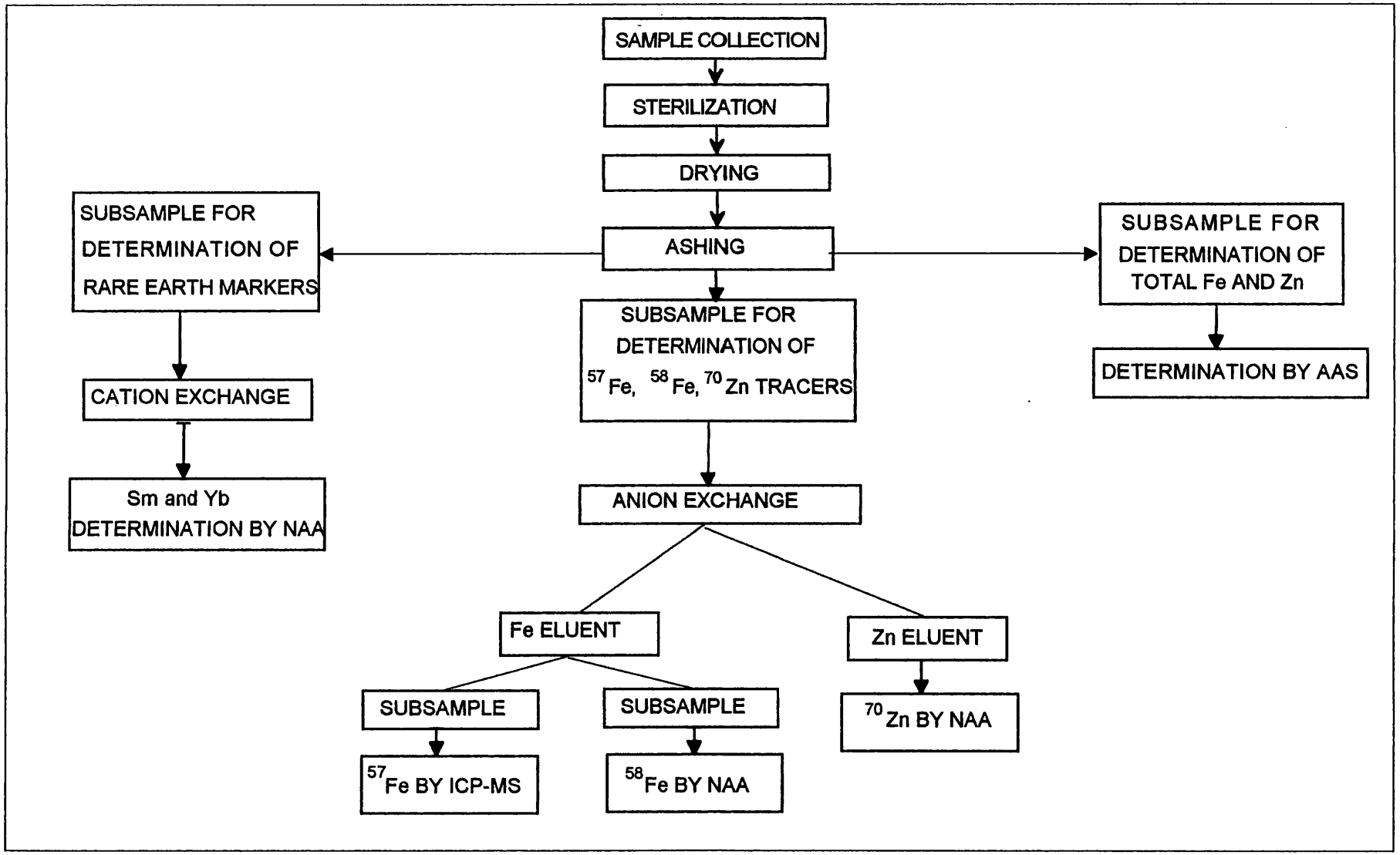


Figure-25 : Outline of overall procedure for determination of markers, tracers, and total elements in faeces

III.2.3.1 DETERMINATION OF RARE EARTH CONCENTRATIONS IN FAECAL SAMPLES BY NEUTRON ACTIVATION ANALYSIS

Procedure:

For cation exchange separation of Sm and Yb, approximately 0.200 g fractions of ashed faecal samples were taken in 50 mL beakers and spiked with 200 µg of Pr as a yield tracer. The samples were dissolved and their Sm and Yb contents separated by cation exchange as previously described. The eluents were evaporated until the volume reduced to 5 mL. The solutions were transferred to 10 mL graduated flasks and the volumes made up.

Duplicate 1 mL fractions of eluent samples, 100 µL of dosing solutions diluted to contain 1.01 µg of Sm and 1.04 µg of Yb, standard solutions containing 1.00 µg of Sm and Yb, and a solution containing 100 µg Pr were pipetted in to polypropylene irradiation vials. A solution containing 250 µg of Fe was added to each of the samples and standards as an internal flux monitor. The solutions were then dried under an IR-lamp. One ashed sample for each of the subjects was also prepared for non-destructive analysis for further confirmation of results. The vials were wrapped in aluminium foils and irradiated in the CVS for 6 h (samples which were obtained from the same subject were irradiated in the same batch). They were then allowed to decay for 1 d (4 d for ashed samples) to reduce exposure from radionuclides with a short half life. The samples were unwrapped and monitored. The dose rate for most of the samples were 5-10 µSv h^{-1} at 20 cm. Samples and standards were counted on a 130 cc HPGe detector at 2.4 cm geometry for 2000-3000 s as convenient.

Results and comments:

Weight of faecal samples and mean concentrations of Sm and Yb found in the ashed samples are provided in **Appendix-5**. The yields for the pre-irradiation separation of Sm and Yb were in a range of 80-100%, as was found in rare earth recovery investigation. Good agreement between duplicate fractions of the column separated and non-destructively analyzed samples showed that the precision of the measurements were satisfactory. There was an insignificant difference between two measurements ($p>0.05$). This agreement can be seen from **Table-51** for Yb for samples which were analyzed by both methods.

Table-51: Yb concentrations found for duplicate fractions of column separated and non-destructively analyzed faecal samples

Yb concentration, µg g^{-1} ashed faeces			
Eluent I	Eluent II	Mean±SDM	INAA
39.7±0.7	40.5±1.1	40.1±0.6	41.8±0.5
32.1±0.1	32.3±0.1	32.2±0.07	31.7±0.3
89.6±1.6	90.9±1.1	90.3±0.9	90.2±0.5
183±1.8	185±1.9	184±1.3	185±1.7
99.4±1.2	101±1.2	100±0.8	96.3±1.1
89.1±0.7	89.6±0.7	89.4±0.4	91.8±0.8
20.8±0.5	21.2±0.5	21.0±0.3	20.6±0.2

Sm and Yb concentrations of dosing solutions obtained with reference to the standards provided evidence for the accuracy of the results (**Table-52**). Insignificant differences were found between the found and expected concentrations ($p>0.05$).

Table-52: Sm and Yb contents of dosing solutions with reference to specpure standards

Batch no	Sm (µg mL^{-1})	Yb (µg mL^{-1})
1	980.4	967.0
2	940.0	977.4
3	1061	946.2
4	1021	998.2
5	950.1	1071
6	970.3	1009
7	929.8	987.8
8	1031	1123
9	998.2	1001
Mean±SDM	987.1±44.6	1009±55
Expected	1010.7	1039.8

III.2.3.2 DETERMINATION OF IRON AND ZINC BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Determination of luminal disappearance using stable isotopic tracers requires account to be taken of the isotopic constituents of endogenous secretion which contribute to the measured amount of excreted tracer. This can be calculated when the amount of the element of interest in the sample is determined by AAS (section I.4.2).

Procedure:

Approximately 0.1 g of ashed faeces were dissolved by gradual addition of 10 mL of aqua-regia and 2-3 mL of 30% H_2O_2 with heating, and the solutions evaporated to dryness. The residues were redissolved in 5 mL of 11M HCl and transferred to 20 mL graduated flasks, and the volumes made up by addition of demineralised water, to provide stock solutions. A blank solution containing the chemicals used was also prepared to determine the contribution from reagents.

A solution containing bovine liver (SRM 1577a, standard reference material 1577a, National Bureau of Standards, U.S.A) was also prepared to monitor quality control of the measurements. Approximately 1 g of bovine liver was ashed in a furnace at 420 °C for 24 h, dissolved, and contained in a 100 mL of solution as described above.

The iron and zinc contents of the samples were determined with a Philips flame atomic absorption spectrophotometer, FAAS (Model PU9100) at the Department of Chemistry, University of Stratchlyde .The spectrophotometer was adjusted for optimum measurement efficiency for each of the elements, and calibrated with a range of iron and zinc concentrations to provide measurement sensitivity and determination limit (**Table-53**).

Table-53: Instrument parameters for the determination of iron and zinc by flame atomic absorption spectrophotometer

Philips PU9100 Flame Atomic Absorption Spectrophotometer				
Burner:	Air-acetylene flame			
Integration time:	4s			
Nebulizer uptake rate:	6 mL min^{-1}			
Detection wave length (nm)	Slit width (nm)	Lamp current (mA)	Sensitivity [µg (unit abs.)$^{-1}$]	Determination limit (µg mL^{-1})
248.3 (Fe)	0.2	10	0.1	0.004
213.9 (Zn)	0.7	8	0.5	0.02

After appropriate dilution of the solutions to provide concentrations comparable to those used for calibration, samples were introduced from a nebulizer and the average of 3 absorption readings taken for each sample, an online computer integrating the absorbance signal for a period of 4 s. A sample with an internal standard, a blank, and a sample of bovine liver were analyzed with each set of samples. The procedure was repeated twice for each sample and the absorbance data converted to concentrations using a linear regression programme. The mean of 2 concentrations with its standard deviation was then used to determine the total zinc and iron contents of the samples.

Results and comments:

The mean of concentrations of Fe and Zn found in faecal samples are provided in **Appendix-5** and **6**. The concentrations of iron and zinc in blank solutions were below the detection limit, indicating that the contribution from Fe and Zn in reagents was insignificant. Results for one sample from each of subjects to which an internal standard was added are provided in **Table-54** as evidence of precision and accuracy.

Table-54: Precision and accuracy of determination of Fe and Zn by AAS

Fe concentration, µg mL^{-1}±SD			Zn concentration, µg mL^{-1}±SD		
Sample	Samp.+Int. std	Int. std.	Sample	Samp.+Int. std	Int. std.
7.22±0.04	9.31±0.09	2.09	2.55±0.01	3.61±0.01	1.06
7.04±0.01	9.08±0.02	2.05	2.60±0.01	3.71±0.09	1.11
3.41±0.01	5.50±0.01	2.09	1.47±0.01	2.50±0.02	1.02
5.01±0.02	7.07±0.04	2.06	3.18±0.01	4.25±0.01	1.07
4.23±0.03	6.32±0.03	2.08	3.42±0.05	4.41±0.03	0.99
5.75±0.03	7.81±0.04	2.06	2.76±0.03	3.78±0.02	1.02
5.39±0.04	7.33±0.04	1.93	3.21±0.03	4.28±0.05	1.07
Mean		2.05±0.05			1.05±0.04
Expected		2.00			1.00

Good reproducibility was obtained for all samples, with a standard deviation of approximately ±1%. Results for the internal standard confirmed the reproducibility of measurements ($p>0.01$, one-tailed t-test). Concentrations of Fe and Zn in Bovine Liver were found to be 189±7 for Fe and 122±8 for Zn (mean of 8 measurements), which were not significantly different from the certified concentrations of 194±20 and 123±8. This was further evidence for the accuracy of measurements.

III.2.3.3 DETERMINATION OF ^{58}Fe, ^{70}Zn BY NEUTRON ACTIVATION ANALYSIS

I. Determination of ^{58}Fe and ^{70}Zn in dosing solutions

The ^{58}Fe and ^{70}Zn contents of the dosing solutions were confirmed by NAA.

Procedure

1. ^{58}Fe in dosing solution

Replicates of 200 µL of diluted dosing solution containing 1.10 µg of ^{58}Fe and 1 mL of Fe standards of natural composition containing 2.8 µg of ^{58}Fe were pipetted into irradiation vials. After addition of a solution containing 1 µg of Yb

to each of the samples as a flux monitor, they were dried under an IR-lamp. The samples were wrapped in aluminium foils and irradiated for 6 h in the CVS ($4.2x10^{12}$ n cm^{-2} s^{-1}).

The samples were allowed to decay for 1-2 days and were then counted on a 130 cc HPGe detector at contact geometry for 2-5 h. The concentration of ^{58}Fe in the dosing solution was calculated with reference to the Fe standards, with account of flux corrections.

2. ^{70}Zn in dosing solution

Replicates of 200 µL of the diluted dosing solution containing 1.01 µg of ^{70}Zn and 300 µL of Zn standards of natural composition containing 1.8 µg of ^{70}Zn were pipetted into irradiation vials. After addition of 100 µL of 16 M HNO_3 to expel chlorine, they were dried under an IR-lamp. The samples in batches consisting of 1 dosing solution and 1 standard were irradiated for 1 h in the pneumatic system ($2.7x10^{12}$ n cm^{-2} s^{-1}).

After decay of half an hour, the samples were counted on a 130 cc HPGe detector for 2000 s on contact geometry. The concentration of dosing solution was then calculated with reference to the Zn standard.

Results and comments

The enriched isotope contents of dosing solutions, determined by NAA with reference to standards of natural abundance are provided in **Table-55**. As can be seen from the table, there is no significant difference between the concentrations found and those expected ($p>0.05$, one-tailed t-test). The precision of ^{58}Fe measurements were in a range of 2-5% because of counting time. This can be reduced by longer periods of counting, or using increased amounts of the dose fractions.

Table-55: Concentrations of ^{58}Fe and ^{70}Zn in enriched dosing solutions

Sample No	^{58}Fe enriched dose	^{70}Zn enriched dose
	$\mu g\ mL^{-1}$	$\mu g\ mL^{-1}$
1	492±8	520±35
2	494±8	557±36
3	513±13	520±36
4	480±8	494±30
5	494±24	517±28
6	467±10	504±39
7	518±16	567±38
8	507±21	
9	544±11	
10	531±21	
Mean±SDM	504±23	526±27
Expected	505	504

The precision of ^{70}Zn results showed that the contribution to precision from the reference standard with no enrichment were ~5% whilst those from the isotope enriched dose solution were 2-3%. This suggests that the used amount of dose fraction in determination of the enrichment in samples will be satisfactory.

II. Determination of ^{58}Fe and ^{70}Zn in faecal samples

Approximately 0.200 g of ashed samples were dissolved and the Fe and Zn contents were separated by anion exchange as previously described. The eluents obtained were evaporated, with dropwise addition of 5 mL of 16 M HNO_3 to expel chlorine, until the volumes were reduced to 5 mL. Solutions were made up 10 mL in graduated flasks and stored for analysis.

Procedure

1.Determination of ^{58}Fe concentrations in faecal samples

The following samples and standards were prepared for determination of ^{58}Fe concentrations in faeces;

a) Duplicates of 2 mL fractions of column eluents containing Fe^{58} from approximately 40-80 mg of ashed faeces,

b) 200 µL of dosing solution diluted to contain 1.01 µg of ^{58}Fe,

c) 200 µL of iron solution of natural composition containing 0.84 µg of ^{58}Fe,

d) 100 µL of zinc solution of natural composition containing 63.2 µg of ^{64}Zn.

For each subject, representative ashed samples (1) and column-separated samples (2) were analyzed to monitor the separation yield. Accuracy was also confirmed by results obtained for duplicates samples of ashed bovine liver (SRM 1577a).

The sample and standard solutions were pipetted into irradiation vials. After addition a solution containing 1 µg Yb as flux monitor, they were dried under an IR-lamp. The samples obtained from the same subjects and standards were then wrapped in aluminium foils and irradiated for 6 h in a flux of $4.2x10^{12}$ n cm^{-2} s^{-1} (in the CVS).

The gross induced activities in samples and standards were allowed to decay for 1-2 d after irradiation to reduce radiation exposure from radionuclides with short half lives. The samples were then unwrapped and counted on a 130 cc HPGe on contact geometry for 2-5 h for samples and for 10 h for standards. The ^{58}Fe contents of samples were calculated with reference to the Fe^{58} content of dosing solution. Zinc contents of Fe eluents was also examined, with reference to Zn-65, $t_{1/2}$=244.1 d, to confirm complete separation. In most cases Zn was not detected.

2. Determination of ^{70}Zn concentrations in faecal samples

Duplicates of 2 mL of column separated zinc solution and 200 µL of ^{70}Zn enriched dose solution containing 1.01 µg of ^{70}Zn were pipetted into counting vials and dried under an IR-lamp. Two samples from different duplicates and a standard were irradiated as a batch in the pneumatic system for 1 h ($2.7x10^{12}$ n cm^{-2} s^{-1}).

After decay of half an hour, the standard was transferred to a counting bottle with 2 mL 16M HNO_3 and counted on the detector for 2000 s on contact geometry. While the standard was being counted, the samples were subjected to the Mn separation procedure previously described. The samples were then counted for 2000-3000 s and their ^{70}Zn contents determined with reference to the ^{70}Zn content of the dosing solution.

Results and comments

1.Determination of ^{58}Fe

The specific activities at the end of an irradiation of 6 h, for isotope enriched dose solution and iron solution of natural isotopic composition were found to be 1.68±0.39 and 1.65±0.38 cps μg^{-1}±SDM. The difference between the activities is insignificant ($p>0.05$, paired t-test) and indicates that systematic errors (pipetting and evaporation of standards) were insignificant.

The Fe^{58} concentrations found in faecal samples are provided in **Appendix-6**. An example set of Fe^{58} concentrations (one sample for each subject) determined by CNAA and INAA are provided in **Table-56** for quality control of the measurements.

Table-56: ^{58}Fe content of faecal samples determined by CNAA and INAA

Sample	^{58}Fe concentration in ashed faeces, µg g^{-1}±SD				Ratio±SD
	CNAA			INAA	I/CNAA
	Dupl. I	Dupl. II	Mean±SDM		
C1	44.6±0.5	44.1±0.5	44.3±0.4	42.6±1.1	0.96±0.03
C2	134.8±1.5	136.7±1.4	135.8±1.4	137.9±2.2	1.01±0.02
C3	75.1±1.3	76.4±1.2	75.8±0.9	80.1±1.7	1.05±0.02
C4	34.3±1.6	35.7±1.2	34.9±1.0	36.1±0.6	1.03±0.03
C5	15.8±0.3	16.1±0.3	15.9±0.2	16.7±0.4	1.05±0.03
C6	20.8±0.3	20.4±0.5	20.6±0.3	21.3±0.5	1.03±0.03
C7	7.8±0.3	8.0±0.3	7.9±0.2	7.8±0.2	0.99±0.04

The Fe^{58} concentrations found in duplicate samples of faeces agree providing 1-3% precision for duplicate samples where the standard deviations of measurements were also 1-3%. This was obtained with the use of an internal flux monitor. There was insignificant difference ($p>0.05$) between results obtained by chemical NAA (CNAA) and non-destructive NAA (INAA) indicating that the overall chemical procedure was effectively quantitative. Iron concentration in bovine liver (187±4 µg g^{-1}±SDM, mean of 2 measurements) determined by INAA agrees with the certified content of this standard (194±20 µg g^{-1}). This was a further evident for accuracy of the results.

The losses of Zn with Fe eluents and the Fe contribution to Zn eluents were below the limit of determination or occasionally <1%. ^{64}Zn with the activation product of Zn-65 ($t_{1/2}$=244.1 d) also enabled comparison of the Zn contents of ashed and column separated faecal samples, examination of the reliability of Zn concentrations determined by AAS. ^{64}Zn concentrations of samples determined by INAA and CNAA (one sample for each subject) and those derived from elemental concentrations obtained by AAS are provided in **Table-57** for comparison.

Table-57: ^{64}Zn contents of faecal samples determined by CNAA, INAA and AAS (SD for all measurements were 1% or less)

	^{64}Zn concentration, µg g^{-1} in ash			Ratio	
Sample	CNAA	INAA	AAS	I/CNAA	INAA/AAS
C1	1168	1030	1039	0.88	1.01
C2	949	894	926	0.94	1.03
C3	516	525	512	1.02	0.98
C4	765	761	811	0.99	0.94
C5	1451	1423	1318	0.98	0.93
C6	1543	1559	1535	1.01	0.98
C7	1073	1002	1014	0.93	1.01

It was found that there were no significant differences between results obtained from any of the measurements techniques ($p>0.05$). This indicates that the chemical yield of the pre- and post-irradiation separation were quantitative. Zinc concentration in Bovine Liver was found to be 124±3 µg g^{-1} (mean of 2 measurements by INAA) which agrees with the certified content of this standard (123±8 µg g^{-1}). This confirms the accuracy of the results.

2.Determination of ^{70}Zn

The specific activities at the end of irradiation for each of the standards for each irradiation batch were found to be constant through the analysis period, at 0.70±0.03 cps μg^{-1} with ±SDM and all were within the 95% confidence limit. The reproducibility of results indicates that random errors were insignificant.

^{70}Zn concentrations for duplicate samples with no enrichment of isotopic tracer determined by CNAA and those derived from elemental concentrations determined by AAS results are provided in **Table-58**, for one sample for each subject.

Table-58: ^{70}Zn concentrations in faeces at background level obtained by CNAA and AAS

	^{70}Zn concentration, µg g^{-1}±SD in ash				
	CNAA			AAS	Ratio
Sample	Dupl. I	Dupl. II	Mean±SDM		AAS/CNAA
C1	11.9±2.8	15.2±4.0	13.6±2.3	13.4	0.98±0.17
C2	17.3±1.0	18.4±1.5	17.8±0.8	18.0	1.01±0.04
C3	6.9±1.3	8.4±1.6	7.7±1.0	7.2	0.94±0.12
C4	9.6±1.4	10.6±0.8	10.1±0.7	10.3	1.02±0.07
C5	12.8±0.9	14.0±2.1	13.4±0.8	13.9	1.04±0.06
C6	14.4±1.8	15.8±1.2	15.2±1.0	15.8	1.04±0.07
C7	11.1±2.1	13.2±2.2	12.1±1.5	10.9	0.90±0.11

The insignificant difference between results obtained by two different measurement techniques ($p>0.05$) was further evidence for quantitative yield of the overall procedure and confirm accuracy of the results.

The precision of the results can be seen vary between ±10-30% providing 5-20% precision for the mean. This can be attributed to the lower amounts determined, e.g. 0.3 µg in a 40 mg fraction of ashed faecal sample. The ^{70}Zn concentrations found in faecal samples are provided in **Appendix-6**.

III.2.3.4 MEASUREMENTS BY ICP-MS

ICP-MS was employed for determination of the enrichment of ^{57}Fe administered with standard solution. The natural levels of rare earths in faecal and urine samples were also determined by this method. The specifications and operating conditions of the spectrometer are provided in **Table-59**.

Table-59: Specifications and operating conditions of the ICP-MS used for determination of ^{57}Fe and rare earths

Instrument: PlasmaQuad 11 STE (Fisons Instruments Elemental Analysis)	
RF power	:1349 W
Reflected power	:<5 W
Nebuliser	: DeGalan V-groove
Spray chamber	: Scott-type double bypass (cooled to 12 °C)
Sampling cone	: Nickel, 1.0 mm orifice
Skimmer cone	: Nickel, 0.75 mm orifice
Auxiliary gas flow	: 0.75 L min^{-1}
Coolant gas flow	: 14.0 L min^{-1}
Nebuliser flow rate	: 0.899 L min^{-1}
Sampling depth	: 10 mm
Sample uptake rate	: 0.8 mL min^{-1}
Optimization	: The lenses were adjusted to maximize the ^{115}In signal
Acquisition parameters	
Mass range	: 51 to 116.5 amu for Fe, 99-185 amu for RE analysis
Channels per amu	: 20
Dwell time	: 320 µs
Collector	: Dual mode
Acquire time	: 30 s
Acquires per sample	: 3 s

1. Measurement of ^{57}Fe in faecal samples

Procedure

The following samples and standards were prepared and diluted with 2% v/v HNO_3 to determine ^{57}Fe concentrations,

1) Standards containing 22, 44 and 88 µg L^{-1} of ^{57}Fe were prepared from a specpure Fe solution,

2) 1 mL fractions of column separated samples were diluted to 50-100 mL as appropriate for the expected concentrations,

3) 0.1 mL of the dose solution diluted to provide 28.9 μg L^{-1} of ^{57}Fe,

4) Approximately 1 g of bovine liver (dry weight) was ashed, acid digested, and subjected to the column separation procedure as described previously. The iron eluent was finally diluted to 100 mL to provide approximately 40 μg L^{-1} of ^{57}Fe,

4) A blank solution which had been run through the separation procedure was prepared to determine the contribution from reagents.

All samples and standards were spiked with ^{115}In as an internal standard to monitor signal fluctuations and drift.

The system was calibrated with the standards and the regression coefficient of linearity for the ion counts at these concentrations was found to be r=0.9998.

The ^{57}Fe content of the column separated samples, bovine liver, and the blank were determined by 3 measurements for each solution. A wash solution of 2% v/v aristar HNO_3 and one of the calibration standards were run between every 3 sample to minimize inter-sample overlap, and to confirm the stability of the system through the measurement period.

Results and comments

Concentrations of ^{57}Fe found in faecal samples are provided in **Appendix-5**. Examples of measured concentrations for the solutions prepared from column eluents and the dose solution together with concentration determined in the blank and the mean of 4 (3 runs for each) measurements for bovine liver are provided in **Table-60**.

Table-60: ICP-MS measurements of ^{57}Fe in column eluents after a dilution by factor of 1000

	^{57}Fe concentrations, µg L^{-1}			
Sample	Run I	Run II	Run III	Mean ±SDM
C1	32.1	31.1	30.4	31.2±0.9
C2	14.0	13.5	13.4	13.7±0.3
C3	42.2	44.0	43.9	43.4±1.0
C4	22.0	21.2	21.3	21.5±0.4
C5	29.7	28.4	29.8	29.6±0.8
C6	20.6	19.8	19.4	20.0±0.6
C7	24.3	23.7	23.5	23.8±0.4
Blank	3.26	3.20	3.30	3.27±0.03

Derived concentrations

^{57}Fe in dosing solution, µg mL^{-1}				**Expected**
305	295	290	297±7.6	289
Fe in bovine liver, µg g^{-1} in dry				
(Mean of 4 measurements)		**Certified**		
190±1.5		194±10.3		

Repeatability of 3 measurements of concentrations for the solutions was found to be 2-3%. The constant concentration for the blank solution showed that the contribution from the contents of chemicals used was measured with 1-2% precision. The result for dosing solution was found to be very close to the expected value with 2.5% precision suggesting the results were accurate. The Fe content of bovine liver derived from ^{57}Fe determinations (as the mean of 4 measurements) also confirmed the accuracy of the results.

2. Measurement of rare earths in faecal and urine samples by ICP-MS

Natural background levels of rare earths were determined in faecal and urine samples provided before administration of the standard solution. Urine samples provided after consumption of the standard solution and meal were

also analyzed to investigate if any absorption had occurred at levels which were below the determination limits of NAA.

Procedure

Approximately 0.1 g of ashed faecal samples were digested in 10 mL of 16M aristar HNO_3 by heating and the solutions evaporated to near dryness. Solutions of digested faecal samples were made up to contain 0.1%, w/v solid concentrations in 2% v/v HNO_3.

The system was calibrated for the measured isotopes using multi-element standards containing 0.2, 1 and 10 ng mL^{-1} of the rare earths with consideration of their natural abundances. The regression coefficients for the calibrations were found to be 0.9979-1.0000. The following samples and standards were analyzed with 3 runs for each,

1. A blank solution provided from the faecal sample preparation,
2. Acid digested faecal samples,
3. A blank solution provided from the urine sample preparation,
4. Previously prepared acid treated urine samples containing 25% v/v urine in final solution (Section III.2.2.3.),
4. BHVO-1 geological standard (Watkins and Nolan, 1992) for quality control of rare earth analysis

After addition of ^{115}In as internal standard to monitor signal fluctuations and drift, analysis of the samples was carried out starting from the blank (for urine), followed by urine samples, to minimize possible contamination. Since the urine samples contained a significant amount of solid, after 3 runs for each of the samples one wash solution was also processed to clean the instrument. The analysis procedure was continued with the blank and solutions of acid digested faecal samples. A multi-element calibration standard containing 1 ng mL^{-1} of rare earths was analyzed with 2-3 sample intervals to monitor stability of the measurement system.

Results and quality control

Rare earth concentrations in faecal and urine samples, taking account of dilution, are provided in **Appendix-7.A** and **B**.

Examples of measured concentrations in solutions derived from faecal and urine (provided 24 h after the meal consumption) samples for subject C2, and a blank for each are provided in **Table-61** for quality control. Rare earth concentrations determined for BHVO-1 geological standard are also provided in the table to confirm accuracy of the results.

As can be seen from the table, the measured concentrations (blank subtracted) of rare earths in the solution of faecal sample were usually significantly higher than those found in the blank solution. Reproducibility of the concentrations measured in faecal samples (mean of 3 measurements) were reasonably good, providing up to 10% standard deviation.

Concentrations of the light rare earths in urine samples were significantly higher than those measured in the blank solution. The levels of heavy rare earths were very low and close to the concentrations determined in the blank solution. The reproducibility of measurements were reasonable at such low levels providing 10-50% standard deviation. The insignificant difference between rare earth concentrations determined in BVHO-1 standard and those provided by Watkins and Nolan (1992) was evidence for the accuracy of the results.

Table-61: Measured rare earth concentrations (blank substracted) in solutions of digested faecal and urine samples for subject C2and in BHVO-1 standard

	MEASURED RARE EARTH IN SOLUTION , ng mL^{-1}										RARE EARTH IN BHVO-1, µg g^{-1}		
	FAECAL SAMPLE					URINE SAMPLE							
RE	I.Run	II.Run	III.Run	Mean±SDM	Blank	I. Run	II.Run	III.Run	Mean±SDM	Blank	Found	Lit.[1]	RE
La	2.23	2.29	2.20	2.23±0.05	ND	0.015	0.015	0.015	0.015±0.004	0.020	14.6	15.9	La
Ce	2.43	2.48	2.59	2.50±0.08	0.04	0.031	0.032	0.046	0.036±0.008	0.037	35.4	36.9	Ce
Pr	0.49	0.51	0.51	0.49±0.04	0.02	0.024	0.028	0.029	0.027±0.003	0.039	5.01	5.43	Pr
Nd	1.52	1.49	1.62	1.54±0.07	ND	0.019	0.026	0.024	0.023±0.004	0.028	23.9	25.1	Nd
Sm	0.23	0.18	0.18	0.20±0.02	0.07	0.047	0.062	0.034	0.048±0.014	0.046	5.96	6.48	Sm
Eu	0.05	0.04	0.07	0.05±0.01	0.01	0.015	0.011	0.013	0.013±0.002	0.031	2.05	2.13	Eu
Gd	0.19	0.26	0.19	0.21±0.04	0.11	ND	-	-	-	0.034	6.42	6.46	Gd
Tb	0.03	0.03	0.03	0.03±0.002	0.03	0.019	0.020	0.021	0.020±0.001	0.010	0.90	0.96	Tb
Dy	0.18	0.21	0.17	0.19±0.02	ND	ND	-	-	-	0.015	5.26	5.40	Dy
Ho	0.04	0.03	0.04	0.04±0.01	0.02	0.004	0.006	0.003	0.004±0.001	0.008	0.92	1.06	Ho
Er	0.11	0.10	0.10	0.10±0.01	ND	0.004	0.008	0.004	0.005±0.002	0.011	2.49	2.65	Er
Tm	0.02	0.01	0.02	0.02±0.01	0.03	0.002	0.002	0.002	0.002±0.000	0.006	0.32	0.33	Tm
Yb	0.25	0.31	0.29	0.29±0.03	ND	0.025	0.018	0.022	0.022±0.004	0.020	2.10	2.06	Yb
Lu	0.02	0.02	0.02	0.02±0.002	0.03	0.009	0.012	0.009	0.010±0.002	0.007	0.30	0.30	Lu

1. Watkins and Nolan (1992)

III.3 RESULTS AND DISCUSSION

In this section the recoveries of Sm and Yb are provided for consideration of their kinetic behaviour. Attention is also be given to their possible absorbance in view of concentrations found in urine and final faecal samples. Consideration is given to the determination of luminal disappearance of isotopic tracers by analysis of individual samples and composites of a limited number of faecal samples. The excretion kinetics of excreted fractions of the tracer isotopes are compared with those of the Sm and Yb markers.

III.3.1 RECOVERIES OF THE RARE EARTH MARKERS

Faecal recoveries of Sm and Yb derived from experimental data given in **Appendix-5** and **6** are provided in **Table-62.a-g**.

The tables contain the time elapsed after administration of the dose, recoveries of the rare earths (% of ingested dose in stool) in individual samples and cumulative recoveries obtained by addition of sequential outputs. Standard deviations for individual recoveries represent measurement errors, whilst the deviations for the total recoveries take account of sum of the errors (99% confidence limit).

The recoveries of Sm and Yb with individual samples and composite of sequential outputs are depicted as a function of time post-dosing in **Figure-26.a-g**.

Table-62.a: Faecal recoveries of Sm and Yb markers for subject C1

SUBJECT C1						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	25.0	78.48±0.99	78.5	22.3	11.87±0.17	11.9
2	49.0	25.67±0.37	104.2	46.3	50.89±0.56	62.8
3	73.0	1.08±0.03	105.2	70.3	33.53±0.25	96.3
4	97.0	0.26±0.02	105.5	94.8	2.90±0.04	99.2
5	121.0	0.18±0.01	105.6	118.8	0.29±0.02	99.5
6	145.0	0.12±0.01	105.8	142.8	0.27±0.04	99.8
7	169.5	0.09±0.002	105.9	168.5	0.40±0.03	100.1
8	193.5	0.09±0.003	106.0			
Total recovery, %±3SD		106.0±3.3			100.1±1.8	

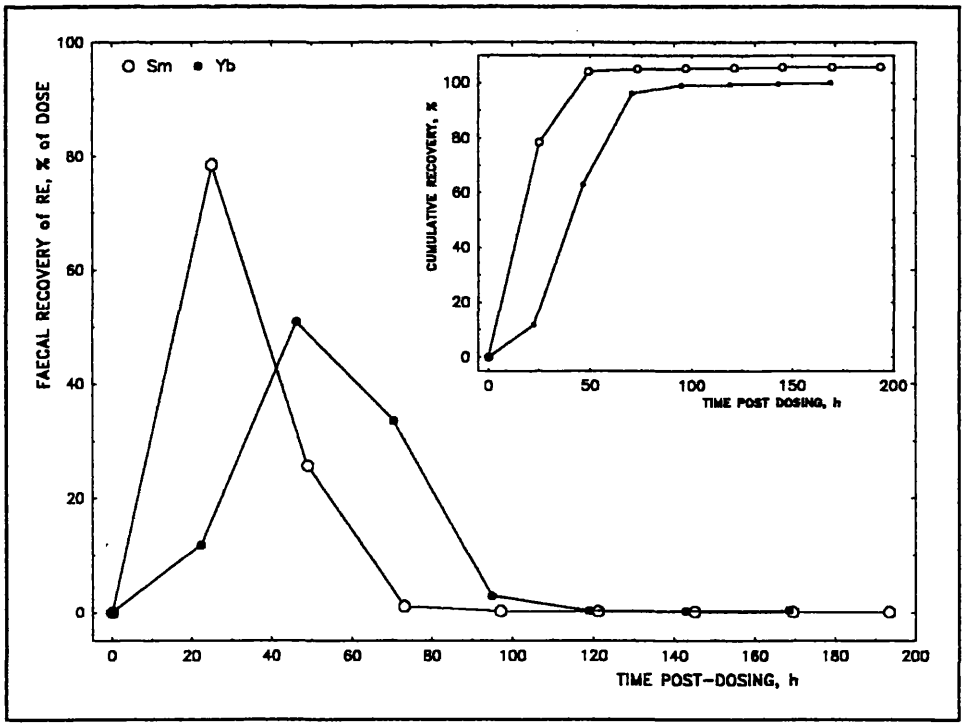


Figure-26.a: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C1

Table-62.b: Faecal recoveries of Sm and Yb markers for subject C2

SUBJECT C2						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	13.3	9.92±0.04	9.9	25.7	21.87±0.06	21.9
2	39.0	25.17±0.18	35.1	62.9	55.51±0.27	77.4
3	63.3	35.40±0.28	70.5	86.7	26.86±0.06	104.2
4	98.4	27.87±0.35	98.4	111.0	0.34±0.01	104.5
5	135.6	0.53±0.03	98.9	145.4	0.09±0.01	104.6
6	159.4	0.48±0.01	99.4	167.0	0.04±0.01	104.7
7	183.7	0.06±0.001	99.5	195.8	0.10±0.01	104.8
8	218.1	0.05±0.001	99.5			
Total recovery, %±3SD		99.5±1.5			104.8±0.9	

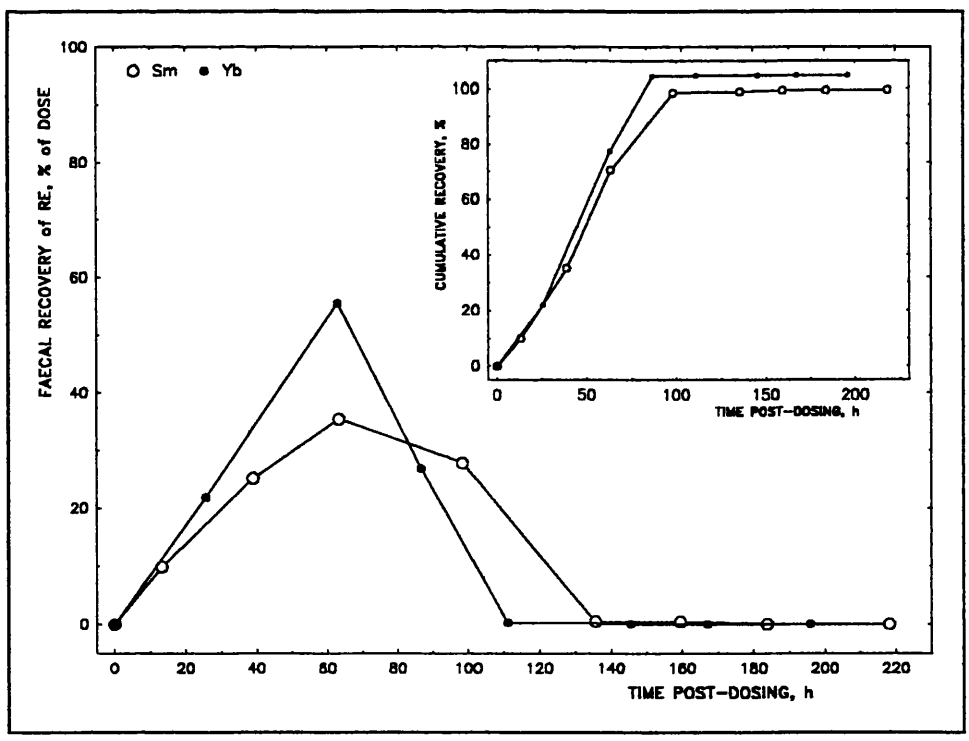


Figure-26.b: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C2

Table-62.c: Faecal recoveries of Sm and Yb markers for subject C3

SUBJECT C3						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	10.3	59.80±0.53	59.8	11.4	6.98±0.05	7.0
2	24.1	34.39±0.52	94.2	23.1	22.80±0.10	29.8
3	48.6	6.50±0.10	100.7	30.0	51.57±0.22	81.4
4	71.6	0.84±0.02	101.5	47.5	13.04±0.11	94.4
5	83.3	0.12±0.03	101.6	70.9	3.17±0.05	97.6
6	95.0	0.06±0.01	101.7	80.3	0.10±0.02	97.7
7	103.9	0.19±0.01	101.9	96.9	0.13±0.03	97.8
8	121.4	0.17±0.01	102.1	124.8	0.14±0.03	97.9
9	144.8	0.20±0.02	102.3	142.6	0.21±0.04	98.1
10	154.2	0.11±0.01	102.4	154.6	0.29±0.06	98.4
Total recovery, %±3SD		102.4±2.4			98.4±0.9	

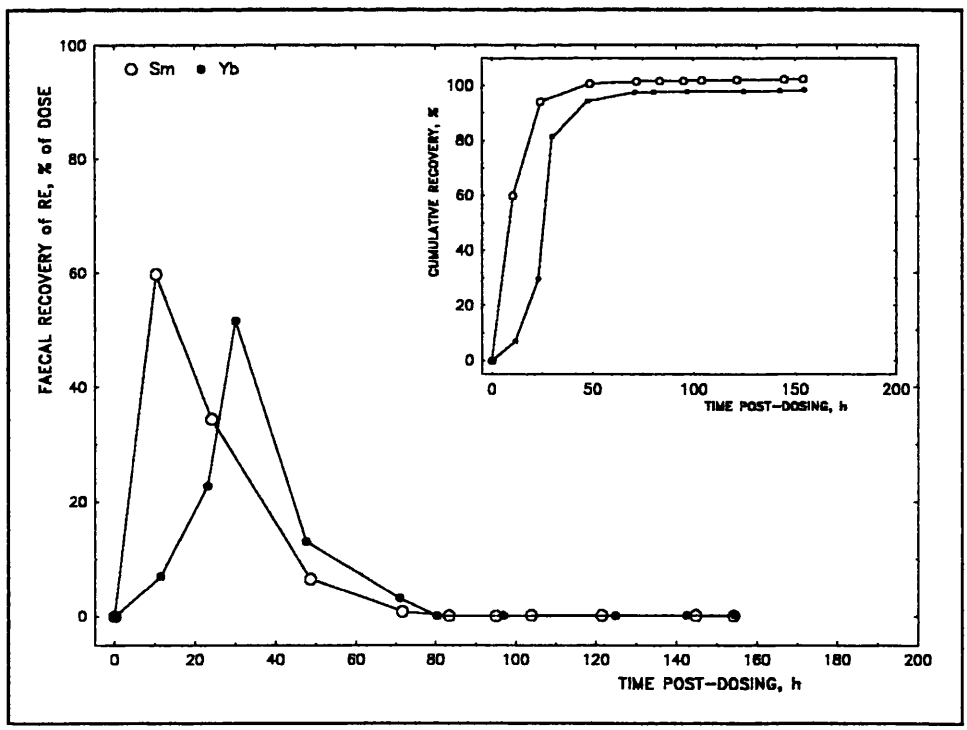


Figure-26.c: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C3

Table-62.d: Faecal recoveries of Sm and Yb markers for subject C4

SUBJECT C4						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	21.9	64.29±0.62	64.3	24.0	90.41±0.64	90.4
2	45.9	39.74±0.39	104.0	47.9	6.87±0.05	97.3
3	69.5	3.38±0.09	107.4	70.7	2.61±0.04	99.9
4	96.2	0.14±0.01	107.5	94.1	0.92±0.02	100.8
5	120.1	0.02±0.01	107.6	118.0	0.10±0.01	100.9
6	142.9	0.04±0.001	107.6	142.3	0.05±0.001	101.0
7	166.3	0.04±0.001	107.6	165.5	0.15±0.01	101.1
8	190.2	0.09±0.001	107.7			
Total recovery, %±3SD		107.7±2.1			101.1±1.8	

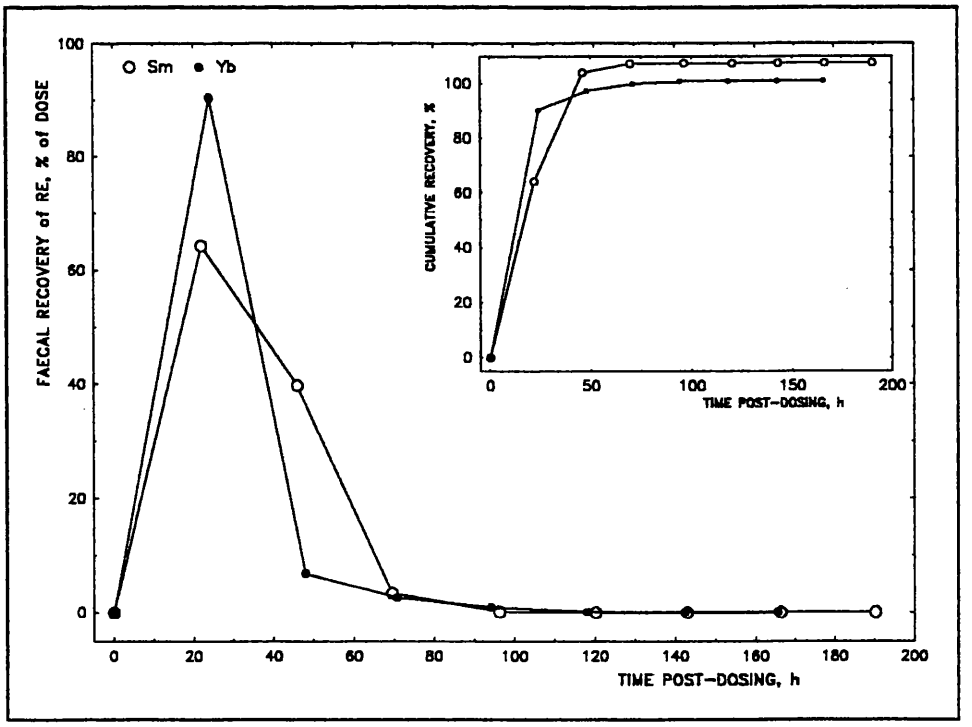


Figure-26.d: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C4

Table-62.e: Faecal recoveries of Sm and Yb markers for subject C5

SUBJECT C5						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	15.3	20.05±0.28	20.1	8.2	43.36±0.22	43.4
2	30.6	74.73±1.05	94.8	33.3	44.68±0.29	88.0
3	60.6	5.78±0.10	100.6	59.9	11.91±0.18	99.4
4	81.2	0.46±0.04	101.0	85.6	0.85±0.05	100.8
5	106.4	0.17±0.02	101.2	97.2	0.31±0.02	101.1
6	133.0	0.24±0.02	101.4	124.2	0.04±0.01	101.1
7	158.7	0.05±0.01	101.5	149.5	0.09±0.01	101.2
8	170.3	0.03±0.001	101.5			
Total recovery, %±3SD		101.5±3.3			101.2±1.2	

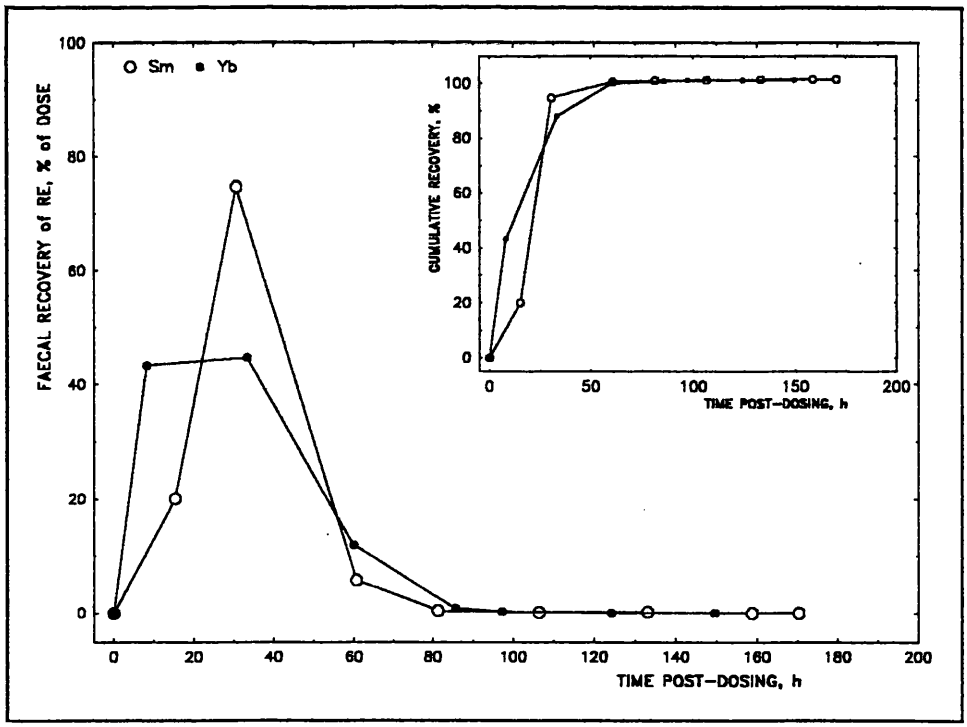


Figure-26.e: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C5

Table-62.f: Faecal recoveries of Sm and Yb markers for subject C6

SUBJECT C6						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	3.5	10.88±0.22	10.9	25.0	48.87±0.17	48.9
2	22.5	77.46±0.11	88.3	48.8	42.98±0.22	91.9
3	48.3	11.40±0.05	99.7	70.3	3.46±0.03	95.3
4	53.5	1.88±0.02	101.6	80.3	0.12±0.01	95.4
5	71.4	1.03±0.02	102.6	94.8	0.15±0.01	95.6
6	97.7	0.12±0.01	102.8	118.8	0.21±0.01	95.8
7	121.5	0.16±0.01	102.9	142.1	0.29±0.02	96.1
8	143.0	0.04±0.002	103.0			
9	153.0	0.11±0.002	103.1			
10	167.3	0.04±0.001	103.1			
Total recovery, %±3SD		103.1±0.9			96.1±0.9	

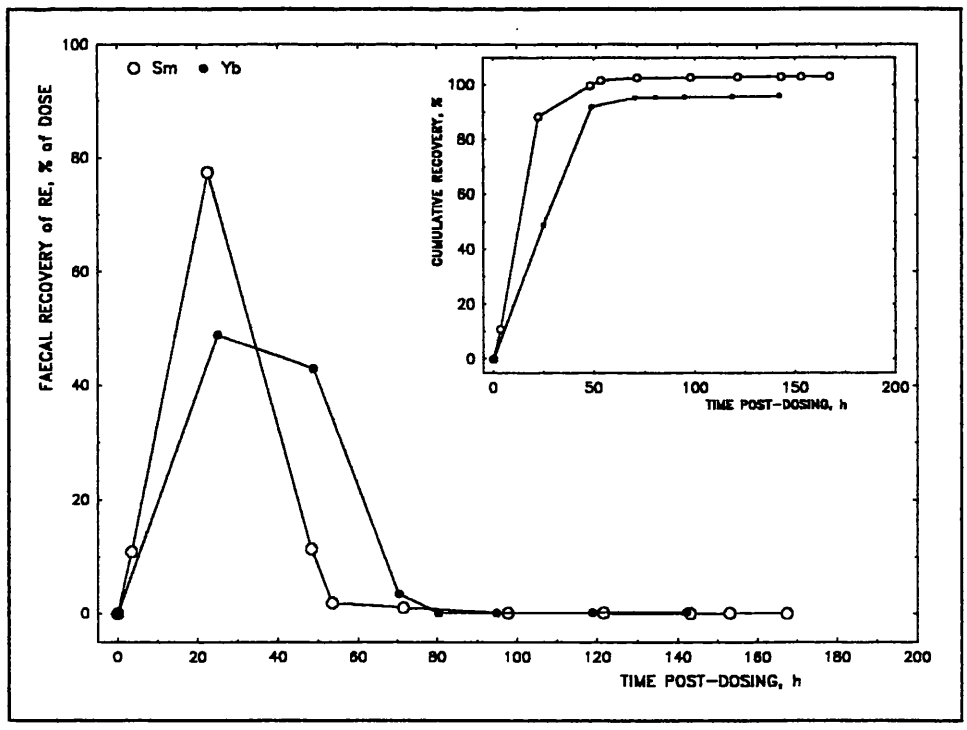


Figure-26.f: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C6

Table-62.g: Faecal recoveries of Sm and Yb markers for subject C7

SUBJECT C7						
FAECAL SAMPLE NO	TIME POST-DOSING, h	Sm RECOVERY		TIME POST-DOSING, h	Yb RECOVERY	
		INDIVIDUAL, %±SD	CUMULATIVE, %		INDIVIDUAL, %±SD	CUMULATIVE, %
1	8.5	10.20±0.23	10.2	23.0	69.18±0.38	69.2
2	23.4	74.02±0.32	84.2	36.8	18.62±0.13	87.8
3	47.8	13.67±0.07	97.9	47.8	6.42±0.12	94.2
4	73.3	1.42±0.08	99.3	72.3	1.71±0.06	95.9
5	95.8	0.36±0.02	99.7	81.6	0.31±0.002	96.2
6	109.6	0.10±0.01	99.8	94.3	0.22±0.01	96.5
7	120.6	0.07±0.01	99.8	118.3	0.23±0.01	96.7
8	145.1	0.08±0.001	99.9	142.3	0.25±0.03	96.9
9	154.4	0.03±0.001	99.9	166.7	0.24±0.03	97.2
10	167.1	0.09±0.001	100.0			
Total recovery, %±3SD		100.0±1.2			97.2±1.2	

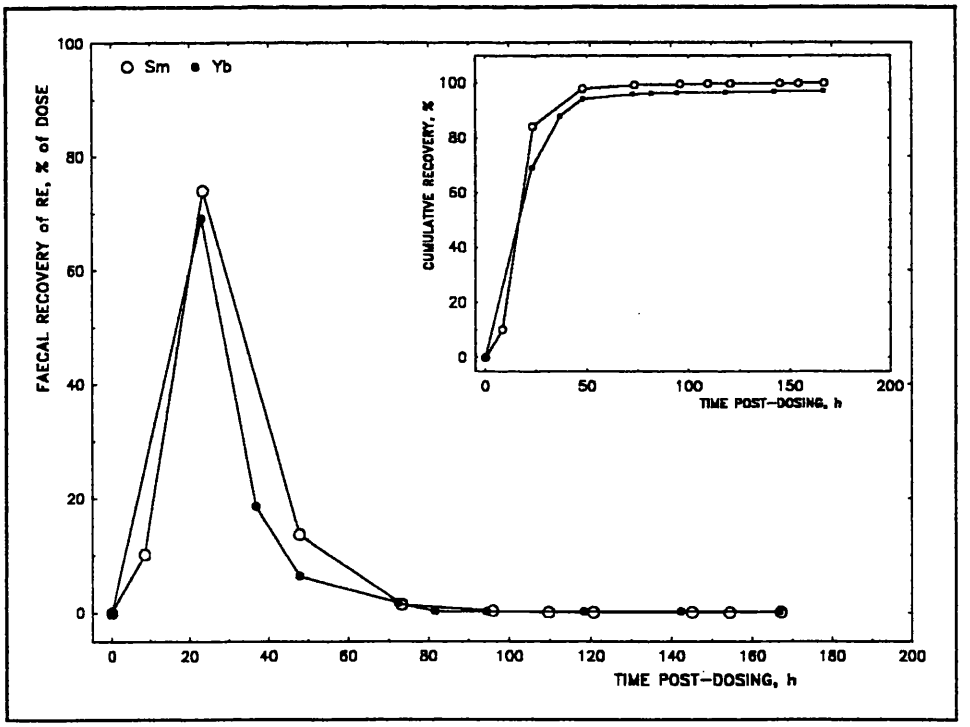


Figure-26.g: Faecal profile of Sm and Yb recoveries for individual samples and composite of sequential outputs for subject C7

III.3.2 DISCUSSION OF RECOVERY OF Sm and Yb MARKERS

III.3.2.1 RECOVERY OF Sm and Yb MARKERS

Recoveries of the rare earth markers were found to be essentially quantitative for all participants for both Sm and Yb despite the fact that their modes of intake were different. The means of marker recoveries were 102.8±3.0% (range of 99.5-107.7) for Sm which was taken with a drink, and 100.1±3.1% (range of 96.1-104.8) for Yb which was taken with a meal (**Table-63**). The mean recoveries for both Sm and Yb were nearer to 100% than those obtained in previous investigations, principally due to inclusion of toilet papers in sample collection, and improvements in sample collection and preparation.

Table-63: Summary of all faecal recoveries of rare earths

		Rare earth recovery, %±SD				
Initial, INAA		Extended, CNAA		S.I. Tracer study		
Subject	RE	Subject	RE	Subject	Sm	Yb
A1	89.5±1.9	B1	96.6±1.1	C1	106.0±1.1	100.1±0.6
A2	98.1±1.8	B2	100.2±1.1	C2	99.5±0.2	104.8±0.3
		B3	91.1±1.3	C3	102.4±0.8	98.4±0.3
		B4	94.2±0.4	C4	107.7±0.7	101.1±0.6
		B5	88.1±1.3	C5	101.2±0.4	103.1±0.2
		B6	94.5±1.3	C6	103.1±0.3	96.1±0.3
				C7	100.0±0.4	97.2±0.4
MEAN±SDM						
93.8±6.1			94.1±4.2		102.8±3.0	100.1±3.1

Quantitative recoveries were obtained after elimination of steps in the sample preparation procedure which caused sample losses by using a modern commode sample collection system, radiation sterilization instead of polythene bags for collection, and autoclave sterilization which resulted in incomplete sample transfer for ashing. This suggests that the lower recovery results were

due to losses during sample collection and processing rather than adsorption or absorption in the intestine.

A comparison of levels of the administered rare earths in natural (baseline) samples obtained before consumption of Sm and Yb and those in samples provided at the end of the collection period are provided in **Table-64** where it can be seen that the Sm and Yb concentrations in the final samples are comparable to natural levels.

Table-64: Comparison of Sm and Yb concentrations in ashed faecal samples provided at the end of collection period with natural levels

	Time post-dosing, h		RE in ash, μg g^{-1}	
Subject	Sm	Yb	Sm	Yb
C1	194	169	0.14±0.01	1.37±0.27
C2	218	196	0.16±0.01	0.21±0.06
C3	154	155	0.12±0.03	0.31±0.12
C4	190	166	0.15±0.01	0.25±0.06
C5	170	150	0.17±0.01	0.18±0.06
C6	167	142	0.28±0.02	0.45±0.09
C7	168	167	0.19±0.01	1.31±0.48
MEAN±SDM			0.17±0.05	0.58±0.52
RANGE			0.12-0.28	0.18-1.37
Natural level in pre-dose sample				
MEAN±SDM			0.17±0.02	0.43±0.32
RANGE			0.15-0.19	0.12-1.01

These results are further evidence that absorption of rare earths is negligible, or any possible absorption must be at a fraction of the natural levels.

III.3.2.2 DISCUSSION OF THE NATURAL LEVELS OF RARE EARTHS IN URINE AND FAECAL SAMPLES

Sm and Yb concentrations in urine samples

Urine samples were provided by 4 of the 7 participants. Samples were collected for 24 h before (for 3 subjects), and after each administration (for 4 subjects) bearing in mind results obtained by Kramsch *et al.* (1980) which indicated that when monkeys were fed a test meal containing 40 mg kg^{-1} body weight of La, the level of La in circulating blood returned to natural levels after 12 h (64.5 µg of La was detected in 24 h urine). Sm and Yb concentrations found in samples provided before and after administrations are compared to evaluate absorbability in **Table-65**.

Table-65: Concentrations of Sm and Yb in urine samples provided before and after consumption of a standard solution containing Sm and a farina meal containing Yb

	Sm or Yb concentration in urine (ng L^{-1} ±SDM)			
a. Sm				
	Mean	**Range**	**n**	**Statistical evaluation[1]**
Natural level	153 ±37	123-193	3	
After drink	1249 ±436	853-1824	4	$p<0.001$
After meal	162 ±82	73-262	4	$p>0.10$
b. Yb				
Natural level	137 ±70	80-287	7	
After meal	93 ±17	72-108	4	$p>0.10$

1. Null hypothesis is that the concentrations do not differs significantly from natural levels

As can be seen from the table, there is a significant difference between Sm concentrations determined in samples provided before administration and after consumption of the standard solution ($p<0.001$). This indicates the occurrence of slight absorption. The difference between the natural level of Sm and the level in samples provided 3 days after its administration (i.e. after the meal) is insignificant ($p>0.10$) which suggested that the Sm concentration returned

to the natural level. There was no significant difference between Yb concentrations in samples provided before or after the meal ($p>0.10$). This indicates that absorption of Yb from meal was not significant.

The slight absorption of Sm may be explained by the fact that when Sm is administered with a solution (with no food) it can not be hydrolysed completely since some intestinal secretion is only stimulated when a meal enters the intestinal system.

It is worth mentioning that Sm can form an absorbable complex with ascorbic acid. Hasegawa *et al.* (1988) showed that ascorbate, as a uninegative anion forms weak complexes with the rare earths. The formation of the Sm complex was calculated to be ~1% of the total Sm dose used in the current investigation (1 mg) using association constant of Sm ascorbate provided by Hasegawa and Sugawara. With the assumption of a steady state between Sm in blood and in urine, and a total blood volume of 5 L, the level found in urine is equivalent to absorption of approximately 0.5%. In conclusion, the slight absorption of Sm can be explained to the fact that a small fraction of the Sm dose remains in an ionic form (non-hydrolysed) and this forms absorbable complexes with ascorbate provided with the drink consumed or chelates provided by intestinal secretion. From data provided by Robinowitz *et al.* (1988), the absorbability of La was calculated to be <1% from concentrations determined in various tissues of rats fed with 40 mg d^{-1} La contained in drinking water. This could be considered as a further evidence of low but determinable absorbability of rare earths when they are administered to subjects who fasted overnight and consumed rare earths with a drink.

Hutcheson *et al.* (1975) found that no absorption occurred when three generations of mice were fed grains containing rare earth oxides and the same conclusion was reported by Luckey *et al.* (1975) for rats fed with pellets labelled with rare earth nitrates. The results for the absorbability of Yb consumed with a meal suggest that absorption of rare earths is very low when

they are administered with food as found by Hutcheson *et al.* (1975).

Rare earth concentrations in urine and faecal samples

The mean of natural levels of rare earths determined in ashed faeces and in urine samples are provided in **Table-66**. The table also contains rare earth concentrationsexpected in faeces calculated from the average intakes with a U.K. diet (MAFF Food Surveillance Paper No. 45, 1994), and La, Ce, and Sm concentrations in ashed faecal samples for a New York population (Linsalata and Eisenbud, 1986).

Table-66: Natural levels of rare earths in ashed faecal samples

		RE IN FAECES, µg g^{-1} ASH				
	RE IN	GLASGOW[1]		MAFF[2]	NEW YORK[3]	
RE	URINE, ng L^{-1}	Mean±SDM	RANGE	RANGE	MEAN	RANGE
La	87±52	1.35±0.52	0.82-2.26	1.25-0.75	0.8±0.5	0.2-1.5
Ce	188±130	1.76±0.68	1.14-3.34	4-1.50	2.3±1.6	0.4-5.4
Pr	142±25	0.31±0.11	0.19-0.52	0.5-<0.25		
Nd	126±43	1.04±0.33	0.69-1.63	1-0.75		
Sm	158±62	0.18±0.03	0.15-0.23	0.5-<0.25	0.12±0.09	0.02-0.28
Eu	81±25	0.09±0.07	0.04-0.24	0.5-<0.25		
Gd	84±33	0.19±0.06	0.11-0.28	0.5-<0.25		
Tb	66±23	0.06±0.05	0.02-0.18	0.5-0.00		
Dy	49±9	0.16±0.04	0.11-0.22	0.5-<0.25		
Ho	21±8	0.03±0.01	0.02-0.04	0.5-0.00		
Er	33±17	0.08±0.03	0.01-0.12	0.5-0.00		
Tm	11±7	0.02±0.003	0.01-0.02	0.5-0.00		
Yb	12±6	0.29±0.26	0.11-1.01	0.5-0.00		
Lu	4±11	0.02±0.004	0.01-0.02	0.5-0.00		

As can also be seen in **Table-66** concentrations of some of the light rare earths agree with those derived for the daily UK input per person (on the basis 4 g d^{-1} ash faecal sample), whilst the range for other rare earths are not

assessable since they are below the limits of detection. Comparison of theresults for current investigation and for those obtained for a New York population indicates that the inputs of rare earths via dietary intakes are comparable. Chondrite normalized rare earth concentrations in faecal and urine samples for this investigation, and those in faecal samples derived from the average intakes with a U.K. diet (MAFF, 1994) and provided by Linsalata and Eisenbud (1986) for a New York population are provided in **Figure-27**.

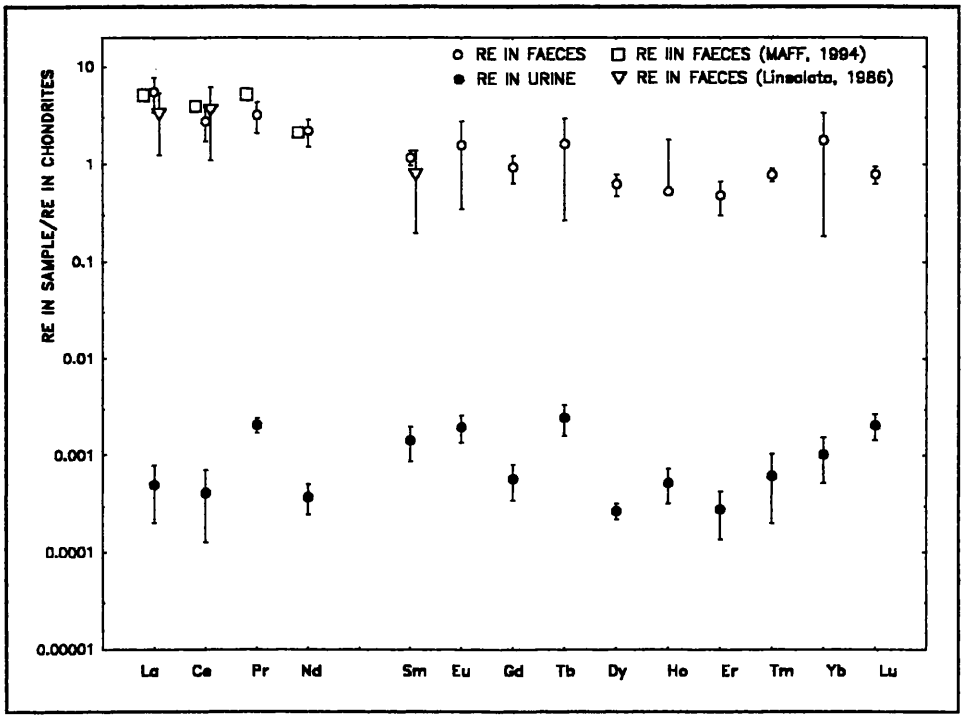


Figure-27: Chondrite normalized rare earth concentrations in ashed faeces and urine

As an be seen from the figure the results for faecal samples provide a smooth trend which is further evidence for the accuracy of the results.

In order to derive evidence of the extend of transfer of rare earths to the body, the total output of rare earths with faeces and urine on a daily basis were compared. By assuming daily faecal output of 4 g (ashed) and a urine output of 1.4 L d^{-1}, and taking account of the indicated levels in outputs, the fraction absorbed via intestinal system is found to be in a range of 0.006-0.19%. Calculated absorbances ranged from 0.006% for La and 0.09% for Eu,

representing light rare earths, and 0.04% for Er and 0.19% for Lu, representing heavier rare earths. Tjioe *et al.* (1983) have shown that there is reduced retention of the heavier rare earths in the human liver or an increased retention of the lighter rare earths. When the results for urine samples are considered alone, the observed anomalies suggest the conclusion of Tjioe *et al.* (1983) for the levels determined in the human liver.

III.3.2.3 FAECAL APPEARANCE PROFILE OF Sm AND Yb

The recovery profiles for Sm and Yb showed a similar kinetic pattern for all subjects. The first appearance time was in 24 h after administration. The patterns have a maximum marker content by 40 h, except for one, and the markers were recovered quantitatively with provision of 3-4 samples in 72 h. For all subjects the content of the rare earths in the fifth sample was below 1% of the doses administered.

Recoveries with the first samples were in a range of 10-80% for Sm, and 7-90% for Yb. Means of doses recovered with the first 2 samples 82±26% for Sm (range of 25.3-98.3, the range was 84.2-98.3% for 6 out of 7 subjects), whilst the mean of recoveries for Yb 75±24% were in a range of 30.3-96.2% containing major fraction of doses administered. The recoveries of both elements are nearly quantitative by collection of the third samples, and after collection of the fourth samples the remainder was below 1%. Recoveries obtained by summation of recoveries in 2,3, or 4 samples per subject are provided in **Table-67** together with the elapsed time between consumption and collection of the final sample. The overall means are compared with those obtained in the previous investigations. Recoveries with number of collection, showing the means of recoveries, are depicted in **Figure-28** as a function of time post-dosing.

Table-67: Recoveries of Sm and Yb obtained by addition of sequential faecal outputs

	2 samples		3 samples		4 samples	
Subject	Recovery, %	Time post-dosing, h	Recovery, %	Time post-dosing, h	Recovery, %	Time post-dosing, h
Results for Sm						
C1	98.3	49	99.3	73	99.5	97
C2	25.3	39	70.9	63	98.9	98
C3	92.0	24	98.3	49	99.2	72
C4	96.6	45	99.7	70	99.8	96
C5	93.4	31	99.1	61	99.5	81
C6	85.7	23	96.7	48	98.5	54
C7	84.2	23	99	48	99.3	73
Mean±SDM	82±26	33±11	95±11	59±11	99±0.4	82±17
Results for Yb						
C1	62.8	46	96.3	70	99.2	95
C2	63.8	63	99.5	87	99.8	111
C3	30.3	23	82.7	30	95.9	48
C4	96.2	48	98.8	71	99.7	94
C5	90.3	37	96.9	48	98.7	72
C6	86.0	33	97.6	60	98.4	86
C7	95.6	49	99.2	70	99.3	80
Mean±SDM	75±24	43±13	96±6	62±18	99±1.3	84±20
Results from the initial INAA investigation (2 subjects)						
Mean±SDM	78±25	38±7	94±10	62±6	98±2.3	85±8
Results from the detailed recovery investigation (6 subjects)						
Mean±SDM	64±26	38±4	89±13	54±8	98±2.1	73±8

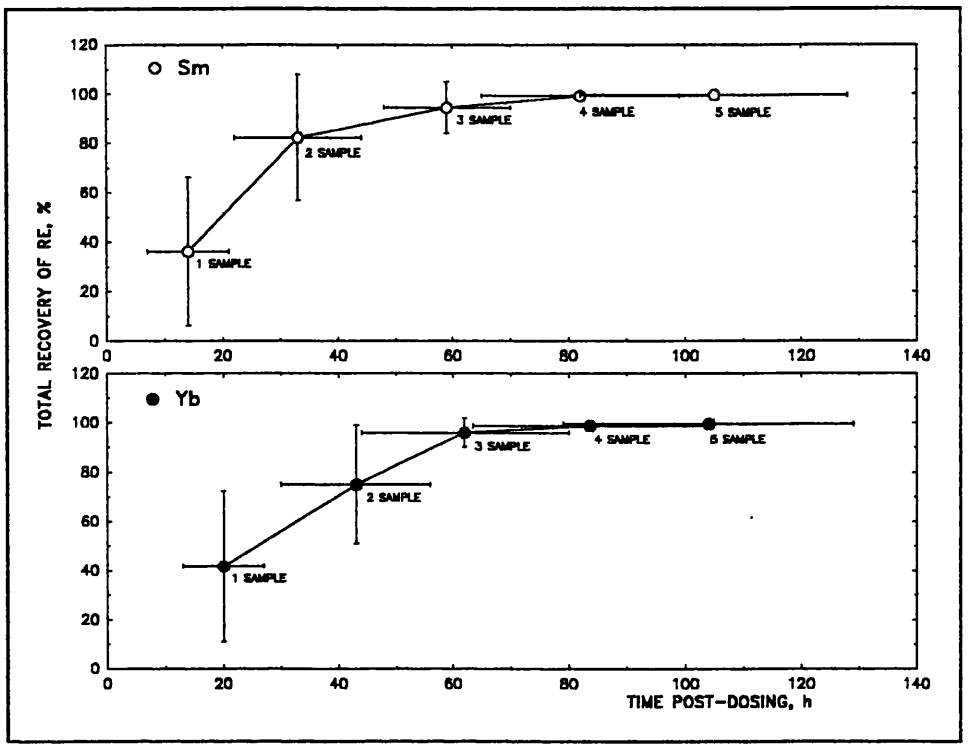


Figure-28: Total recoveries (±SD) of Sm (O), and Yb (●) as the mean of recoveries obtained by addition of sequential faecal outputs as a function of the mean of time post-dosing

As can be seen from the table and the figure, the Sm recovery rate is higher than that of Yb. This suggests that the intestinal passage time of Sm administered with a solution is shorter than that of Yb consumed with a meal. However the mean recovery of Sm with 2 samples was not significantly different from that with 4 samples, as was the case with Yb from 3 samples ($p>0.05$, paired t-test).

Results obtained in a kinetic evaluation of the transit time of Sm and Yb, using a logarithmic relation between fraction of retention and time post dosing (as described in section II.2.6.3) are provided in **Table-68** with regression coefficients for Log retention% and time post-dosing.

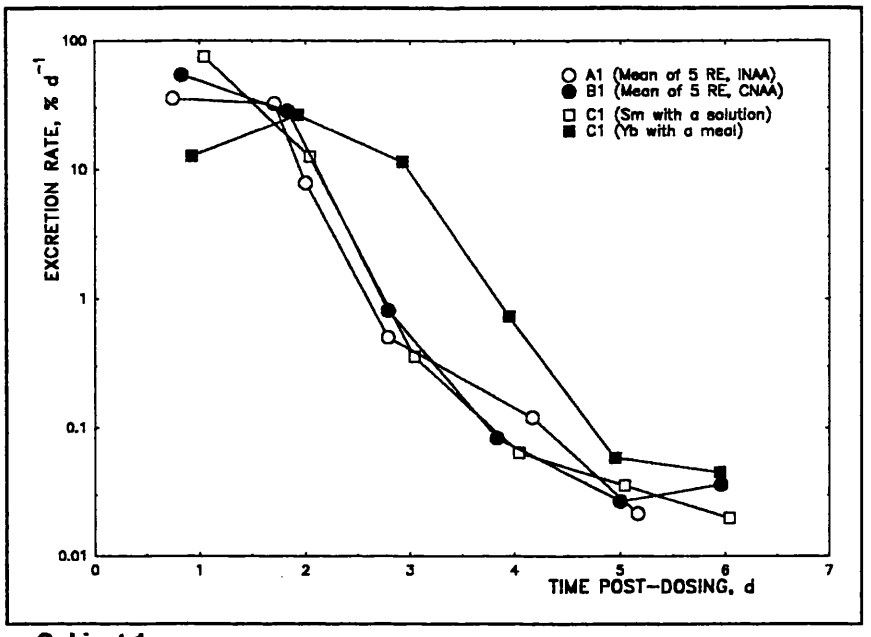
Table-68: Kinetic characteristics of Sm and Yb markers when they are administered with different modes of intake

Subject	First appearance h±SD	G.I. half-life h±SD	Time for total excretion h±SD	r^2
a. Characteristics for Sm consumed with a standard drink				
C1	7.1±2.0	14.7±4.2	58.3±16.6	0.958
C2	24.1±8.4	35.8±12.5	99.5±32.3	0.844
C3	3.8±0.8	9.8±2.0	43.8±8.8	0.953
C4	11.8±1.4	18.5±2.1	56.2±6.5	0.988
C5	7.2±1.5	15.4±3.3	61.5±13.1	0.951
C6	3.4±0.3	10.9±1.1	53.4±5.3	0.974
C7	3.6±0.4	11.9±1.4	59.1±7.2	0.980
MEAN±SDM	8.7±7.4	16.7±8.9	61.7±17.6	
b. Characteristics for Yb consumed with a reference meal				
C1	15.7±2.6	26.1±4.3	84.6±14.1	0.961
C2	28.7±8.7	37.9±11.5	89.8±27.3	0.901
C3	12.3±1.2	20.6±2.1	67.8±6.8	0.968
C4	NA	NA	NA	NA
C5	9.7±0.9	17.5±1.6	61.0±5.4	0.994
C6	7.7±2.0	18.4±4.8	79.1±20.6	0.988
C7	16.5±1.3	24.3±1.9	67.8±5.3	0.995
MEAN±SDM	15.1±7.5	24.1±7.5	75.0±11.1	
Results from the initial INAA investigation (2 subjects)				
MEAN±SDM	21.2±6.8	29.7±9.0	77.3±21.8	
Results from the detailed recovery investigation (6 subjects)				
MEAN±SDM	12.1±1.7	22.7±3.3	82.3±22.5	

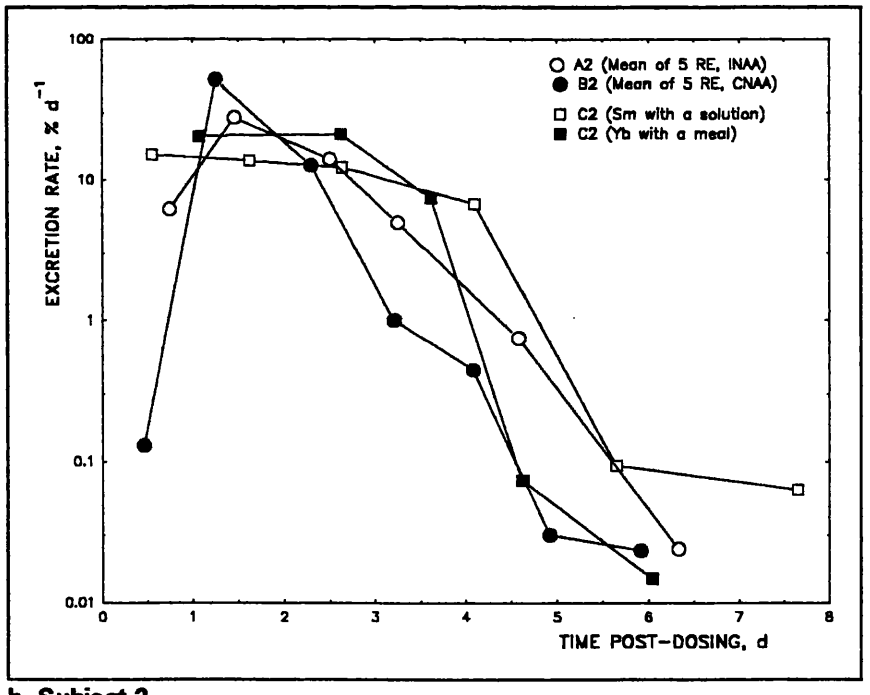
As can be seen from the table, it is confirmed that the first appearance time for Sm given with the standard drink is earlier than for Yb consumed with a meal ($p>0.05$, two-tailed t-test). Such measurements could be used to measure the passage time of diets or pharmaceuticals in the intestine.

The kinetic characteristic of the elements for subjects 1 and 2 who participated in the three investigations were compared, for evidence of

reproducibility, and are provided in **Figure-29.a** and **b**.



a. Subject-1



b. Subject-2

Figure-29: Comparison of faecal excretion rates of rare earths for subjects 1 and 2

The profiles obtained could be considered as characteristic of intestinal

physiology. Luckey *et al.* (1979) has suggested that the rate of excretion varies between individuals. Some have slow, some have fast excretion rates which are not related to age, sex, or life style but could be related to intestinal physiology. The excretion profile for Subject-1 indicates a more regular rate of excretion compared with that for Subject-2 despite the fact that both subjects consumed the rare earths with the same meal and/or solution.

III.3.3 LUMINAL DISAPPEARANCE OF IRON DETERMINED WITH ^{57}Fe TRACER AND Sm MARKER, CONSUMED AS A STANDARD SOLUTION

In this section, recoveries of marker and tracer are discussed with reference to their kinetic behaviour. The use of a rare earth marker to determine the luminal disappearance of Fe from a drink with single or limited faecal sampling is considered by comparing the results with those obtained from total collections of stools.

The recoveries of Sm and ^{57}Fe were determined for 5 consecutive faecal samples provided after administration. The number of samples collected were sufficient to recover non-absorbable marker quantitatively so that the kinetic behaviour of the marker and tracer could be evaluated.

Presentation of results

Recoveries and luminal disappearances have been calculated by applying **Equation-5.b** (Section I.5) to experimental data provided in **Appendix-5**. The recoveries of Sm marker and ^{57}Fe tracer, and luminal disappearance derived from recoveries are provided in **Table-69.a-g**.

Faecal recovery (%±SD) indicates the recovery of Sm marker and ^{57}Fe tracer with individual samples with ±SD derived from errors of measurement. Tracer recoveries take account of the endogenous contribution,

Luminal disappearance (%±SD) indicates the luminal disappearance with ±SD derived from the recoveries obtained for marker and tracer for **individual** samples (where applicable, otherwise indicated with **n.a.**) and for **cumulative** collections which are composites of the samples accumulated consecutively. Luminal disappearance derived from recovery of tracer, assuming no losses occurred during collection, are provided at the end of each table.

It can be seen that the luminal disappearances obtained for single samples are only provided for initial outputs, where the recoveries of the marker and tracer are significant, since those from later collections are low and include possibly re-excretion of tracer initially taken up. Profiles of faecal outputs for marker and tracer for individual samples and for total pools which are composites of summation of sequantial outputs are also provided in accompanying figures (**Figure-30.a-g**).

Table-69.a: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 1					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	25.0	78.48±0.99	35.40±1.51	**54.9±2.5**	54.9±2.45
2	49.0	25.67±0.37	17.12±0.54	**33.3±1.2**	**49.6±1.60**
3	73.0	1.08±0.03	2.17±0.05	n.a.	**48.0±1.49**
4	97.0	0.26±0.02	1.78±0.03	n.a.	**46.5±1.40**
5	121.0	0.18±0.01	1.07±0.03	n.a.	**45.5±1.46**
Total, %		**105.7±1.1**	**57.5±1.6**		
100 - Total ^{57}Fe recovery **42.5±1.6%**					

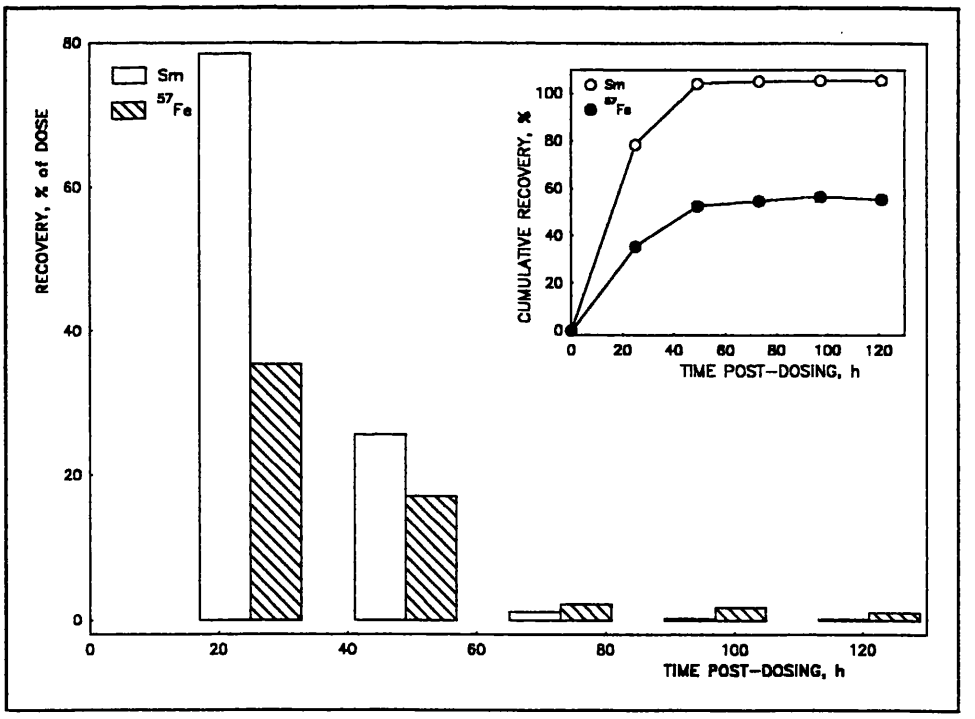


Figure-30.a: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output (Cumulative) for Subject-1

Table-69.b: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 2					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	13.3	9.92±0.04	7.57±0.22	**23.7±2.5**	23.7±2.5
2	39.0	25.17±0.18	18.98±0.51	**24.6±1.2**	24.3±1.6
3	63.3	35.40±0.28	23.87±0.54	n.a.	28.5±1.5
4	98.4	27.87±0.35	25.02±0.75	n.a.	23.3±1.4
5	135.6	0.530.03	5.30±0.18	n.a.	**18.4±1.5**
Total, %		**98.9±0.5**	**80.7±1.1**		
100 - Total ^{57}Fe recovery **19.3±1.1%**					

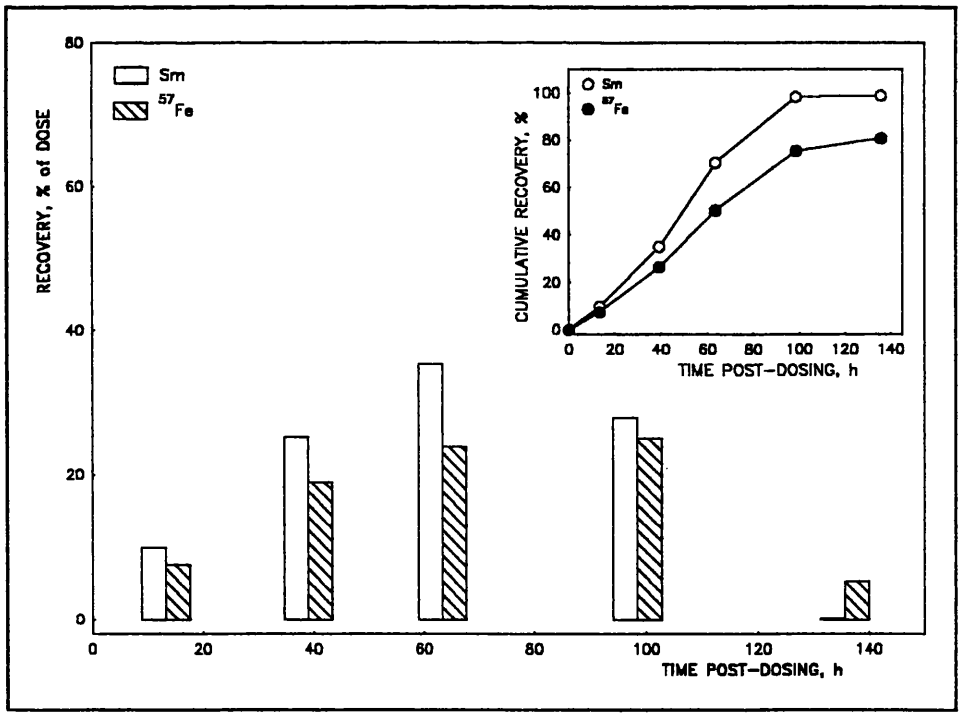


Figure-30.b: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output (Cumulative) for Subject-2

Table-69.c: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 3					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	10.3	59.80±0.53	39.66±0.81	**33.7±0.4**	33.7±0.7
2	24.1	34.39±0.52	20.65±0.55	**40.0±1.2**	36.0±0.6
3	48.6	6.50±0.10	8.21±0.22	n.a.	31.9±0.5
4	71.6	0.84±0.02	11.16±0.23	n.a.	21.5±0.3
5	83.3	0.12±0.02	2.24±0.05	n.a.	**19.4±0.3**
Total, %		**101.6±0.7**	**81.9±1.0**		
100 - Total ^{57}Fe recovery **18.1±1.0%**					

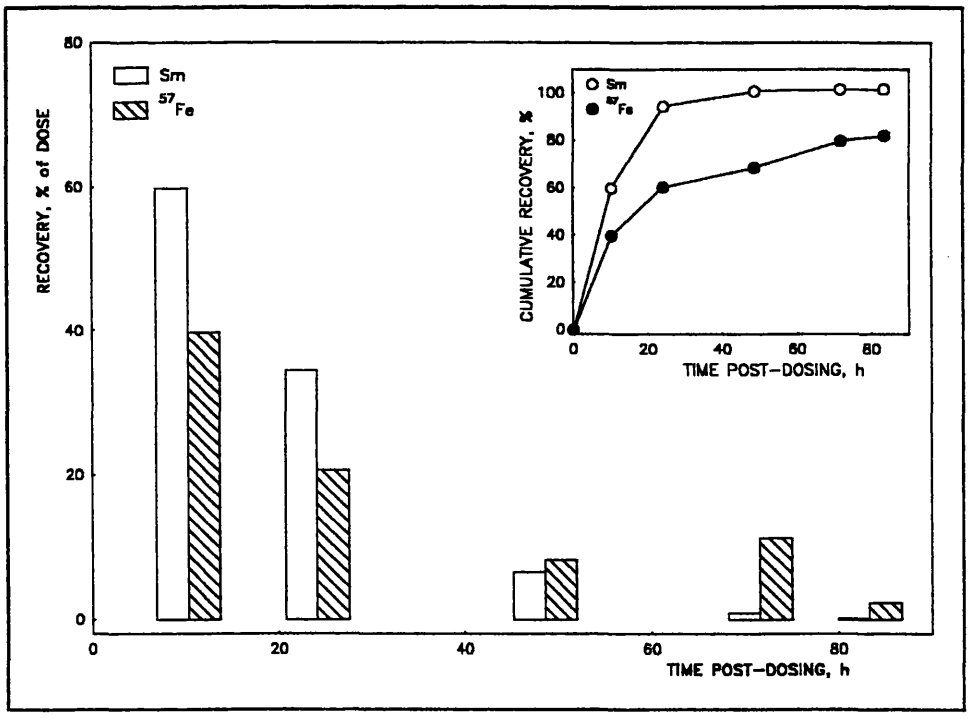


Figure-30.c: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output for (Cumulative) Subject-3

Table-69.d: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 4					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	21.9	64.29±0.62	55.18±1.42	14.2±0.3	14.2±0.4
2	45.9	39.74±0.39	29.52±1.26	25.7±0.4	18.6±0.4
3	69.5	3.38±0.09	5.85±0.15	n.a.	15.7±0.3
4	96.2	0.14±0.01	6.61±0.14	n.a.	9.6±0.2
5	120.1	0.03±0.01	1.66±0.04	n.a.	8.1±0.2
Total, %		107.6±0.7	98.8±1.0		
100 - Total ^{57}Fe recovery 1.2±1.0%					

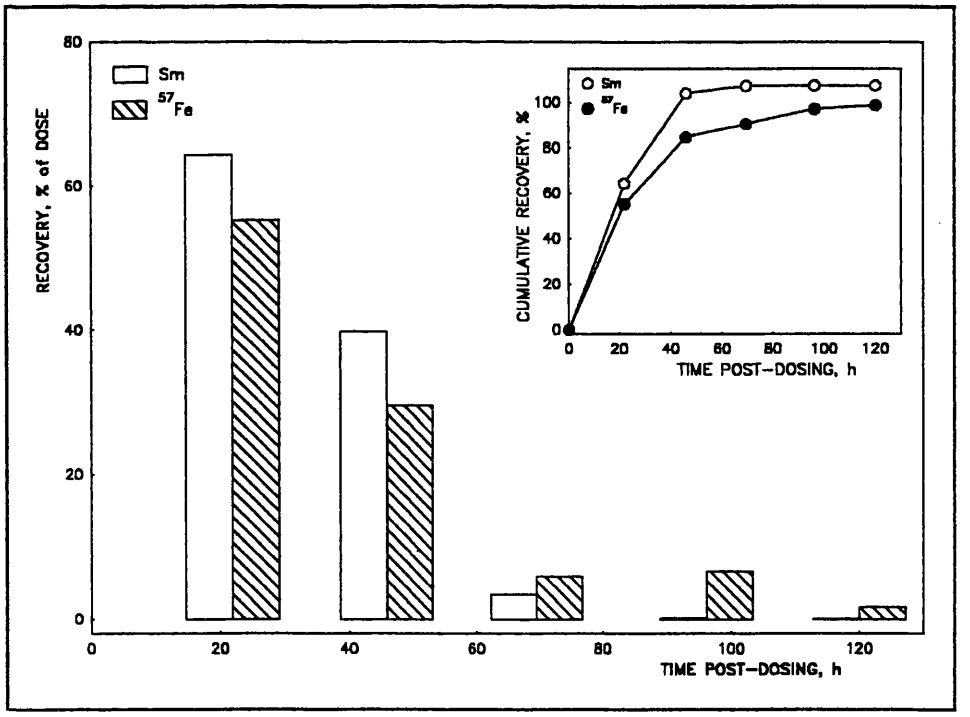


Figure-30.d: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output for (Cumulative) Subject-4

Table-69.e: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 5					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	15.3	20.05±0.28	16.69±0.57	16.7±0.6	16.7±0.6
2	30.6	74.74±1.05	33.53±0.57	55.1±1.2	47.0±0.9
3	60.6	5.78±0.10	10.25±0.27	n.a.	39.9±0.7
4	81.2	0.46±0.04	0.95±0.05	n.a.	39.2±0.7
5	106.4	0.17±0.02	0.87±0.02	n.a.	38.4±0.7
Total, %		101.2±1.1	62.3±0.9	37.7±0.5	
100 - Total ^{57}Fe recovery 37.7±0.9%					

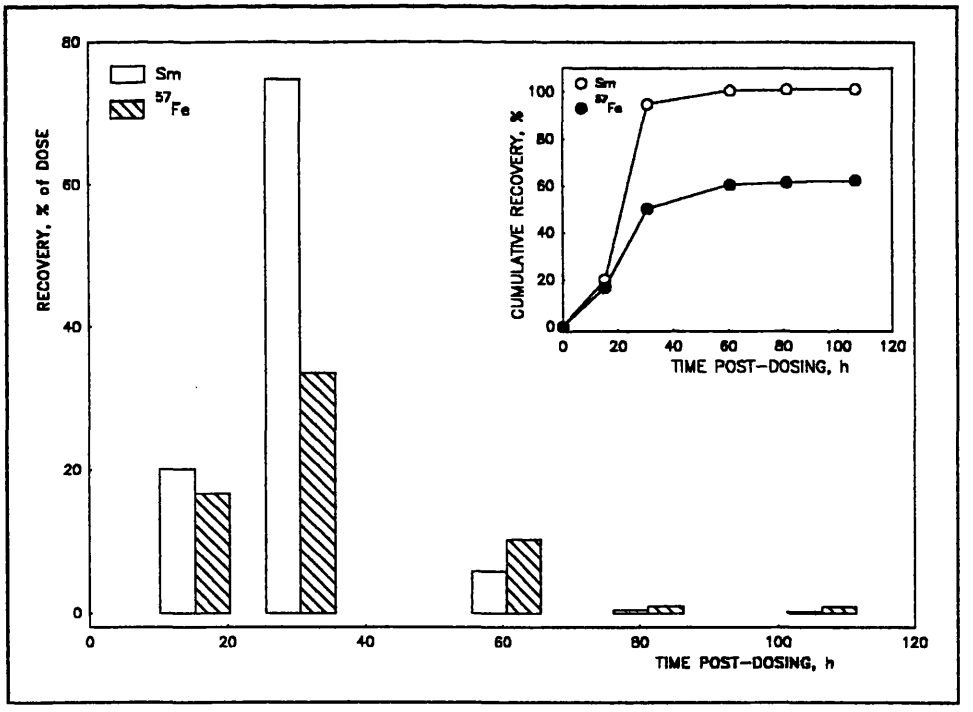


Figure-30.e: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output (Cumulative) for Subject-5

Table-69.f: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 6					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	3.50	10.88±0.22	7.92±0.26	27.3±1.05	27.3±1.0
2	22.5	77.46±0.11	72.18±1.58	6.8±0.15	9.3±0.2
3	48.3	11.40±0.04	10.13±0.29	11.2±0.32	9.5±0.2
4	53.5	1.88±0.02	3.38±0.06	n.a.	7.9±0.1
5	71.4	1.03±0.01	2.30±0.04	n.a.	6.6±0.1
6	97.7	0.12±0.02	1.83±0.04	n.a.	4.9±0.1
Total, %		102.8±0.3	97.7±1.6		
100 - Total ^{57}Fe recovery 2.3±1.6%					

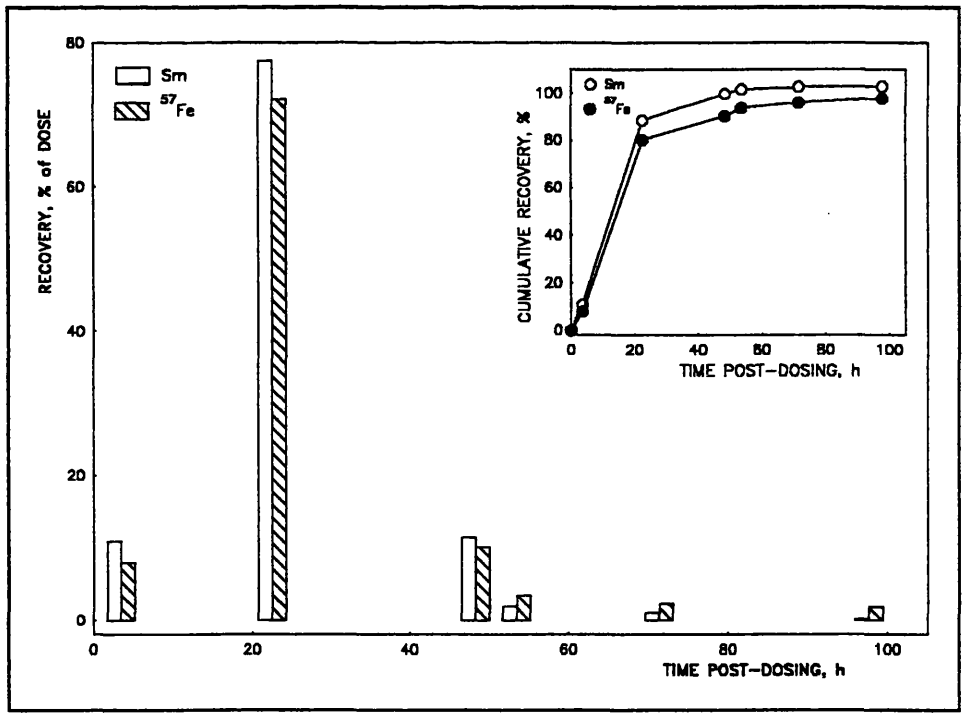


Figure-30.f: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output for (Cumulative) Subject-6

Table-69.g: Faecal recoveries of Sm marker, ^{57}Fe tracer, and luminal disappearance of iron, when consumed with a reference drink

SUBJECT 7					
Faecal sample No	Time post-dosing, h	Faecal recovery, %±SD		^{57}Fe Luminal disappearance, %±SD	
		Sm	^{57}Fe	Individual	Cumulative
1	8.5	10.20±0.23	9.58±0.31	**6.1±0.24**	6.1±0.2
2	23.4	74.02±0.32	39.55±1.69	**46.6±2.00**	41.7±1.5
3	47.8	13.67±0.07	10.01±0.41	**26.8±1.10**	39.6±1.2
4	73.3	1.42±0.08	2.71±0.09	n.a.	37.7±1.1
5	95.8	0.36±0.02	0.02±0.001	n.a.	**37.9±1.1**
Total, %		**99.7±0.4**	**61.9±1.1**		
100 - Total ^{57}Fe recovery **38.1±1.1%**					

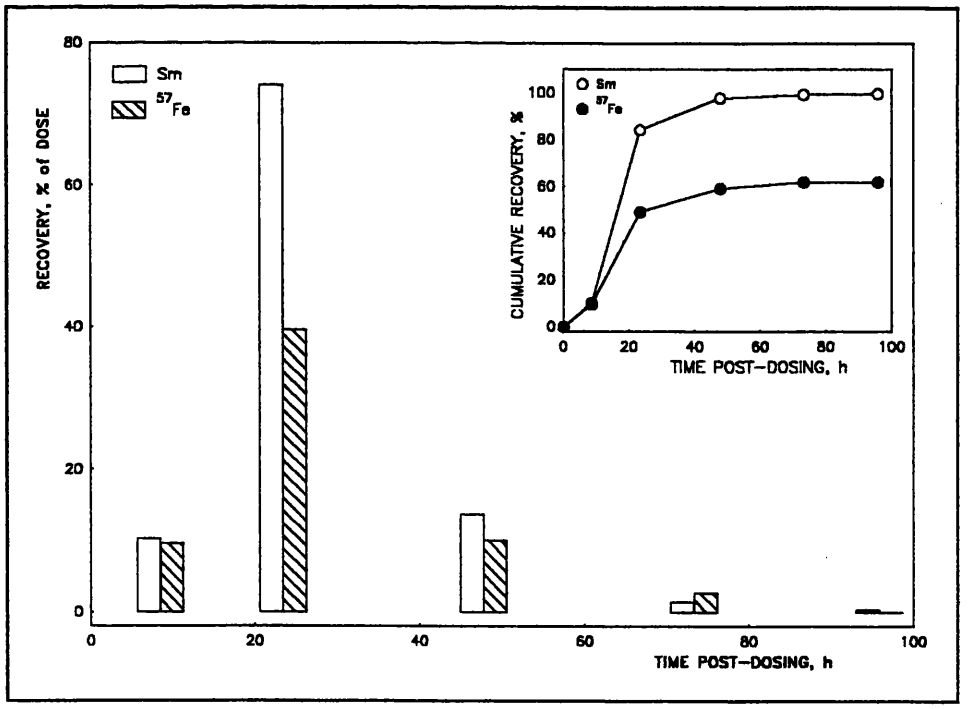


Figure-30.g: Faecal profile of Sm marker and ^{57}Fe tracer for individual samples and composites of sequential output (Cumulative) for Subject-7

III.3.4 DISCUSSION

III.3.4.1 RECOVERY KINETICS OF ^{57}Fe AND Sm

As can be seen from **Table-69.a-g** luminal disappearances derived from composites of the first 2-3 stools are different from those derived from the complete collection (composites of 5 samples). This can be explained by the intestinal passage time of marker and tracer being different or the tracer initially retained (mucosal uptake+mucosal surface adsorption) being released later and appearing in stools after the marker, i.e. the tracer and the marker have become dissociated.

For comparison of kinetic behaviour of marker and isotopic excreted tracer, the ratio of tracer to marker recoveries for individual samples and those derived from composites of sequential outputs for all subjectsare provided in **Figure-31** .

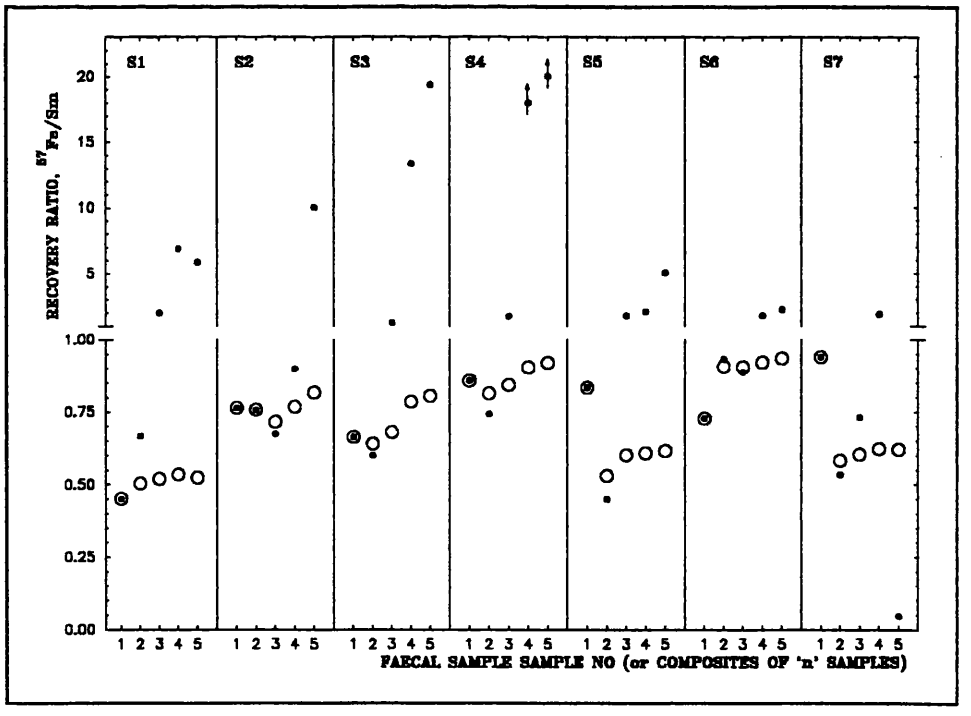


Figure-31: Ratios of faecal recoveries of ^{57}Fe tracer and Sm marker for individual samples, ●, and for composites of sequential outputs, ○, for all subjects

For the first faecal samples, the ratio of recoveries of the marker and tracer differs significantly from that derived from composites of the first two samples. Deviations of the first samples, as obtained for 3 subjects, are associated with marker recoveries less than 20%, and may be indicative of streaming. This suggests that the first faecal sample collected from an individual after consumption may not contain marker and tracer from which initial luminal disappearance can be determined so that at least the first two samples should be collected to evaluate the fraction initially retained.

The ratio of recoveries after the second samples show a trend to increase. This increase which is more obvious for individual samples suggests a slower loss of the tracer and could be indicative of release of ^{57}Fe which has been retarded in the gut perhaps by surface adsorption or mucosal uptake and release in desquamated cells. However Hayes *et al.* (1964) (by using radioactive La-140 marker and Fe-59 tracer) and Boender & Verloop (1969) (by using radioactive Ba-131 marker and Fe-59 tracer) found similar results where the participants consumed a standard solution. Ratios of recoveries derived from the results of Hayes *et al.* and Boender & Verloop are provided in **Figure-32**.

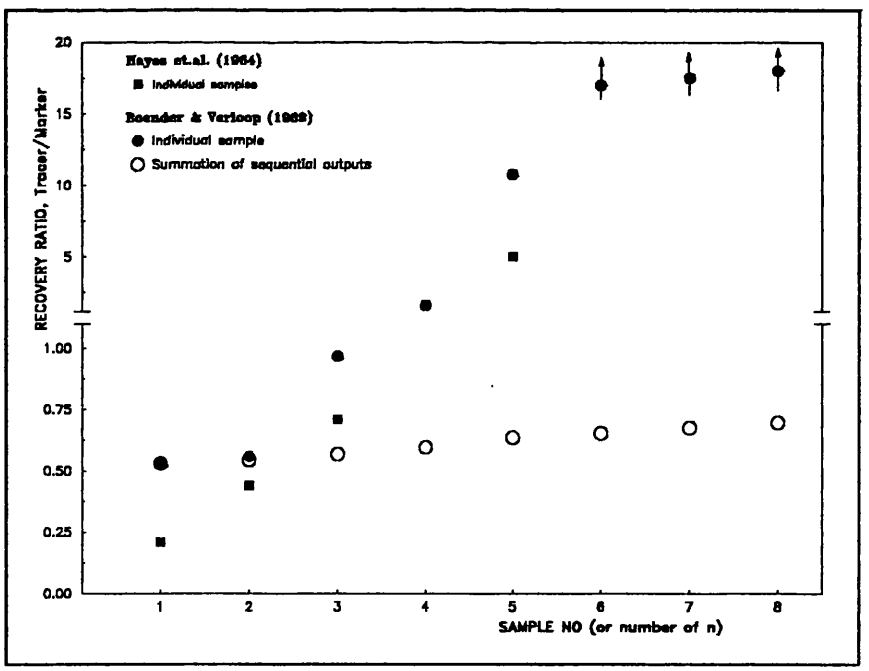


Figure-32: Ratio of faecal recoveries of tracer and marker derived from the results of Hayes *et al.* (1964) (data available for recovery ratio only) and Boender and Verloop (1969)

That the ratios of recoveries obtained in this investigation and those from Boender and Verloop (1969) agree indicates that retarded excretion of tracer becomes significant after the first 2-3 outputs.

The magnitude of the contribution of 're-excreted' tracer to the outputs obtained in composites of the third to fifth sequential faecal samples can be illustrated by the difference between fractions of the total recoveries of tracer and marker in individual faecal samples. The differences should be negative for samples in which tracer is initially retained or positive for samples in which 're-excretion' of tracer occurs, with an equal integrated area for both sides. The differences for individual samples obtained by this approach as a function of the sequence in which faecal samples were collected after consumption of marker and tracer are depicted in **Figure-33**.

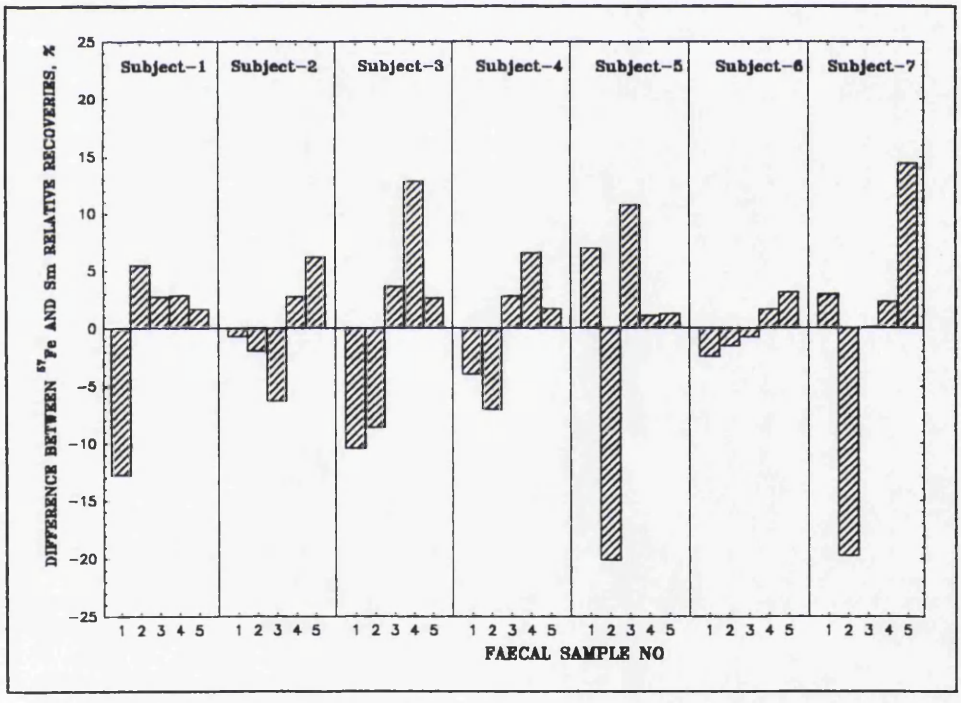


Figure-33: Differences between relative recoveries (percent of total output) of Sm marker and ^{57}Fe tracer for all subjects

For all subjects the net differences between relative recoveries of tracer and marker are negative for composites of the first 2 samples composite but is positive for the following samples which contains a fraction of tracer initially retained.

Evaluation of the kinetic behaviour of Sm marker and ^{57}Fe tracer with the above approaches suggests that net luminal disappearance obtained from the fractions of marker and tracer recovered with composites of the initial two or three faecal samples minimizes the contribution of 're-excreted' tracer. Linear relation between outputs of marker and non-retained tracer, derived from recoveries for all subjects, should provide the number of faecal samples required to obtain the net luminal disappearance in which contribution of 're-excreted' tracer is minimal.

The linear relation between the recoveries of marker and tracer with composites of the first two samples (since the recovery rates of marker and tracer with the first samples are not reliable) and with the following individual outputs, as percent of their total recoveries for composites of the first three to five faecal samples, for all subjects are provided in **Table-70**. The linearity for the composites of the first two samples is derived from the relative recoveries of marker and tracer obtained for composites of the first three samples. The linear relation is also derived for the collection of five stools for which the first and second outputs are considered as individual samples, to show the effect of the relative recoveries of marker and tracer obtained for the first samples, and provided in the table. The table contains linearity between the recoveries and its correlation coefficient.

Table-70: Linearity between the relative recoveries of Sm marker and ^{57}Fe tracer [y=(A±a)x+(B±b), where y and x are recovery of tracer and marker, and A and B are the slope and intercept]

Composites	A±a	B±b	R^2
3 samples (2 samples composites & 3. samples)	0.90±0.02	4.75±1.46	0.991
(2 samples composites)	0.89±0.07	6.54±5.87	0.971
4 samples (2 samples composites and 3. and 4. samples)	0.85±0.02	4.90±1.22	0.985
5 samples (2 samples composites and 3., 4., 5. samples)	0.82±0.02	4.25±0.98	0.980
5 samples (From the first to fifith individual samples)	0.77±0.03	4.51±1.00	0.945

As can be seen from **Table-70** that slopes of the linear relations which are the closest to the unity are obtained for the samples which the relative recoveries obtained from composites of the first three samples or composites of the first two stools of the three samples composites. This indicates that recoveries obtained for composites of the 2 or 3 samples are the most appropriate for calculation of luminal disappearance with minimum effect of 're-excretion' of isotope initially retained.

III.3.4.2 LUMINAL DISAPPEARANCE AND SIGNIFICANCE OF RETARDED EXCRETION

Having evaluated the excretion kinetics of marker and tracer, the difference between luminal disappearances derived from various composites of sequential faecal samples were considered to see the extent to which they were possibly effected by 're-excretion' of initially retained tracer. The mean of luminal disappearance obtained for individual samples for which it could be calculated, for composites of 1,2,3 and 4 samples, and for total collections together with marker recoveries for the corresponding groups of samples are provided in **Table-71**.

The mean of luminal disappearances obtained for the first two or three single samples provided high coefficients of variations for 6 of the 7 subjects due to different kinetic behaviour of marker and tracer in the first samples. The difference between the mean of luminal disappearances obtained for composites of sequential two and three samples, in which major fraction of marker was recovered were found to be insignificant ($p>0.10$), whilst the differences between those obtained for the composites of 1-4 samples, and the total collection (5 samples) were significant, $p<0.05$. This indicates the effect of 're-excretion' of tracer initially retained, as discussed above.

The differences in luminal disappearances obtained for sequential composites, SC, (2SC and 3SC, 3SC and 4SC, and 4SC and 5SC) are provided in **Figure-34** for each subject. The differences between those obtained for composites of sequential outputs and total collection (2SC and 5SC, 3SC and 5SC, and 4SC and 5SC) are provided in **Figure-35** for each subject.

Table-71: Luminal disappearences of ^{57}Fe tracer for single (the means of luminal disappearances obtained for the first, second, and third individual faecal samples, n_1) and composites of sequential (from 1 to 5) outputs, and recoveries of Sm marker

Subject	LUMINAL DISAPPEARANCE, %						
	For single samples %±SDM	n_1	Composites of 'n' sequential samples				
			1 Sample	2 Samples	3 Samples	4 Samples	5 samples (Total collection)
1	40.9±15.3	2	54.9	46.7	45.1	43.4	42.5
2	24.1±0.6	3	23.7	24.3	28.5	23.3	18.4
3	36.8±4.4	2	33.7	36.0	31.9	21.5	19.4
4	13.6±9.2	2	7.6	12.4	9.3	2.8	1.2
5	35.9±27.2	2	16.7	47.0	39.9	39.2	38.4
6	12.7±11.1	3	25.2	6.8	7.0	5.3	2.3
7	26.5±20.2	3	6.1	41.7	39.6	37.7	37.9
Mean±SDM	27.2±11.3		24.0±16.8	30.7±16.4	28.8±15.1	24.8±16.3	22.9±17.2
Sm recovery, %±SDM	not applicable		36±30	86±24	98±12	102±3	102±3
#Time±SDM	n.a.		13.9±7.4	34±11	59±11	81±17	109±18

The mean of time post-dosing (h) for provision of n^{th} sample

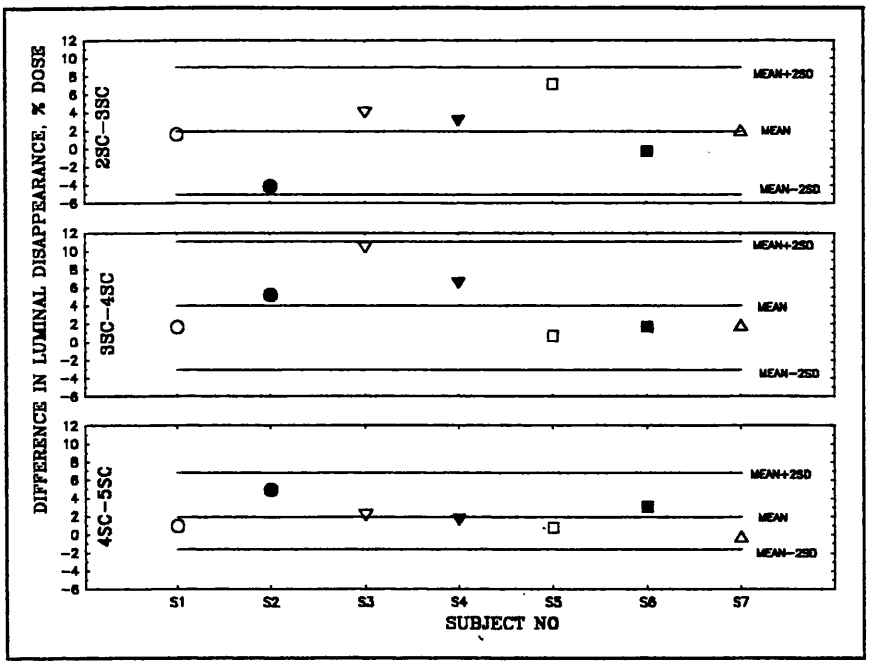


Figure-34: Differences between luminal disappearances obtained for composites of sequential 2-5 samples with means and 95% confidence limits

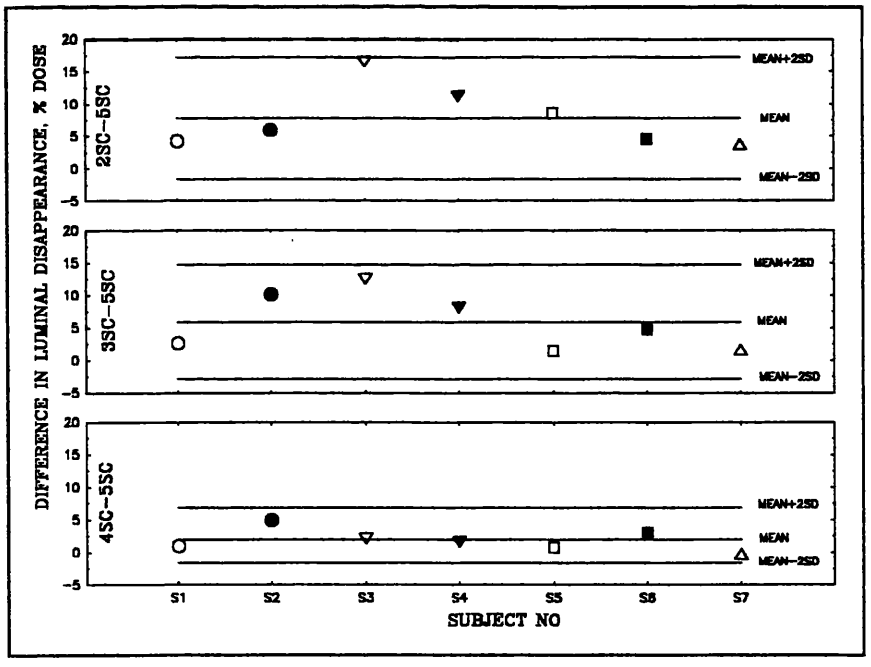


Figure-35: Differences between luminal disappearances obtained for composites of sequential 2-4 samples and total collection with means and 95% confidence limits

The differences between luminal disappearances obtained for composites of sequential the first two and three, three and four, and four and five samples were 1.9±3.5, 4.0±3.5, and 1.9±1.7% of the dose administered respectively. The differences were not significantly different from each other ($p>0.05$). Differences between luminal disappearances of composites of sequential the first two to four samples and of total collection (five samples composite) were 7.8±3.5, 5.9±3.5, and 1.9±1.7% of the dose for the first two and five, three and five, and four and five.

Initial luminal disappearance that can not be measured by faecal monitoring, in which the contribution of tracer initially retained is the least can be derived from the differences described above. The differences as a function of the mean of time post-dosing for provision of the first two, three, and four samples (34, 59, and 81 h respectively) are provided in **Figure-36.A** and **B**.

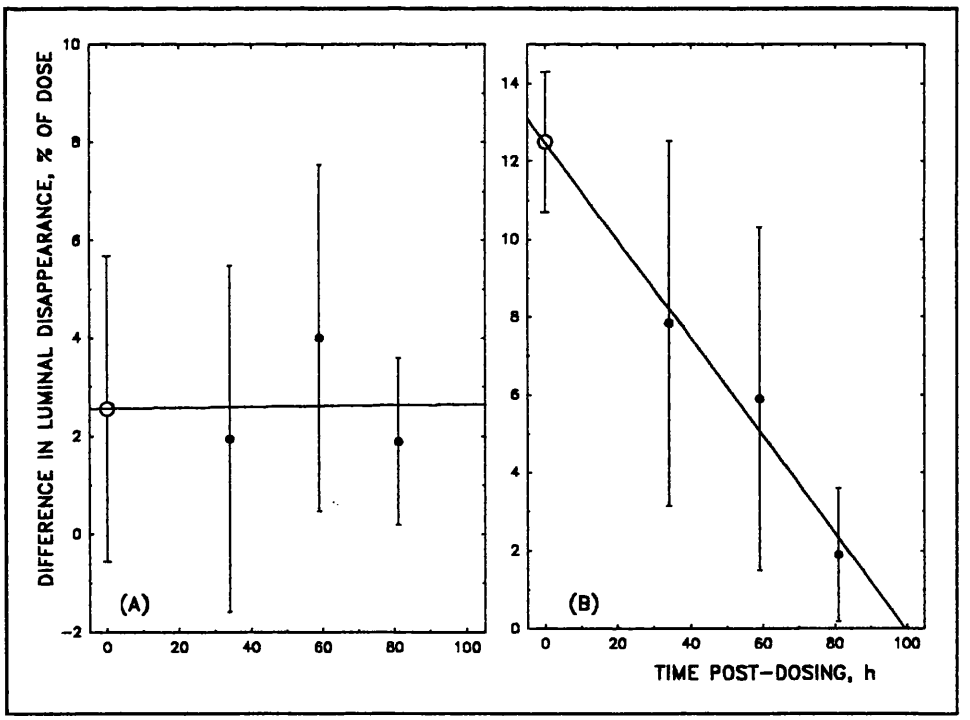


Figure-36: The mean of differences between luminal disappearances of composites of sequential pairs in 2-5 samples (A), [$Y=(8.2x10^{-4}\pm0.05)X+(2.6\pm3.1)$, $R^2=0.0003$], and between those of composites of sequential 2-4 samples and total collection (B), [$Y=-(0.1\pm0.03)X+(12.5\pm1.8)$, $R^2=0.946$] (O indicates the differences at t=0 by extrapolation)

From the relations between the differences and time post dosing, the difference between luminal disappearances of the initial and of composites of the first two sequential samples can be calculated to be 2.6±3.1%, and that between luminal disappearances of the initial and of total collection is 12.5±1.8%. By addition of these differences to the luminal disappearances obtained for composite of the first two sequential samples (30.7±16.4%) and of total collection (22.9±17.2%), the initial luminal disappearances are found to be 33.3±16.5% and 35.4±17.3%. The initial luminal disappearance found with both approaches are not significantly different from that determined for composites of the first two sequential samples, $p>0.05$.

Marx (1979) suggested that the net luminal disappearance can be measured accurately only shortly after ingestion of a test dose. In normal subjects when the tracer retained is measured 72 h after ingestion the values are lower than the initial measurement due to excretion of iron initially retained. The results of this investigation confirm this conclusion. A similar effect has been observed in earlier studies where La, Cr, and Ba were used as radioactive markers by Hayes *et al* (1964) (La-140), Powell *et al* (1970) (Cr-51), and Boender and Verloop (1969) and Jasani *et al* (1971) (Ba-131) respectively where the marker and Fe-59 tracer were consumed in a drink.

By considering the retarded excretion of tracer as a fraction of the dose consumed (3 mg of iron), approximately 30% of 1 mg initially retained iron is excreted within 5 days (mean luminal disappearance was ~20%) of administration, at 0.06 mg per day. When the losses from initial retention are considered with reference to the biological half-life of iron (483 d, Hunt *et al.*, 1994), the results obtained, when a tracer is consumed with a drink, indicate that the amount initially retained in the intestinal mucosa is not absorbed or transferred into the body through the mucosa.

The mean and range of luminal disappearances obtained in this study and those obtained for the same mode of intake but using radioactive marker and

tracer (**Table-72**) are similar and so suggest the applicability of rare earths as marker in investigations of Fe absorption from a standard Fe solution by faecal monitoring. With radioactive tracers net luminal disappearance (with the collection of initial 2-3 samples) has been determined in patients with iron deficiency anaemia, idiopathic haemosiderosis (genetic haemochromatosis) where the mucosal transfer index is close to 1, i.e. the fraction of Fe-59 tracer initially taken up by the intestinal mucosa is the same with that retained in the body when measured 2 weeks after the administration (Marx, 1979). The kinetic evaluation of results of this current investigation also suggest that initial luminal disappearance can be obtained from composites of the two or three samples so that similar applications with stable rare earth markers should be possible.

Table-72: Summary of measurements of luminal disappearance of iron when consumed as a drink (**FM**, faecal monitoring and **WBC**: whole body counting)

Subject no (sex)	Iron uptake %±SD & range	Dose, mg in intake mode	Tracer, marker and method	Reference
6 (M)	29.3±11.1 (17-47)	1 mg in drink	Fe-59, Ba-131 (FM)	Boender & Verloop, 1969
15 (M)	37.6±16.8 (3.6-60.7)	1 mg in drink	Fe-59, Cr-51 (FM and WBC)	Marx, 1979
10 (F)	42.8±16.2 (13.1-68.3)			
11 (M)	22.8±9.4 (3-38)	8.8 mg in drink	^{58}Fe, Carmine (FM and NAA)	Fairweather-Tait et al, 1983
10 (4M, 6F)	29±5.1	10 mg in drink	^{58}Fe, Carmine (FM and NAA)	Fairweather-Tait and Minski 1986
7 (6M, 1F)	22.9±17.2 (1.2-45.5)	3 mg in drink	^{57}Fe, Sm (FM, NAA, ICP-MS)	This study

III.3.4.3 LUMINAL DISAPPEARANCE OF ^{57}Fe IN RELATION TO IRON STATUS

Marx (1979) described iron absorption as occurring in two phases: firstly, mucosal uptake from the intestinal lumen and secondly transfer of iron through the mucosal cells towards the blood. A transferrin-like protein identified as isoferritin is responsible for the rapid transfer of iron from brush border to the blood. The amount of this protein in mucosal cells binds various amounts of iron, and eventually releases this iron to the blood at a rather slow rate. In normal subjects a considerable amount of iron is apparently not released from the ferritin but desquamated with these cells into the lumen of the gut, resulting in relatively low values for the mucosal transfer fraction of iron.

A proportion of the iron entering the mucosal cells is transferred to the portal circulation within minutes. Transfer continues at a much slower rate for 12-24 h, but some of the iron is sequestered as ferritin and is eventually lost when the mucosal cell exfoliates. The relative proportions following these alternative pathways depend on the requirement for iron, transfer being enhanced when iron deficiency is present and ferritin formation being maximal when the body is replete with iron (Charlton and Bothwell, 1983).

A linear relationship between serum ferritin concentration and body iron stores has previously been demonstrated by Cook *et al.* (1974) and Lynch *et al.* (1989). A regulatory function for both serum and mucosal ferritin in iron absorption had been proposed by Halliday *et al.* (1978). Subsequently with immunologic studies, it is consistent that ferritin being a storage protein which incorporates iron to prevent oxidative damage to the cell rather than it being a regulator of iron absorption (Conrad and Umbreit, 1993). However Conrad *et al.* (1993) postulated that mucosal uptake of iron from the gut lumen was mediated via integrin-mobilferrin pathway; 'Dietary nonheme iron is solubilized at acidic pH of the stomach. At acid pH, it combines with mucins that maintain the iron soluble and available in the more alkaline small intestine. Integrins

that traverse the luminal surface of the absorptive cell may facilitate the uptake of iron into the mucosal cell in association with mobilferrin. Mobilferrin is a cytosol protein that is probably important in the regulation of iron absorption.'

A significant correlation between serum ferritin (as an index of iron status) and luminal disappearances obtained for both composite of the first 2 samples ($r=-0.807$, $p<0.05$), and completion of collection ($r=-0.725$, $p<0.10$) was found (**Figure-37**). These correlations might suggest that luminal disappearance of ferrous iron is related with ferritin, as postulated by Walters *et al.* (1975). Agreement between the correlations obtained for the luminal disappearances of the first 2 samples composite and total collection (5 samples) could also be considered as evidence that a fraction of iron initially retained in the intestine is excreted with exfoliated mucosal cells in which ferritin is associated with the tracer retained in the mucosa (Marx, 1979).

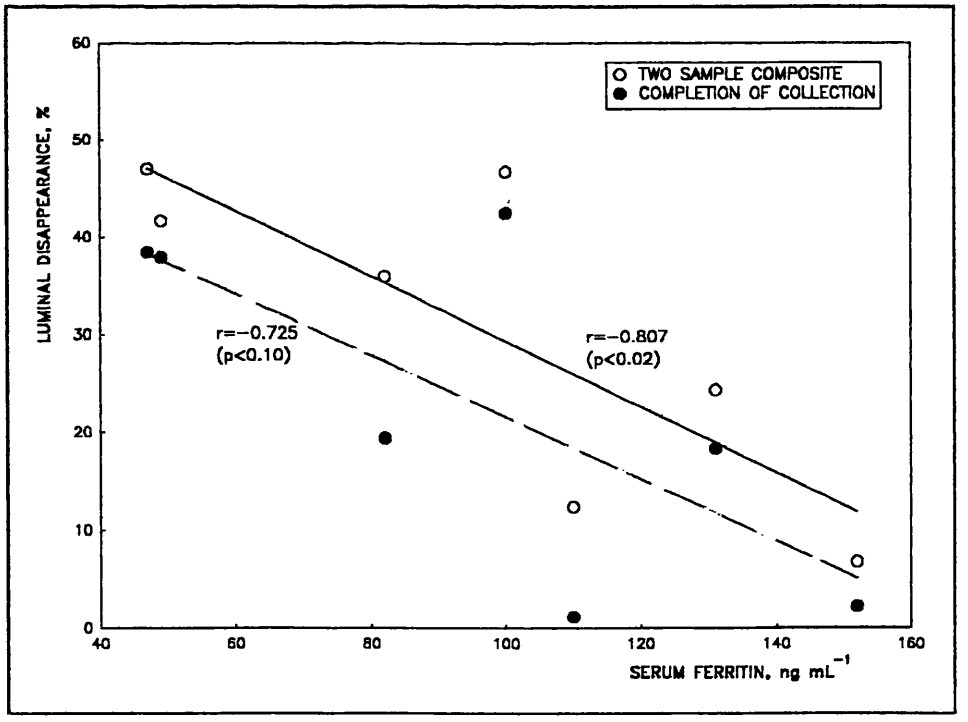


Figure-37: Correlation between serum ferritin and luminal disappearances obtained for composite of the first two stools (solid line), and total collection (dashed line)

III.3.5 LUMINAL DISAPPEARANCE OF IRON CONSUMED WITH A FARINA MEAL, DETERMINED WITH ^{58}Fe TRACER AND Yb MARKER

Faecal recoveries of Yb marker and non-retained ^{58}Fe tracer and luminal disappearance of tracer derived from the recoveries are provided in this section. Luminal disappearances determined for individual and composites of sequential samples, and for total collection following consumption of a standard farina meal are provided and considered with reference to the intestinal kinetic profiles of marker and tracer.

Recoveries and luminal disappearances have been calculated by applying **Equation-5.b** (Section I.5) to experimental data provided in **Appendix-6**. Recoveries of Yb marker and ^{58}Fe tracer and luminal disappearance derived from recoveries are provided in **Tables-73.a-g**. The nature of the contents of the tables are explained in the previous section. Recoveries of marker and tracer with single and composites of sequential collections are depicted as a function of time and provided in the accompanying **Figures-38.a-g**.

Table-73.a: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 1					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	22.3	11.87±0.17	10.30±0.11	13.3±0.2	13.3±0.2
2	46.3	50.89±0.56	43.86±0.29	13.8±0.2	13.7±0.2
3	70.3	33.53±0.25	27.81±0.20	17.1±0.2	14.9±0.1
4	94.8	2.90±0.04	3.30±0.09	n.a.	14.0±0.1
5	118.8	0.29±0.02	0.84±0.08	n.a.	13.4±0.1
6	142.8	0.27±0.04	0.60±0.17	n.a.	13.1±0.1
7	168.5	0.40±0.03	0.52±0.09	n.a.	12.9±0.1
Total, %		100.1±0.6	87.2±0.7		
100 - Total ^{58}Fe recovery 12.8±0.70%					

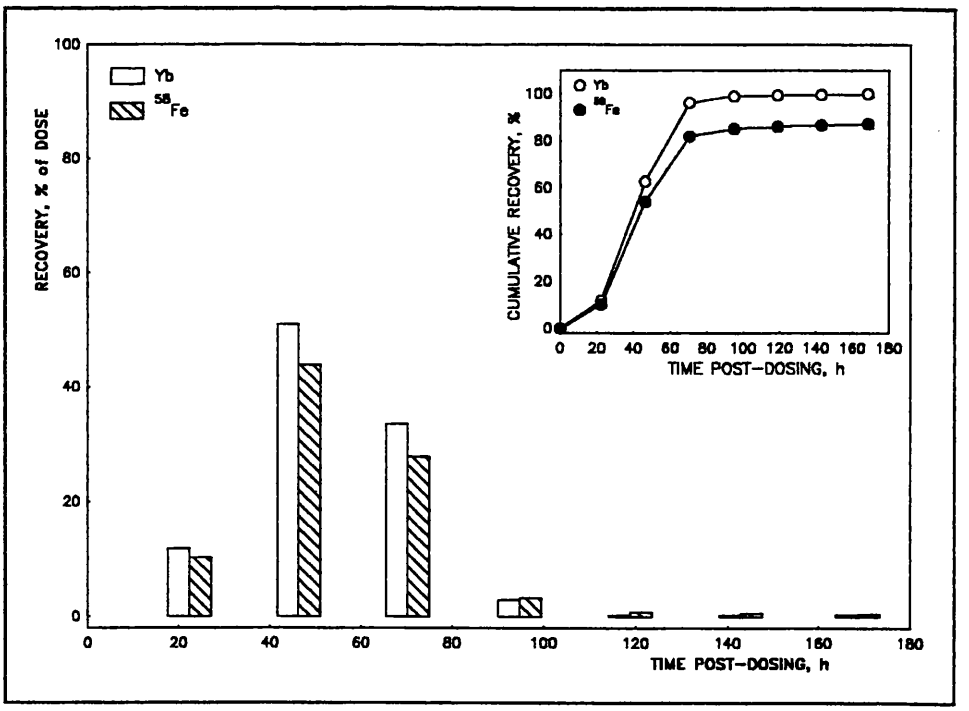


Figure-38.a: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-1

Table-73.b: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 2					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	25.7	21.87±0.05	16.75±0.21	23.4±0.3	23.4±0.3
2	62.9	55.51±0.27	40.30±0.32	27.4±0.3	26.3±0.2
3	86.7	26.86±0.06	20.65±0.22	23.1±0.3	25.5±0.2
4	111.0	0.34±0.01	0.63±0.03	n.a.	25.1±0.2
5	145.4	0.09±0.01	0.43±0.03	n.a.	24.7±0.2
6	167.0	0.04±0.005	0.36±0.05	n.a.	24.4±0.2
7	195.8	0.10±0.01	0.41±0.06	n.a.	24.1±0.2
Total, %		104.8±0.3	79.5±0.5		
100 - Total ^{58}Fe recovery 20.5±0.5%					

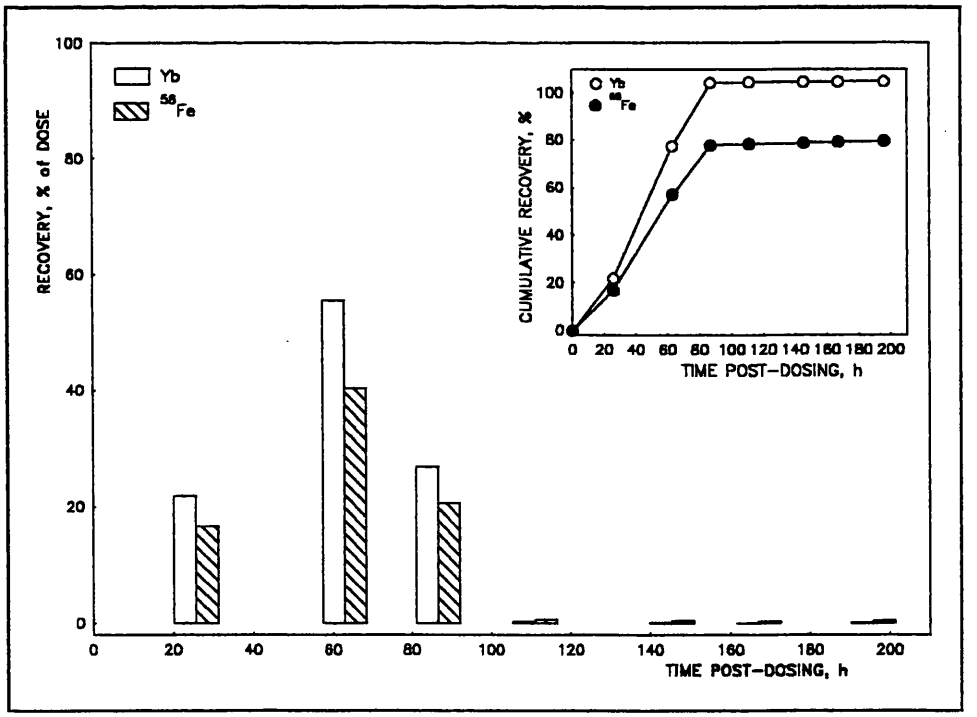


Figure-38.b: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-2

Table-73.c: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 3					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	11.4	6.98±0.05	5.80±0.10	**16.9±0.3**	16.9±0.3
2	23.1	22.80±0.10	18.29±0.22	**19.8±0.3**	18.3±0.2
3	30.0	51.57±0.22	42.96±0.56	**16.7±0.2**	17.6±0.2
4	47.5	13.04±0.11	9.19±0.15	n.a.	19.2±0.2
5	70.9	3.17±0.05	5.00±0.17	n.a.	16.7±0.1
6	80.3	0.10±0.02	0.95±0.06	n.a.	15.8±0.1
7	96.9	0.13±0.03	0.26±0.04	n.a.	15.7±0.1
8	124.8	0.14±0.03	0.48±0.04	n.a.	15.3±0.1
9	142.8	0.21±0.04	0.16±0.09	n.a.	15.3±0.1
10	154.6	0.29±0.05	0.61±0.10	n.a.	**15.0**±0.1
Total, %		**98.4±0.3**	**83.7±0.7**		
100 - Total ^{58}Fe recovery **16.3±0.7%**					

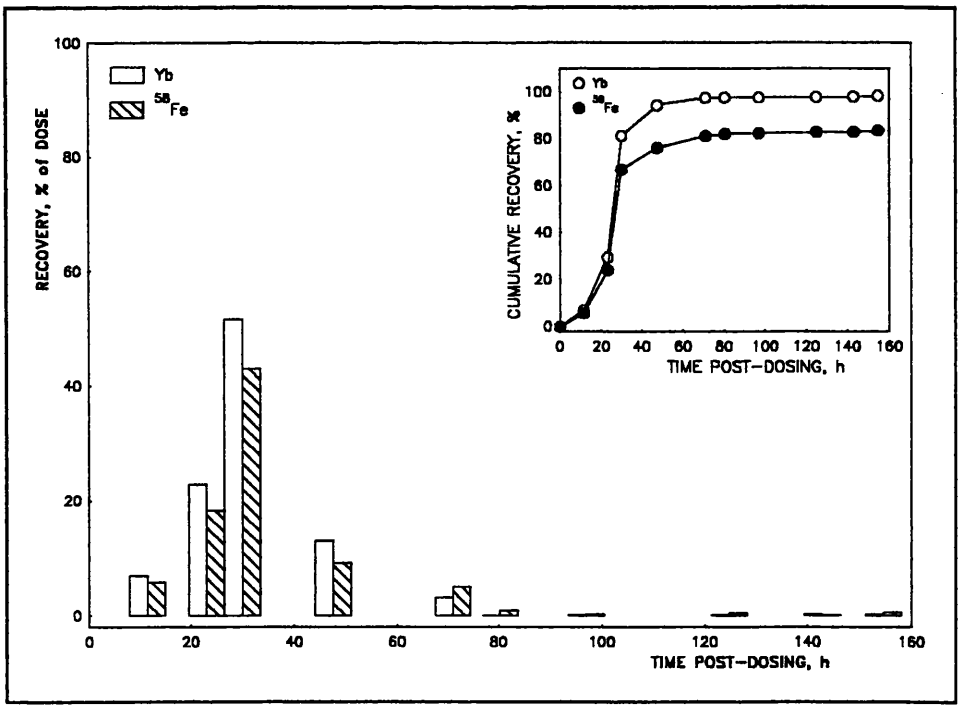


Figure-38.c: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-3

Table-73.d: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 4					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	24.0	90.41±0.64	66.43±0.90	**26.5±0.4**	26.5±0.4
2	47.9	6.87±0.05	5.85±0.21	n.a.	25.7±0.4
3	70.7	2.61±0.04	2.85±0.06	n.a.	24.8±0.3
4	94.1	0.92±0.02	2.51±0.07	n.a.	23.0±0.3
5	118.0	0.10±0.01	0.89±0.12	n.a.	22.2±0.3
6	142.3	0.05±0.002	0.84±0.12	n.a.	21.4±0.3
7	165.5	0.15±0.01	0.13±0.02	n.a.	**21.4±0.3**
Total, %		**101.1±0.6**	**79.5±0.9**		
100 - Total ^{58}Fe recovery **20.5±0.9%**					

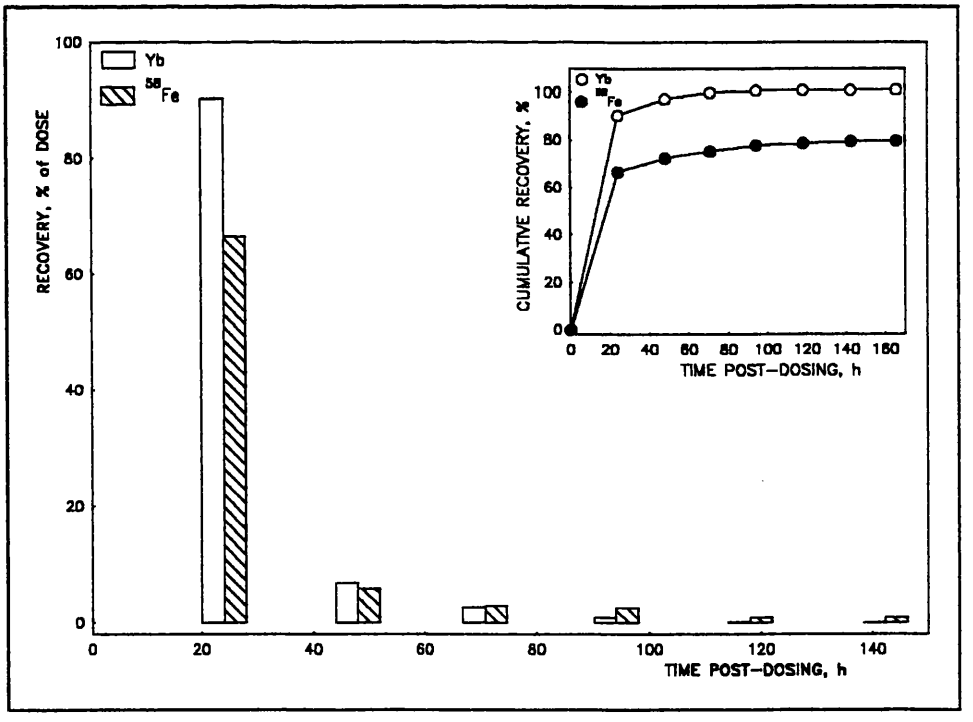


Figure-38.d: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-4

Table-73.e: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 5					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	8.2	43.36±0.22	34.31±0.35	**20.9±0.2**	20.9±0.2
2	33.3	44.68±0.29	35.31±0.47	**21.0±0.3**	20.9±0.2
3	59.9	11.91±0.18	11.43±0.22	n.a.	18.9±0.2
4	85.6	0.85±0.05	1.11±0.03	n.a.	18.5±0.2
5	97.2	0.31±0.02	1.28±0.09	n.a.	17.5±0.2
6	124.2	0.04±0.01	0.35±0.02	n.a.	17.2±0.2
7	149.5	0.09±0.01	1.01±0.06	n.a.	**16.2±0.1**
Total, %		**101.2±0.4**	**84.8±0.6**		
100 - Total ^{58}Fe recovery **15.2±0.6%**					

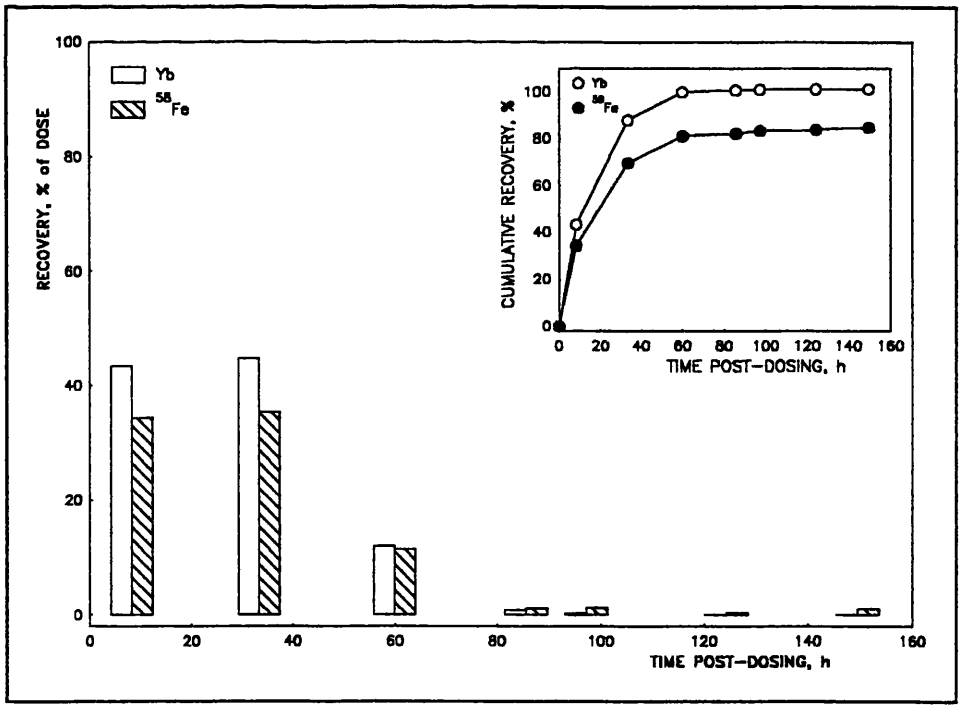


Figure-38.e: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-5

Table-73.f: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 6					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	25.0	48.87±0.17	32.45±0.31	**33.6±0.3**	33.6±0.3
2	48.8	42.98±0.22	29.82±0.37	**30.6±0.4**	32.1±0.3
3	70.3	3.46±0.03	2.28±0.05	**34.1±0.8**	32.2±0.3
4	80.3	0.12±0.01	0.31±0.02	n.a.	32.0±0.3
5	94.8	0.15±0.01	0.04±0.01	n.a.	32.0±0.3
6	118.8	0.21±0.01	1.00±0.08	n.a.	31.2±0.3
7	142.1	0.29±0.02	0.72±0.06	n.a.	**30.6±0.3**
Total, %		**96.1±0.3**	**66.6±0.5**		
100 - Total ^{58}Fe recovery **33.4±0.5%**					

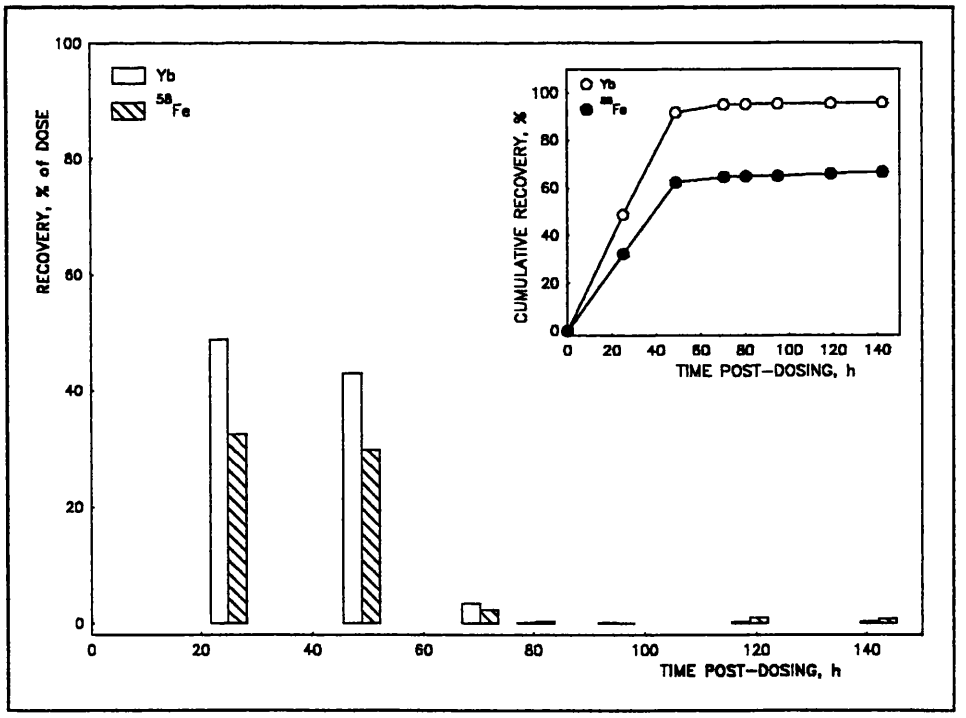


Figure-38.f: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-6

Table-73.g: Faecal recoveries of Yb marker, ^{58}Fe tracer, and luminal disappearance of iron, when consumed with a farina meal

SUBJECT 7					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{58}Fe Luminal disappearance, %±SD	
		Yb	^{58}Fe	Individual	Cumulative
1	23.0	69.18±0.38	48.70±0.93	**29.6±0.6**	29.6±0.6
2	36.8	18.62±0.13	14.25±0.29	**23.5±0.5**	28.3±0.5
3	47.8	6.42±0.12	4.91±0.14	**23.5±0.8**	28.0±0.4
4	72.3	1.71±0.06	2.52±0.04	n.a.	26.6±0.4
5	81.6	0.31±0.002	0.66±0.01	n.a.	26.2±0.4
6	94.3	0.22±0.01	0.91±0.04	n.a.	25.4±0.4
7	118.3	0.23±0.01	0.62±0.02	n.a.	24.9±0.4
8	142.3	0.25±0.03	0.67±0.07	n.a.	24.5±0.3
9	166.7	0.24±0.03	0.30±0.02	n.a.	**24.3±0.3**
Total, %		**97.2±0.4**	**73.5±1.0**		
100 - Total ^{58}Fe recovery **26.5±1.0%**					

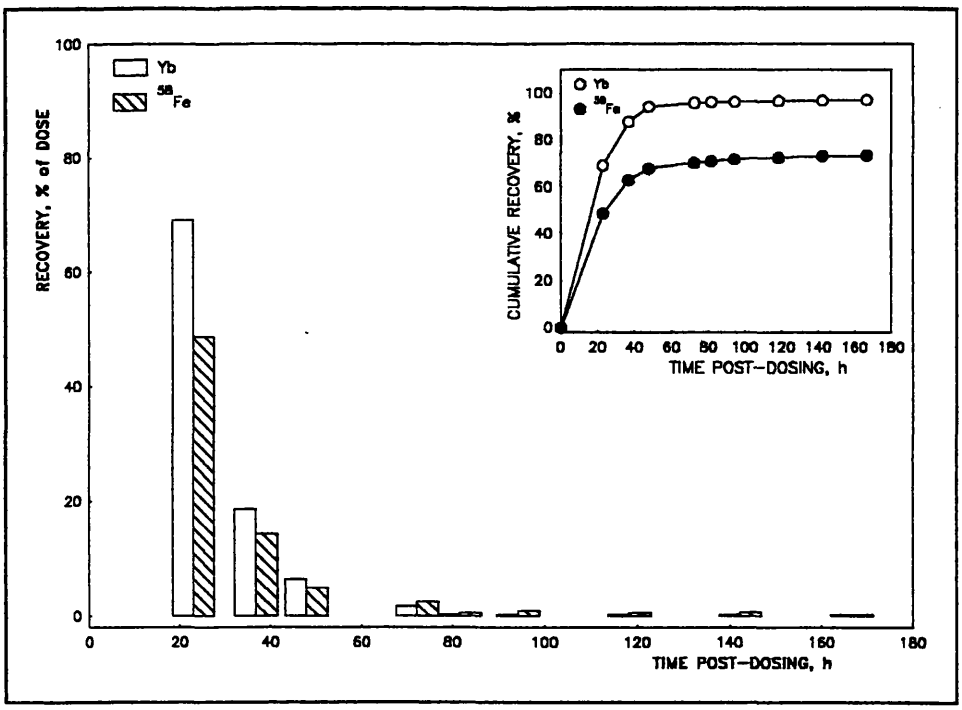


Figure-38.g: Faecal profile of Yb marker and ^{58}Fe tracer for individual samples and composites of sequential output (Cumulative) for subject-7

III.3.6 DISCUSSION

III.3.6.1 RECOVERY KINETICS OF ^{58}Fe AND Yb

As can be seen from **Table-73.a-g** luminal disappearances (intestinal uptakes) derived from composites of the initial sequential samples are different from those derived from completion of collection (composites of 7 samples). This can be explained by the tracer initially retained being released at a slower rate after recovery of marker is completed.

For comparison of kinetic behaviour of marker and isotopic tracer excreted, the ratio of tracer to marker recoveries for individual samples and those derived from composites of sequential outputs for all subjects are provided in **Figure-39** .

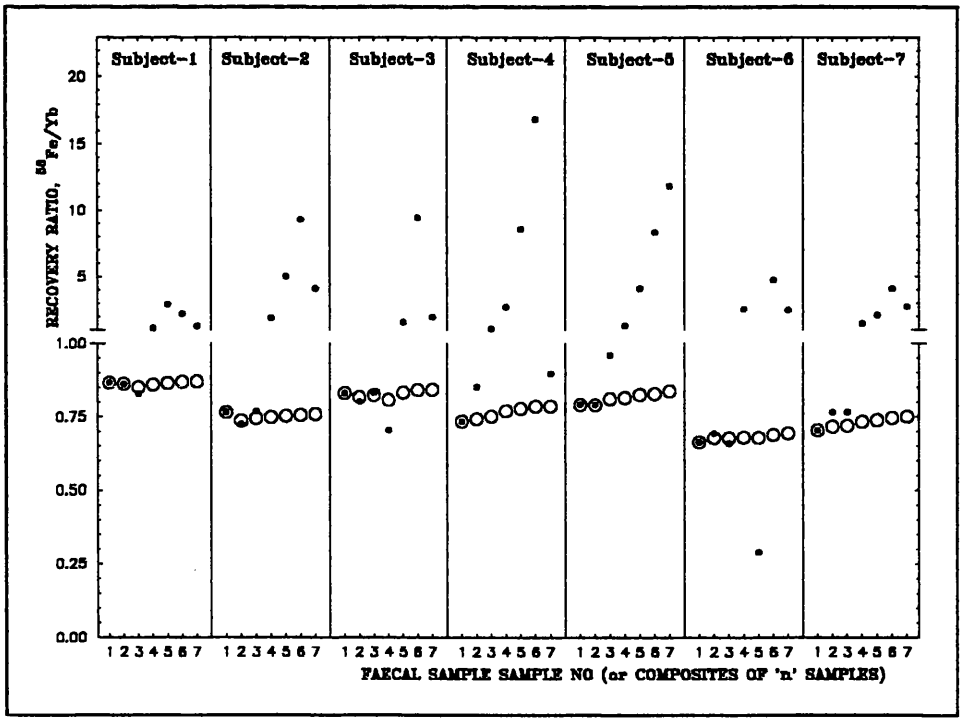


Figure-39: Ratios of recoveries of ^{58}Fe tracer and Yb marker for individual samples, ●, and for composites of sequential outputs, O, for all subjects

As can be seen from the above figures, for most of the subjects the ratio of recoveries after the second or third samples show an increasing trend which is more obvious for individual samples. This increase illustrates the 're-excretion' of the tracer. Deviations from the expected increasing trend for individual samples could indicate irregularities in 're-excretion' of tracer but they were only for samples in which recoveries of marker or tracer was less than 0.5%.

The magnitude of the contribution of 're-excreted' tracer to outputs obtained in composites of 3-7 samples can be illustrated by the difference between the fractions of total recoveries of tracer and marker in individual samples as described previously (Section III.3.4.1). The differences for individual samples as a function of the sequence in which faecal samples were collected are provided in **Figure-40**.

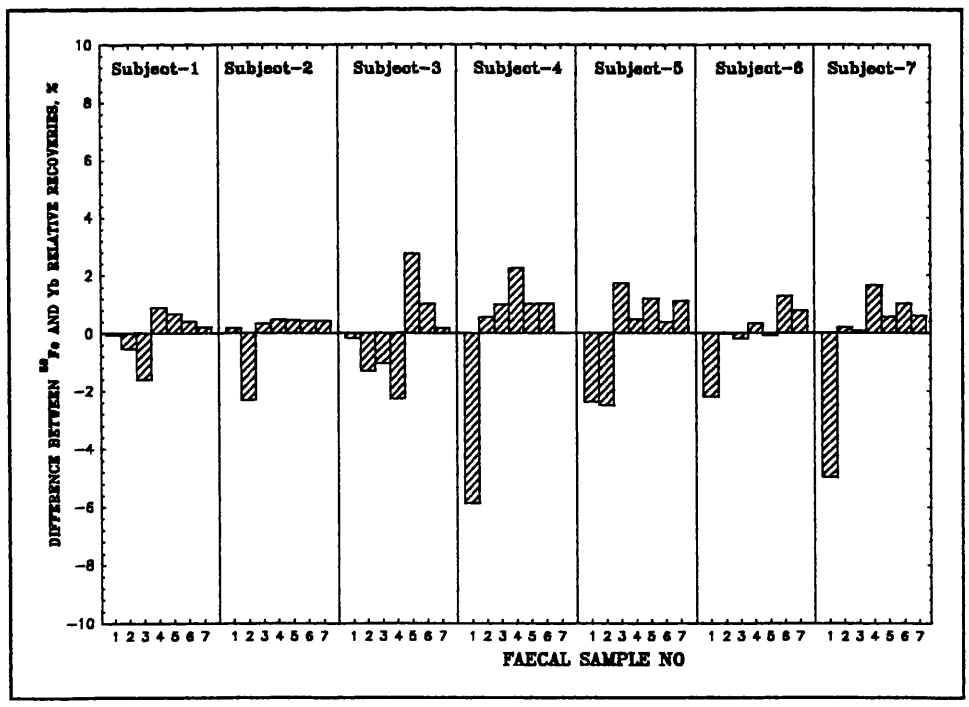


Figure-40: Difference between relative recoveries (percent of their total outputs) of Yb marker and ^{58}Fe tracer for all subjects

For all subjects the net differences between relative recoveries of tracer and marker

are negative for composites of the first two samples but is usually positive for the following samples which contains a fraction of tracer initially retained.

Evaluation of the kinetic behaviour of Yb marker and ^{58}Fe tracer with the above approaches, which are illustrated in **Figure-39** and **40**, suggest that the initial luminal disappearance, obtained from the fractions of marker and tracer recovered with composites of the initial two or three faecal samples, minimizes the contribution of retarded excretion of tracer.

The linear relation between the relative recoveries of marker and tracer with composites of the first two samples (since the recovery rates of marker marker and tracer with the first samples may not be reliable) and with the following individual outputs, as percent of their total recoveries for composites of the first three to five faecal samples and for total collection (7samples), for all subjects are provided in **Table-74**. The linearity for the composites of the first two samples is derived from the relative recoveries of marker and tracer obtained for composites of the first three samples. The linearity is also derived for the collection of five stools for which the first and second samples are considered as individual samples, to show the effect of the relative recoveries of marker and tracer obtained for the first samples, and provided in the table.The table contains the linearity between the recoveries and its correlation coefficient.

Table-74: Linearity between recoveries of Yb marker and ^{58}Fe tracer for all subjects [y=(A±a)x+(B±b), where y and x are recoveries of tracer and marker, and A and B are the slope and intercept]

Composites	**A±a**	**B±b**	R^2
3 samples (2 samples composites & 3. samples)	0.98±0.01	0.85±0.42	0.999
(2 samples composites)	0.99±0.02	0.14±1.38	0.998
4 samples (2 samples composites & 3. and 4. samples)	0.97±0.01	0.93±0.35	0.989
5 samples (2 samples composites & 3., 4., 5. samples)	0.96±0.01	0.84±0.26	0.999
[5 samples (The first to fifith individual samples)	0.95±0.01	0.99±0.23	0.998]
Total collection (2 samples composites & 3.,...,7. samples)	0.95±0.05	0.82±0.17	0.999

As can be seen from **Table-74** that slopes of the linear relations which are the closest to the unity are obtained for the samples, which the relative recoveries obtained from composites of the first three samples or composites of the first two stools of the three samples composites. This indicates that recoveries obtained for composites of the two or three samples are the most appropriate for calculation of luminal disappearance with minimum effect of 're-excretion' of isotope initially retained.

III.3.6.2 LUMINAL DISAPPEARANCE AND SIGNIFICANCE OF RETARDED EXCRETION

The means of luminal disappearance obtained for individual (single) samples for which it could be calculated, for composites of the first one to six samples, and for total collections, together with marker recoveries for the corresponding groups of samples are provided in **Table-75**.

The mean of luminal disappearances obtained for the first two or three single samples provided coefficients of variation of 6-15% for 6 of the 7 subjects. This can be explained by slight difference between kinetic behaviour of marker and tracer in the first samples or contribution of re-excreted tracer to the third samples. The difference between the mean of luminal disappearances obtained for composites of two and three or four samples were found to be insignificant ($p>0.10$), whilst the differences between those obtained for the composites of 2-6 samples and total collection (7 samples) were significant, $p<0.05$. This indicates the effect of retarded excretion of tracer initially retained, as discussed above.

The differences in luminal disappearances obtained for composites of sequential samples, SC, (2SC and 3SC, 3SC and 4SC,.., and 6SC and 7SC) are provided in **Figure-41**, and the differences between those obtained for composites of sequential samples and total collection (2SC and 7SC, 3SC and 7SC,.., and 6SC and 7SC) are provided in **Figure-42** for each subject.

Table-75: Luminal disappearences of ^{58}Fe tracer for single (means for n_1) and composites of sequential 1-7 samples, and recoveries of Yb marker

Subject	LUMINAL DISAPPEARANCE, %								
	For single samples %±SDM	n_1	Composites of 'n' sequential samples						
			1 Sample	2 Samples	3 Samples	4 Samples	5 Samples	6 Samples	7 Samples (Total collection)
1	14.7±2.0	3	13.3	13.7	14.9	14.0	13.4	13.1	12.9
2	24.6±2.4	3	23.4	26.3	25.5	25.1	24.8	24.4	24.1
3	17.8±1.7	3	17.0	18.3	17.6	19.2	16.7	15.8	15.7
4	26.5	1	26.5	25.7	24.8	23.0	22.2	21.4	21.4
5	20.9±0.1	2	20.9	20.9	18.9	18.5	17.5	17.2	16.2
6	32.8±1.9	3	33.6	32.2	32.2	32.0	32.0	31.2	30.6
7	25.5±3.5	3	29.6	28.3	28.0	26.6	26.2	25.4	25.0
Mean±SDM	23.3±6.0		23.5±7.1	23.6±6.3	23.1±6.2	22.6±5.9	21.8±6.4	21.2±6.3	20.8±6.2
Yb recovery, %±SDM	not applicable		42±31	76±23	96±7	99±4	100±3	100±3	100±3
#Time±SDM	n.a.		20±7	42±14	61±20	84±22	105±27	125±32	148±33

The mean of time post-dosing (h) for provision of n^{th} sample

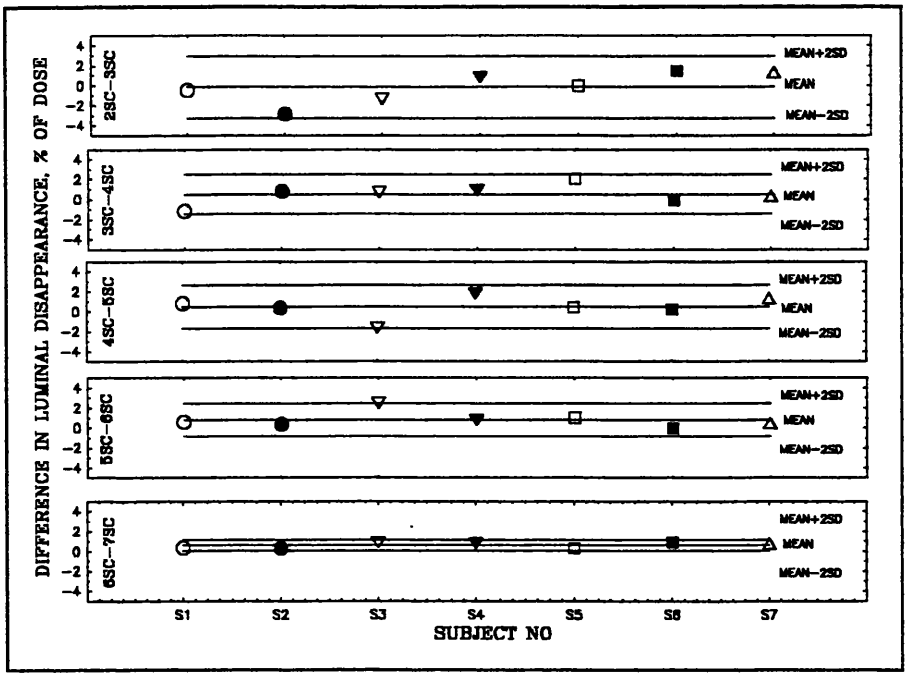


Figure-41: Differences between luminal disappearances obtained for composites of two to seven sequential samples with means and 95% confidence limits

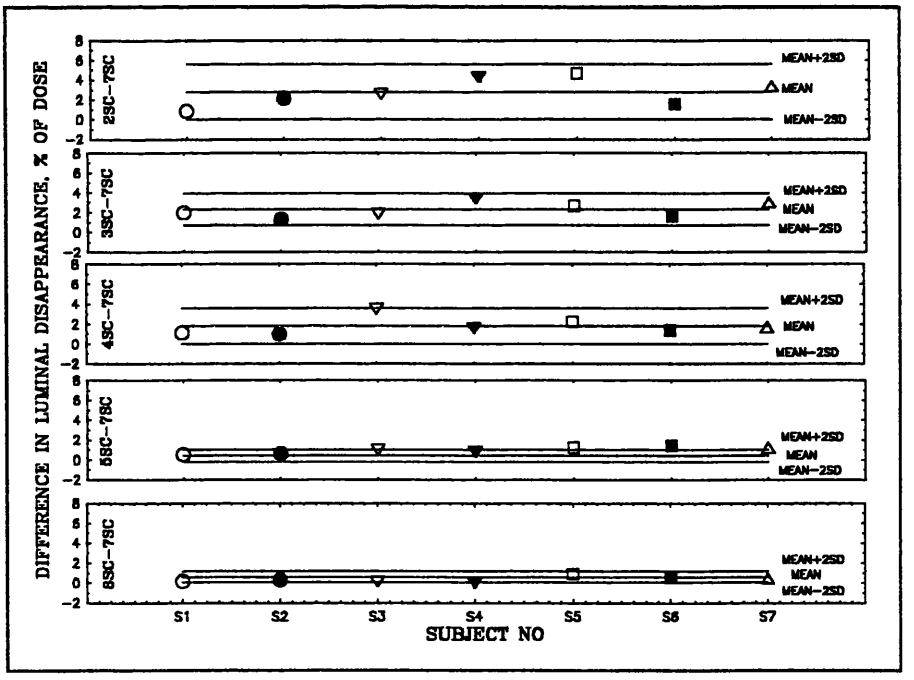


Figure-42: Differences between luminal disappearances obtained for composites of sequential two to seven samples and total collection with means and 95% confidence limits

The mean differences between luminal disappearances obtained for the first 2 and 3, 3 and 4,...,and 6 and 7 samples were found to be 0.5±1.0, 0.5±1.1, 0.8±0.8, 0.6±0.3, and 0.4±0.3% of the dose administered respectively. The differences were not significantly different from each other ($p>0.05$). Differences between luminal disappearances of composites of sequential two to six samples and of total collection (7 samples composite) were 2.8±1.4, 2.3±0.8, 1.8±0.8, 1.0±0.3, and 0.4±0.3% of the dose.

The initial disappearance, in which the contribution of tracer initially retained is the least can be derived from the differences described above. The differences as a function of time of the mean of time post dosing for provision of the first 2,3,.., and 6 samples (42, 61, 84, 105, and 125 h respectively) are provided in **Figure-43.A** and **B**.

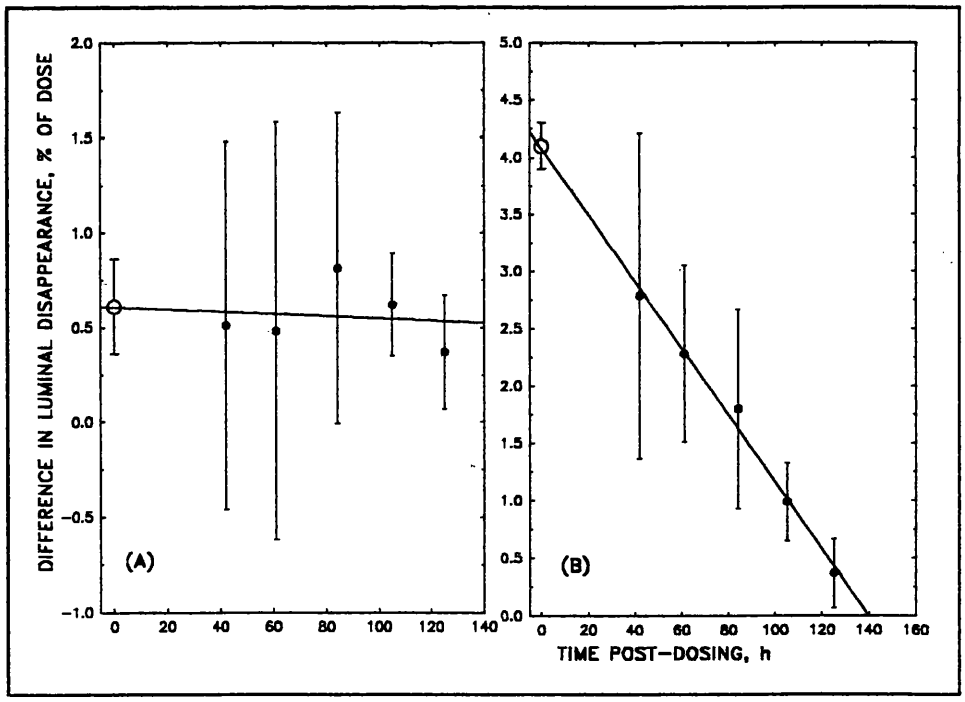


Figure-43: The mean of differences between luminal disappearances of composites of sequential pairs in 2-7 samples (A), [$Y=-(5.9\times10^{-4}\pm2.9\times10^{-3})X+(0.6\pm0.3)$, $R^2=0.013$], and between those of composites of sequential 2-6 samples and total collection (B), [$Y=(0.03\pm0.002)X+(4.1\pm0.2)$] (O indicates the differences at t=0 by extrapolation)

From the relations between the mean differences and time post dosing, the difference between luminal disappearances of the initial and of composites of 2 sequential samples can be calculated to be 0.6±0.3%, and that between initial and of total collection is 4.1±0.2% by extrapolating the linear regressions to t=0. By addition of these differences to the luminal disappearances obtained for composite of the first 2 sequential samples (23.6±6.3%) and of total collection (20.8±6.2%), the initial luminal disappearances are found to be 24.2±6.3% and 24.9±6.2%. The initial luminal disappearances found with both approaches are not significantly different from that determined for composites of 2 sequential samples.

By considering the re-excreted fraction of the dose consumed (3 mg of iron), approximately 10% of 0.7 mg initially retained iron is excreted within 6 days of administration, at 0.01 mg per day. Dubach *et al.* (1955) found the average daily excretion over a period of 100 days was 0.01% of the dose when 5-14 mg of iron labelled with Fe-59 was injected into 4 healthy individuals. When the average of daily losses from initial retention obtained for this investigation (~0.5%) is compared with that found by Dubach *et al.* (0.01%), the losses found should not be the fraction of iron transferred to the blood and back to the lumen. This should be the fraction taken up by the intestinal mucosa and eventually desquamated with shedded mucosal cells. If the difference between luminal disappearances of composites of the first two and seven samples (23.6±6.3% and 20.8±6.2% of the dose) was the fraction retained temporarily, ~90% of iron initially retained (equivalent to 0.6 mg of iron) should be absorbed.

III.3.6.3 LUMINAL DISAPPEARANCE OF ^{58}Fe IN RELATION TO ITS MODE OF ADMINISTRATION AND IRON STATUS

Jasani *et al.* (1971) demonstrated that there was no temporary retention of radioactive iron (Fe-59 in ferric chloride, or citrate form) when it was given to healthy subjects with a meal, the absorption being measured by monitoring the subjects for 14 days using whole body counting. Permanent retention of iron was proved with reference to initial retention of iron measured with the aid of non-absorbable $BaSO_4$ marker labelled with radioactive Ba-131. It was speculated that the percentage of Fe-59 temporarily retained when retention of Ba-131 of less than 1% was below the detection limit for 9 out of 10 healthy subjects (the exception was an individual who retained 5.7%). Jasani and Fletcher (1972) confirmed that iron absorption from a meal (which was labelled with radioactive Fe-59 as ferric chloride or citrate, and Ba-131 as marker) measured by whole body counting in 14 subjects, 10 days after administration was insignificantly different from that determined by monitoring faecal samples composites of samples collected for 3 days.

The results of this investigation show that temporary retention of iron is ~3% of the dose when the mean of luminal disappearances obtained for composites of the initial two samples and that obtained for composites of samples collected for a week is compared. Insignificant differences were found between luminal disappearances obtained for composites of the first two, three, and four samples in which marker recovery is major or nearly quantitative. This suggest that the initial luminal disappearance can be obtained from the first two, three, and four samples (2-3 days collection) with minimal effect of the fraction of iron temporarily retained and agrees with conclusion drawn by Jasani and Fletcher (1972).

Bjorn-Rasmussen *et al.* (1972) showed that absorption from an uncooked wheat based meal (dough) containing 1.5 mg of iron labelled intrinsically with Fe-55 and extrinsically with Fe-59 as ferric chloride were not significantly

different. The study was performed on 5 healthy subjects and they consumed the meal with tap water. The mean of absorption measured from blood samples withdrawn 2 weeks after administration and by whole body counting was 29.3±12.4% (range 12.9-36.9%). In this current investigation, luminal disappearance from a wheat based farina meal (cooked) labelled with 3 mg of Fe containing ^{58}Fe tracer was determined for 7 healthy subjects. Luminal disappearances were 23.6±6.3 (range 13.7-32.2%) for 2 faecal sample composites and 20.8±6.2 (range 12.9-30.6%) for 7 sample composites. The absorption results for both investigations agree with similar mean and range for comparable nature of input.

Luminal disappearances found for composites of sequential 2-7 samples and serum ferritin levels of participants were also subjected to statistical evaluation to see if there is any significant correlation between them. Unlike the situation for ferrous iron consumed with a standard solution, no significant correlation was found ($p>0.05$).

III.3.6.4 LUMINAL DISAPPEARANCE OF FERROUS VERSUS FERRIC IRON

There was a significant inverse correlation between luminal disappearance of ferrous-iron derived from the first two and five samples composites and serum ferritin concentrations of the subjects. This can be explained by the amount of ferrous-iron temporarily retained in the intestinal lumen, or the absorbed fraction being related to serum ferritin concentrations. Unlike ferrous-iron, there was no significant correlation when intestinal uptakes for ferric-iron consumed with a meal were subjected to the same evaluation, as reported by Mason *et al.* (1990). In contrast a negative relation was found by Turnlund *et al.* (1990) but it was not significant, intestinal uptakes being obtained by the use of isotopically labelled ferric-iron with faecal monitoring. These contradictory correlations between serum ferritin concentration and the intestinal uptake for ferrous- and ferric-iron may indicate that the regulatory mechanism for uptake of iron depends on the oxidation state of iron or the

composition of input (solution or meal).

Forbes *et al.* (1989) demonstrated that ferric-phosphate was less bioavailable than ferrous-sulphate (both labelled with radioactive tracer) when they were consumed with a farina meal, and that their absorption was enhanced by addition of ascorbic acid. The absorption determined for blood samples was inversely related with iron status (serum ferritin level). Siegenberg *et al.* (1991) reported that the inhibitory effects of polyphenols and phytates on non-haem iron absorption could be prevented by ascorbic acid. In contrast, results based on a 10 week investigation of women with low iron stores carried out by Hunt *et al.* (1994) revealed that ascorbic acid supplementation had a more modest effect on iron absorption from a diet with poorly bioavailable iron or a typical western diet than was indicated by studies with single meals. The results also suggested that serum ferritin was not a good indicator of short-term improvements in iron stores. However, Núñez *et al.* (1994) speculated that a human intestinal epithelium cell line mediated the reduction of ferric-iron in the apical medium. Conrad *et al.* (1993) and Conrad and Umbreit (1993) concluded that ferritin is a storage protein which incorporates iron to prevent oxidative damage to the cell rather than a regulator of iron absorption.

In the light of the above considerations, the intestinal uptake of ferrous-iron, inversely correlated with serum ferritin level and possibly encouraged by added ascorbic acid, should have been more consistent at the amount initially taken up if the fraction taken up was transferred to circulating blood with an irreversible regulatory absorption mechanism based on reduction-oxidation chemistry. This could be explained by iron in the ferrous state being readily bioavailable. It is taken up by epithelial cells in the intestine and transferred into circulating blood [serum enrichment reaching a maximum approximately 2 h after oral administration (Barrett et al., 1994)] whilst a considerable fraction initially retained with epithelial cells is exfoliated with shedded cells. Marx (1979) explained this mechanism by regulation of iron absorption taking place at least at 2 sites; at the brush border a transferrin like protein is

responsible for rapid transport of iron from brush border to the blood (this pathway was defined as mucin-mobilferrin-integrin by Conrad and Umbreit, 1993), whilst a considerable amount of iron is bound by ferritin within mucosal cells and is not released but desquamated with these cells in to the lumen of the gut. The results obtained from this investigation which indicate significant temporary retention support the interpretation of Marx.

The intestinal uptake of ferric-iron (chloride) was not correlated with serum ferritin but the amount initially retained was more consistent. The difference is related to its oxidation state or the composition of the mode of intake. Jasani *et al.* (1971) and Jasani and Fletcher (1972) demonstrated that temporary iron uptake from a solution containing ferric-chloride or -citrate (labelled with radioactive Fe-59 as tracer and Ba-131 as marker) and ascorbic acid was not significant when the solution was consumed with a standard meal (chicken, tomato, lettuce, and banana), but it was high when tracer was given with haemoglobin. Absorption of iron determined by faecal monitoring with marker method and by whole body counting suggested that the absorption of haemoglobin iron was not well regulated and occurred more slowly so that absorbed iron appears in the plasma later when compared with of inorganic iron, providing good agreement between results of the two measurements (for whole body counting, even 10 days after consumption). Jasani *et al.* (1971) found that temporary retention and the amount of finally absorbed ferric iron was the same as the amount initially retained by the intestinal mucosa. The intestinal uptake results for the present investigation, in which a farina meal labelled with stable isotopic tracer in ferric form was used, demonstrate that the major fraction (~90%) of iron initially taken up should be transferred into circulating blood. In the context of administration of iron with a meal, the conclusion of this investigation agrees with that drawn by Jasani *et al.* (1971).

In comparison of intestinal uptake results obtained for ferrous and ferric iron, ferrous iron was found to be readily absorbed in an amount depending upon

iron status, but absorption was not as well regulated as ferric iron. The absorption of ferric-iron phosphate and of ferrous-iron sulphate from a farina meal, as indicated by Forbes *et al.* (1989) showed that ferrous iron is four times more absorbable (3.30% of 3 mg iron) than ferric phosphate (0.83% of 3 mg iron) due to the lower solubility of iron (III) phosphate, whilst absorption of dissolved iron phosphate from a cocoa drink was 25% of 10 mg iron (Fairweather-Tait *et al.*, 1983). The intestinal uptake of iron from a farina meal was found to be approximately 0.6 mg (20% of a 3 mg dose). This is less than the uptake of 2.5 mg (25% from 10 mg of ferric phosphate) from a cocoa drink described by Fairweather-Tait *et al.* (1983), although the fractional uptakes are comparable. This suggests that highly soluble iron (III) chloride should be more absorbable than iron phosphate in amounts comparable with iron sulphate. However, recent investigations have focused the use of NaFe(III)EDTA as iron additive (Davidsson *et al.*, 1994) instead of $Na_2Fe(II)EDTA$ (MacPhail *et al.*, 1994).

III.3.7 LUMINAL DISAPPEARANCE OF ZINC CONSUMED WITH A FARINA MEAL, DETERMINED WITH ^{70}Zn TRACER AND Yb MARKER

Recoveries of Yb marker and non-retained ^{70}Zn tracer and luminal disappearance of tracer derived from the recoveries are provided in this section. Luminal disappearances determined for individual and composites of sequential samples, and for total collection following consumption of a standard meal (farina) are provided and considered with reference to the intestinal kinetic profiles of marker and tracer.

Recoveries and luminal disappearances have been calculated by applying **Equation-5.b** (Section I.5) to experimental data provided in **Appendix-6**. Recoveries of Yb marker and ^{70}Zn tracer and luminal disappearance derived from recoveries are provided in **Tables-76.a-g**. The nature of the contents of the tables is explained in the previous section. Recoveries of marker and tracer with single and composites of sequential collections are depicted as a function of time and provided in the accompanying **Figures-44.a-g**.

Table-76.a: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal.

SUBJECT 1					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	22.3	11.87±0.17	7.20±0.36	**39.3±2.0**	39.3±2.0
2	46.3	50.89±0.56	33.49±0.89	**34.2±1.0**	35.2±0.9
3	70.3	33.53±0.25	22.18±0.63	**33.8±1.0**	34.7±0.7
4	94.8	2.90±0.04	n.d.	n.a.	36.6±0.7
5	118.8	0.29±0.02	n.d.	n.a.	**36.8±0.7**
Total, %		**99.5±0.6**	**62.9±1.1**		
100 - Total ^{70}Zn recovery **37.1±1.1%**					

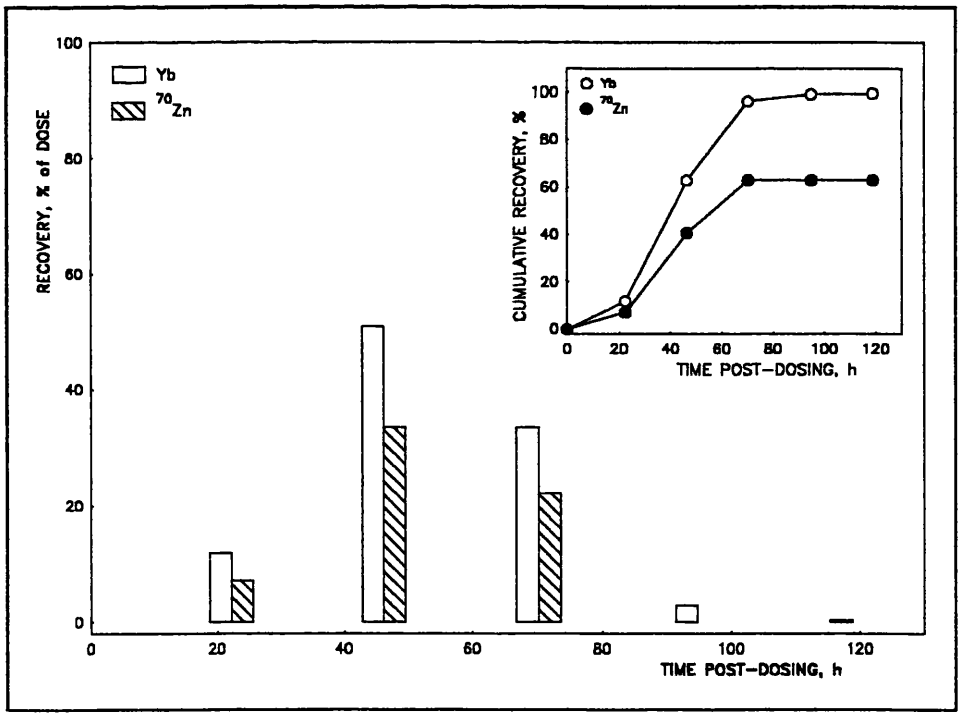


Figure-44.a: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-1

Table-76.b: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 2					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	25.7	21.87±0.05	13.79±0.50	**36.9±1.3**	36.9±1.3
2	62.9	55.51±0.27	36.97±0.96	**33.4±0.9**	34.4±0.7
3	86.7	26.86±0.06	17.18±0.53	**36.0±1.1**	34.8±0.6
4	111.0	0.34±0.01	0.85±0.07	n.a.	34.2±0.6
5	145.4	0.09±0.01	n.d.	n.a.	**34.3±0.6**
Total, %		**104.7±0.3**	**68.8±1.2**		
100 - Total ^{70}Zn recovery **31.2±1.2%**					

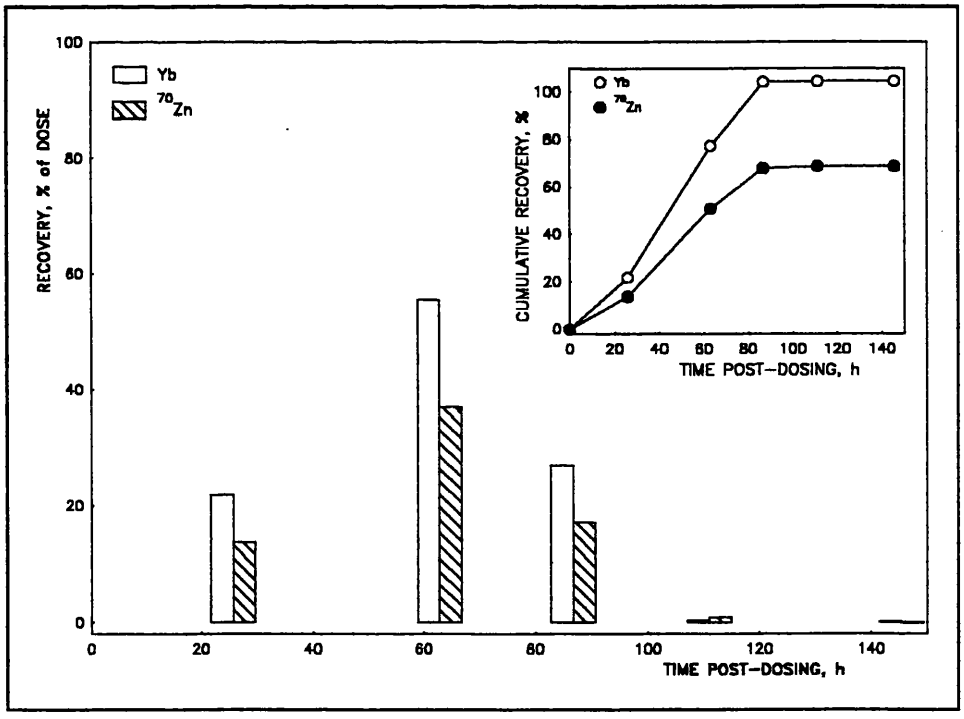


Figure-44.b: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-2

Table-76.c: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 3					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	11.4	6.98±0.05	4.29±0.55	**38.6±5.0**	38.6±5.0
2	23.1	22.80±0.10	15.98±0.52	**29.9±1.0**	31.9±1.2
3	30	51.57±0.22	35.11±1.33	**31.9±1.2**	31.9±0.9
4	47.5	13.04±0.11	8.38±0.33	**35.7±1.5**	32.5±0.8
5	70.9	3.17±0.05	n.d.	n.a.	34.7±0.9
6	80.3	0.10±0.02	n.d.	n.a.	34.7±0.9
Total, %		**97.7±0.3**	**63.8±1.6**		
100 - Total ^{70}Zn recovery **36.2±1.6%**					

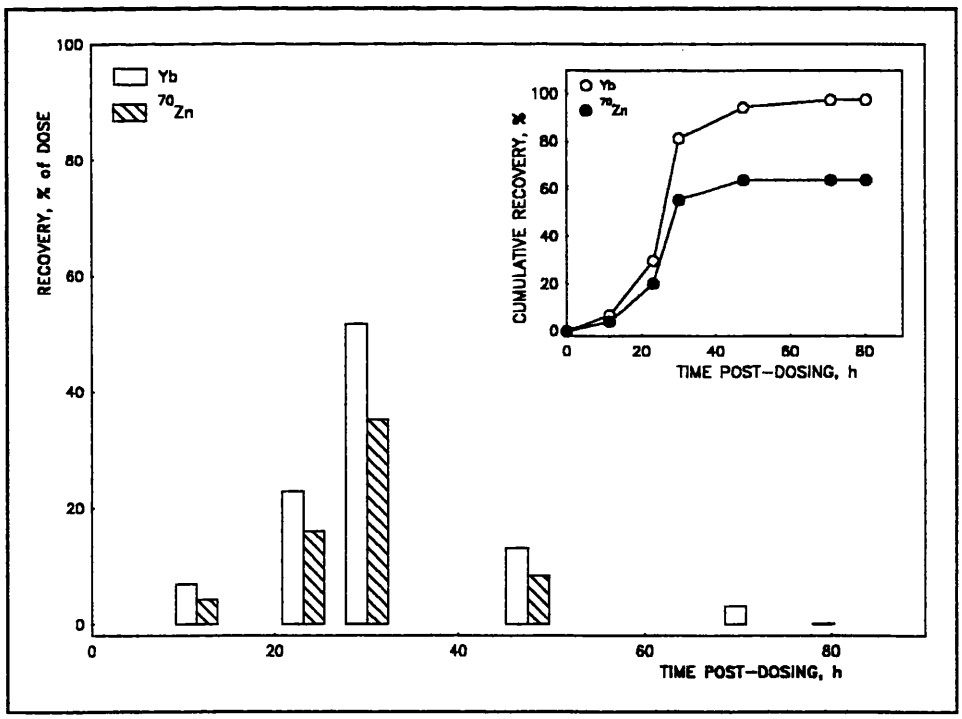


Figure-44.c: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-3

Table-76.d: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 4					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	24	90.41±0.64	51.60±1.11	**42.9±1.0**	42.9±1.0
2	47.9	6.87±0.05	3.92±0.11	**42.9±1.3**	42.9±0.9
3	70.7	2.61±0.04	0.93±0.05	n.a.	43.5±0.9
4	94.1	0.92±0.01	0.64±0.03	n.a.	43.4±0.9
5	118	0.10±0.01	n.d.	n.a.	**43.4±0.9**
Total, %		**100.9±0.6**	**57.1±1.1**		
100 - Total ^{70}Zn recovery **42.9±1.1%**					

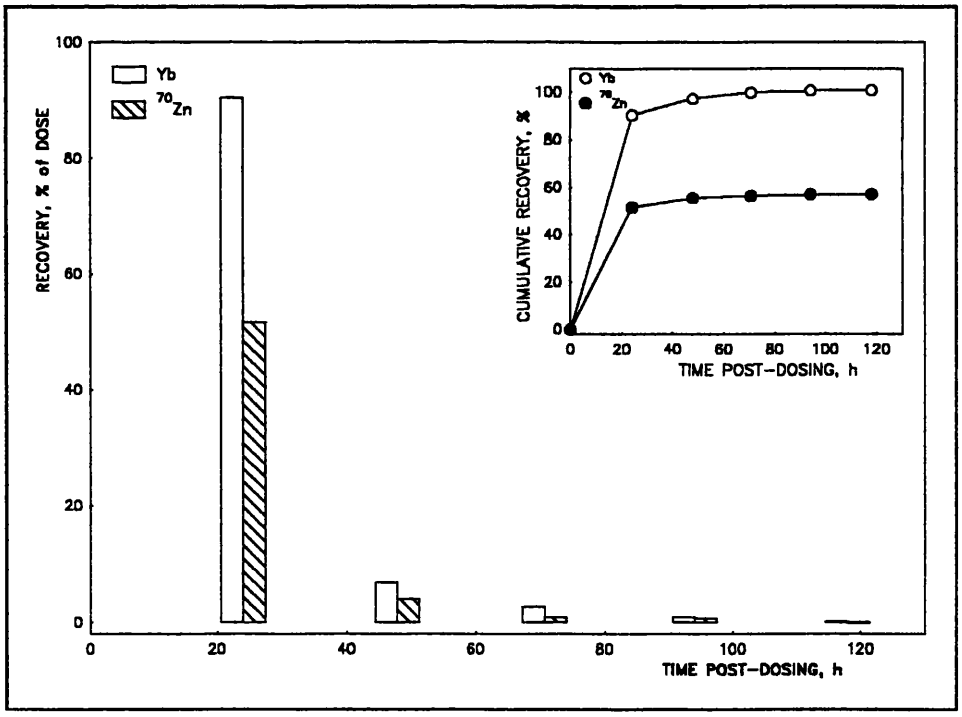


Figure-44.d: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-4

Table-76.e: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 5					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	8.2	43.36±0.22	21.86±0.48	**49.6±1.1**	49.6±1.1
2	33.3	44.68±0.29	19.45±0.34	**56.5±1.1**	53.1±0.8
3	59.9	11.91±0.18	5.79±0.23	**51.4±2.2**	52.9±0.7
4	85.6	0.85±0.05	0.42±0.03	**50.6±5.0**	52.9±0.7
5	97.2	0.31±0.02	n.d.	n.a.	**53.0±0.7**
Total, %		**101.1±0.4**	**47.5±0.6**		
100 - Total ^{70}Zn recovery **52.5±0.6%**					

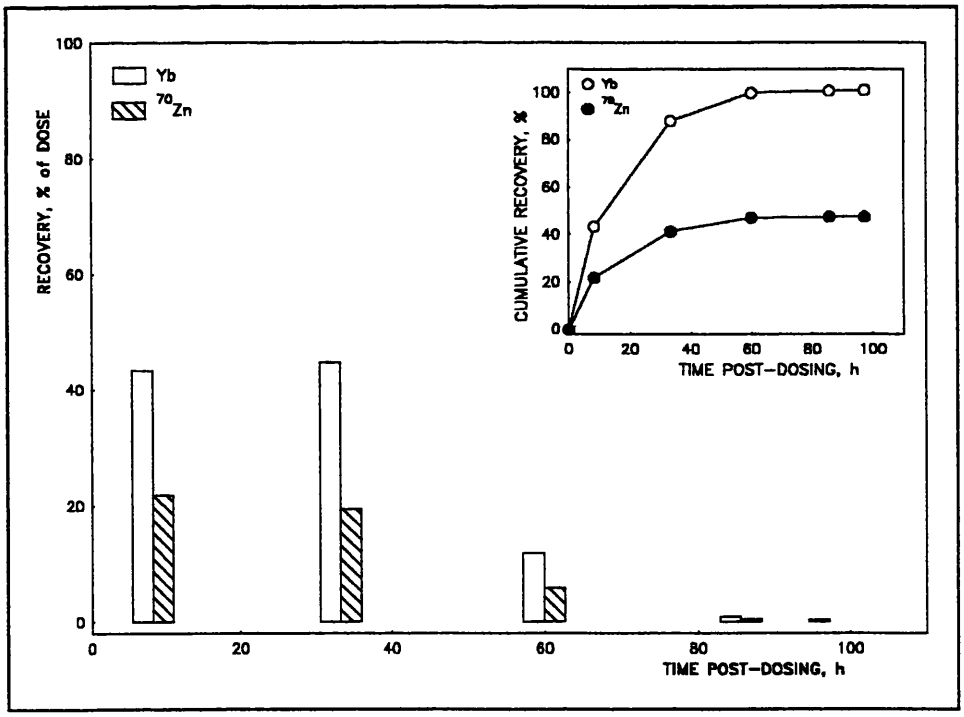


Figure-44.e: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-5

Table-76.f: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 6					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	25	48.87±0.17	27.59±0.76	**43.5±1.2**	43.5±1.2
2	48.8	42.98±0.22	25.52±0.70	**40.6±1.1**	42.1±0.8
3	70.3	3.46±0.03	1.48±0.1	n.a.	42.7±0.8
4	80.3	0.12±0.01	n.d.	n.a.	42.7±0.8
5	94.8	0.15±0.01	n.d.	n.a.	**42.8±0.8**
Total, %		**95.6±0.3**	**54.6±1.0**		
100 - Total ^{70}Zn recovery **45.4±1.0%**					

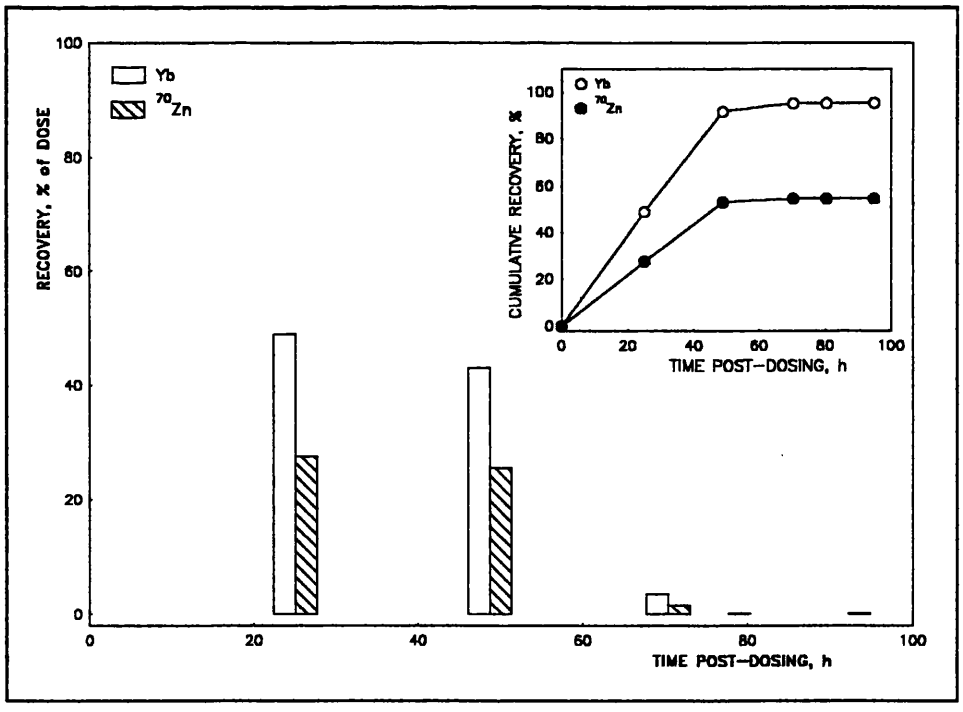


Figure-44.f: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-6

Table-76.g: Faecal recoveries of Yb marker and ^{70}Zn tracer, and luminal disappearance of zinc, when consumed with a farina meal

SUBJECT 7					
Faecal sample No	Time post-dosing, h	Recovery, %±SD		^{70}Zn Luminal disappearance, %±SD	
		Yb	^{70}Zn	Individual	Cumulative
1	23	69.18±0.38	47.58±1.41	**31.2±0.9**	31.2±0.9
2	36.8	18.62±0.13	12.50±0.44	**32.9±1.2**	31.6±0.8
3	47.8	6.42±0.12	4.32±0.27	**32.6±2.1**	31.6±0.7
4	73.3	1.71±0.06	1.75±0.14	n.a.	31.0±0.7
5	81.6	0.31±0.002	0.11±0.01	n.a.	**31.1**±0.7
Total, %		**96.2±0.4**	**66.3±1.5**		
100 - Total ^{70}Zn recovery **33.7±1.5%**					

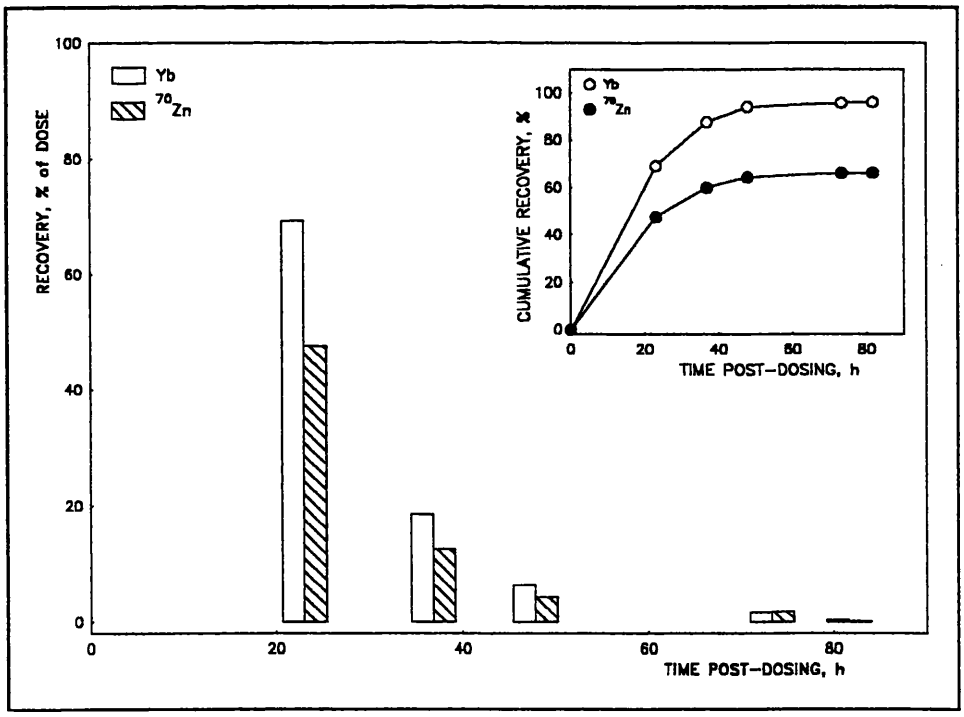


Figure-44.g: Faecal recovery profile of Yb marker and ^{70}Zn tracer for individual samples and composites of sequential output (Cumulative) for Subject-7

III.3.8 DISCUSSION

III.3.8.1 RECOVERY KINETICS OF ^{70}Zn AND Yb

As can be seen from **Table-76.a-g** luminal disappearances (intestinal uptakes) obtained for the second, third, and even fourth individual samples are not significantly different from those of composites of 2-5 sequential samples. The deviations obtained for the first samples, particularly for the samples in which recovery of marker is less than 40%, suggest that at least two the first two samples should be collected to evaluate the fraction initially retained.

For comparison of kinetic behaviour of marker and isotopic tracer excreted, the ratio of tracer to marker recoveries for individual samples and those for composites of sequential outputs for all subjects are provided in **Figure-45**.

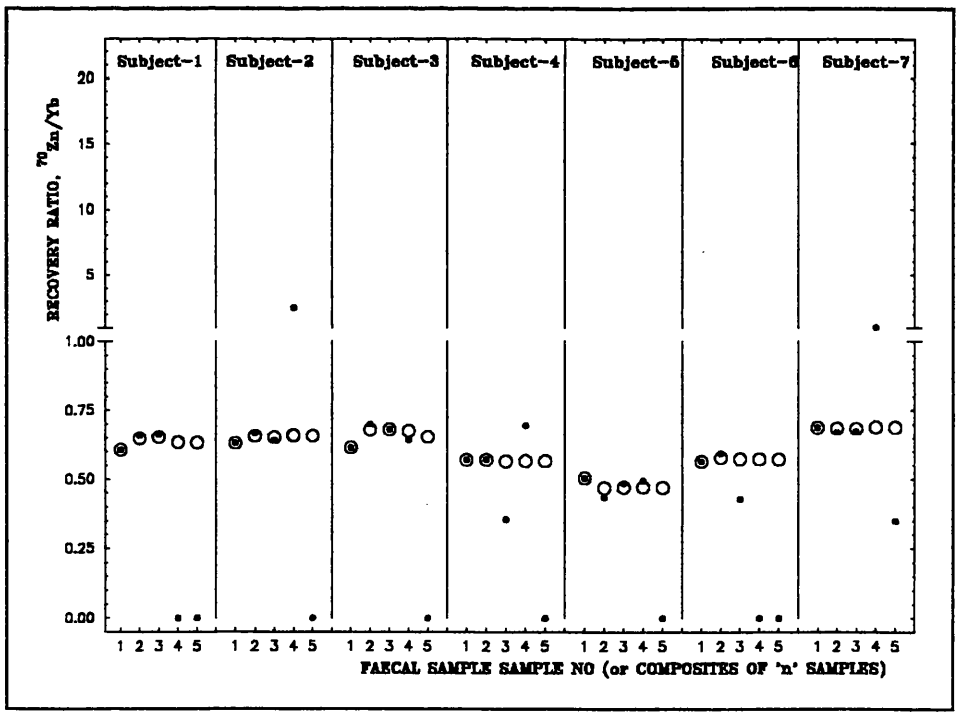


Figure-45: Ratios of recoveries of ^{70}Zn tracer and Yb marker for individual samples, ●, and for composites of sequential outputs, ○, for all subjects

The ratio of recoveries are constant for composites of the first two to five sequential samples. Irregular deviations for individual samples (usually after the third samples) can be explained by the enrichment of isotopic tracer over natural level of the isotope being below the limit of detection or that determined less than 1% of the dose, with recovery of marker being only a few percent of the dose. The magnitude of the differences between marker and tracer as a function of the sequence in which faecal samples were collected after consumption of marker and tracer are provided in **Figure-46**, as described previously.

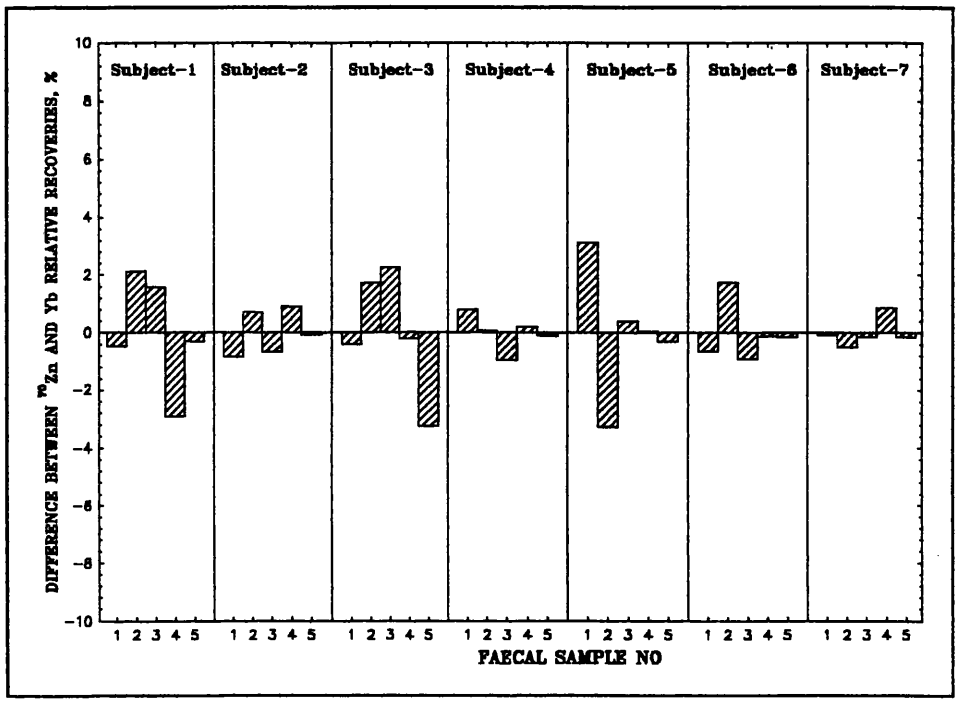


Figure-46: Differences between recoveries (as percent of their total outputs) of ^{70}Zn tracer and Yb marker for all subjects

As can be seen from the figures differences are much less than was obtained for iron and do not show a regular trend which could be indicative of re-excretion of tracer initially retained. The magnitude of differences also indicate the differences in kinetic behaviour of marker and tracer are small and insignificant.

The linear relation between the relative recoveries of Yb marker and ^{70}Zn tracer with composites of the first two samples (since the recovery rates of marker and tracer

with the first samples may not be reliable) and with the following individual outputs, as percent of their total recoveries for composites of the first three to five faecal samples for all subjects are provided in **Table-77**. The linearity for the composites of the first two samples is derived from the relative recoveries of marker and tracer obtained for composites of the first three samples. The linearity is also derived for the collection of five stools for which the first and second samples are considered as individual samples, to show the effect of the relative recoveries of marker and tracer obtained for the first samples, and provided in the table. The table contains the linearity between the recoveries and its correlation coefficient.

Table-77: Linearity between recoveries of Yb marker and ^{70}Zn tracer for all subjects [y=(A±a)x+(B±b), where y and x are recoveries of tracer and marker, and A and B are the slope and intercept]

Composites	A±a	B±b	R^2
3 samples (2 samples composites & 3. samples)	1.01±0.01	-0.62±0.18	1.000
(2 samples composites)	1.01±0.01	-0.70±0.58	1.000
4 samples (2 samples composites & 3. and 4. samples)	1.01±0.01	-0.31±0.28	0.999
5 samples (2 samples composites & 3., 4., 5. samples)	1.01±0.01	0.30±0.25	0.999
[5 samples (The first to fifith individual samples)	1.02±0.01	-0.38±0.29	0.997]

As can be seen from **Table-77** that slopes of the linear relations which are the closest to the unity are obtained for all composites. This suggests that luminal disappearances derived from the recoveries obtained for composites of the two or three samples should not be significantly different from those obtained for the following composites.

III.3.8.2 DISCUSSION OF LUMINAL DISAPPEARANCE OF ZINC

The results for intestinal uptakes (luminal disappearances) of ^{70}Zn tracer determined with Yb yield correction are provided in **Table-78**. The table contains the results for individual samples where available and for composites of the first to fifth sequential samples. The recoveries of marker with the corresponding composites are also provided.

Table-78: Luminal disappearences of ^{70}Zn tracer for single (means for n_1) and composites of sequential 1-5 samples, and recoveries of Yb marker

Subject	LUMINAL DISAPPEARANCE, %						
	For single samples %±SDM	n_1	Composites of 'n' sequential samples				
			1 Sample	2 Samples	3 Samples	4 Samples	5 samples (Total collection)
1	35.8±3.1	3	39.3	35.2	34.7	36.6	36.8
2	36.5±3.3	3	36.9	34.4	34.8	34.2	34.3
3	34.0±3.9	4	38.5	31.9	31.9	32.5	34.7
4	42.9±0.02	2	42.9	42.9	43.5	43.4	43.4
5	52.5±3.6	4	49.6	53.1	52.9	52.9	53.0
6	42.1±2.1	2	43.5	42.1	42.7	42.7	42.8
7	32.2±0.9	3	31.2	31.6	31.6	31.0	31.1
Mean±SDM	39.4±7.0		40.3±5.8	38.9±7.8	39.0±7.7	39.4±7.5	40.2±7.9
Yb recovery, %±SDM	not applicable		42±31	76±23	96±7	99±4	100±3
#Time±SDM	n.a.		20±7	42±14	61±20	84±22	105±27

The mean of time post-dosing (h) for provision of n^{th} sample

As can be seen from the table the mean of luminal disappearances for the first two samples composites is 38.7±7.8. This is not significantly different from the means for composites of the first one, three, four, and five samples and individual stools, $p>0.10$. The initial two sample composites were chosen as the reference for comparison since the mean of uptakes for the first samples showed a slight positive bias when compared with that for the composites of initial two sample, which may be explained by temporary mucosal trapping of the uptake of tracer from the lumen.

For 2 samples composites, intestinal uptake of 38.7% is equivalent to uptake of 1.1 mg of Zn for an input of 3 mg. For consumption of 3 meals per day containing comparable levels of Zn (or a total of 9 mg), the daily uptake of zinc would be 3.3 mg. The amount taken up is comparable to the amount endogenously secreted and so obviously replaces the losses, as found by Turnlund et *al.* (1984).

The range of absorption from different dietary intakes using different methods of measurement, as provided by different authors are provided in **Table-79**. The agreement between the absorption results provided by Couzy *et al.* (1993) and this investigation (39.2±9.8% range 27.5-54.2% and 38.7±7.8% range 31.6-53.1%) are worthy of note. The dietary intake had similar constituents with comparable levels of zinc.

In conclusion, intestinal uptake of zinc from a farina meal labelled with ^{70}Zn tracer and Yb marker was determined from composites of sequential two to five faecal samples. Difference between intestinal uptakes obtained for the composites were insignificant, which agreed with the findings provided by Flanagan *et al.* (1985) where radioactive tracer (Zn-65) and marker (Cr-51) were used. The best approach for determination of intestinal uptake is the determination in the first 2 consecutively accumulated stool samples (recovery of marker as the mean of Yb recoveries for 7 subjects was 76±23% provided in 42±14 h post-dosing), which minimise the possible effects of temporary retention and re-excretion.

Table-79: Summary of measurements of absorption of zinc (**WBC**: whole body counting, **FM** and **UM**: faecal and urine monitoring)

Subject no (sex)	Absorption,%±SD (Range)	Dose, mg Intake mode	Method of analysis	Reference
5 (M)	29.6±4.9 (21.7-39.5)	Zn-65 (Breakfast)	Zn-65 in blood	Spencer, 1965
8 (F)	46.0±14.5*	Basic formula 11mg/d labelled with mg ^{70}Zn	FM by NAA	King, 1978
5 (M)	61* (41-79)	2 mg of $ZnSO_4$ labelled with ^{69m}Zn in solution	^{69m}Zn in blood	Molokhia, 1980
5*	58±26*	3 mg Zn labelled with Zn-65	WBC and FM	Payton, 1982
10 (M)	57.1±18.5 (16-89)	7-11 mg/d Zn with chicken etc. labelled with ^{70}Zn and ^{68}Zn	FM by NAA	Janghorbani et al, 1982
8 (M)	82.1±10.6 (61-93)	1.5-15 mg dose dependent input labelled with ^{70}Zn	FM by NAA	Istfan et al, 1983
1 (M)	38.7±13.4 (20.7-57.8)	5 mg Zn labelled with ^{67}Zn , intravenous	FM by TIMS	Jackson et al, 1984
4 (M)	34.0±12.5 (21.9-51.3)	15 mg/d labelled with ^{67}Zn, egg albumin protein diet	FM by TIMS	Turnlund et al, 1984
15 (F)	27.4±8.7 (14.3-43.2)	5.7±1.0 mg/d Zn labelled with ^{67}Zn and ^{70}Zn, milk and formula diet	FM by ICPMS	Egan, 1991
4 (2M, 2F)	61.0±3.0 (57-65)	2 mg ^{70}Zn in solution	FM by NAA	Wastney et al, 1991
4 (3M, 1F)	49.8±22.9 (24-70)	9±4 mg/d Zn labelled with 3mg of ^{70}Zn or 4mg of ^{68}Zn, oral and iv simultaneously	UM by FAB-SIMS	Friel et al , 1992
11 (M)	31.1±11.8 (10-57)*	3 mg Zn labelled with 2 mg ^{67}Zn in chicken sandwich and 1.5 mg ^{70}Zn intravenous	FM by TIMS	Fairweather-Tait et al, 1992
9 (M)	38.9±9.8 (27.5-54.2)	1.84 mg Zn labelled with 0.8 mg ^{70}Zn in milk, butter and jam with rolls	FM by TIMS	Couzy et al, 1993
7 (6 M, 1F)	38.7±7.8 (31.6-53.1)	3 mg Zn labelled with 1 mg of ^{70}Zn, in Farina meal	FM by NAA	This study

* No absorption results available for each subject

Payton et al. (1982) determined the 'absorption' of zinc, administered as solution, from the first three consecutive faecal samples, re-entry of initially absorbed dose being assumed to be 1% d^{-1} and insignificant. The authors used radioactive Zn-65 as tracer and Cr-51 as non-absorbable marker. Flanagan et al. (1985) used the same tracer and marker to investigate zinc uptake from a solution and a test meal by taking account of the marker and tracer contents of the first stool samples which contained >10% of marker. Results of both studies showed that intestinal uptake of zinc could reliably be determined by faecal monitoring and results were confirmed by whole body counting. However the associated exposure to radiation was a continuing limitation (Flanagan et al., 1985 estimated the radiation dose to be 500 μSv assuming 50% of Zn-65 was absorbed).

The absorbed amount of the extrinsic addition should be the same as the natural zinc content of the food. Janghorbani *et al.* (1982) compared absorption of extrinsically added zinc with that of chicken meat tagged intrinsically. It was concluded that the extrinsic tag approach was valid for measurement of bioavailibility of dietary zinc in human subjects where the difference between intrinsic and extrinsic labels was relatively small (6-11%) and could be attributable to a systematic bias inherent in NAA. Fairweather-Tait *et al.* (1991) showed that despite the food itself having a more profound effect on apparent zinc absorption, extrinsically and intrinsically labelled foods may provide different answers. In contrast, the results based on determination of zinc absorption from milk based diets in adult human subjects indicated that the technique of extrinsic labelling could be valid (Egan et al., 1991). Egan *et al.* found no significant difference between intrinsic and extrinsic labels, even though inter-subject variation was relatively high. It is also worth mentioning that Couzy *et al.* (1993) showed that the different amounts of zinc in two test meals (2.74 vs 1.84 mg Zn) had no significant influence on results unless the extrinsically added zinc exceeded 4.5 mg. The study was based on the comparison of zinc absorption (in elderly and young men) from a high bioavailibility test meal with a low bioavailibility test meal.

Additional questions related with the physiological process of intestinal uptake of zinc when determined with isotopic tracers (radioactive or stable) are: 1) the amount of the endogenous contribution to total faecal output during the sample collection period and 2) the degree of uptake of the dietary mineral from the lumen which is temporarily trapped in the mucosal cells and subsequently excreted in faeces.

The endogenous contribution to total faecal output is considerable, ranging from 2.2 mg to 5.4 mg, which is approximately equivalent to daily zinc absorption (Turnlund et al., 1982, 1984, and 1986). The average faecal excretion of intravenously administered radioactive Zn-65 has been found to be 12.8% over 12 days, whereas the average excretion in urine was 1.6% (Spencer et al., 1965). The mean excretion of intravenously administered ^{70}Zn was found to be 7.5% in collection of faeces for 5d and 0.9% d^{-1} seven day post-injection whereas it had fallen to 0.3% at 20-21 d in healthy adult men (Fairweather-Tait et al., 1992). By the use of faecal tracer excretion results from the former study, the authors and Swanson *et al.* (1983) indicated the difference between apparent and true absorption was minimal and insignificant when endogenous losses are small. In order to avoid overestimation of the net absorption of tracer, which required complete collection of faecal samples containing all unabsorbed isotope, Solomons *et al.* (1982) suggested that collection of five consecutive stools after oral administration would be sufficient to collect all unabsorbed stable isotopic tracer. Changes in the amount of isotopic tracer in faecal samples due to re-entry to the faecal pool of absorbed tracer in this period would not be detectable.

Solomons et al., 1982 have suggested that the fraction of zinc trapped in mucosa may be involved in normal intestinal regulation of zinc absorption. In an investigation considering the relation of zinc absorption with the vitamin B-6 content of diet, Turnlund et al. (1991) found that the serum zinc increased linearly with vitamin B-6. It was concluded that zinc was sequestered within the gastro intestinal tract or elsewhere, and not transferred to the blood when

vitamin B-6 was depleted. This suggests that a fraction of zinc taken up might be trapped in the intestinal mucosa, depending on regulatory factors of zinc absorption.

The results of above considerations for physiological influences in determination of 'zinc absorption' assume 'apparent absorption' to be nearly equivalent to 'true absorption'. However almost all authors agree that the endogenous losses (secretion) play an important role in the homeostasis (Turnlund et al., 1982, Sian et al., 1993, and Lee et al., 1993). Considering this, it seems that determination of zinc uptake from a diet labelled with stable isotopic tracer based on determination of faecal disappearance in composite of the first 2 samples, the effects of both the endogenous contribution and temporary mucosal retention would be minimized.

Results for the intestinal uptake of zinc were consistent and the results obtained from the first 2 sample composites were not significantly different from those obtained from 3,4, or 5 sample composites. Unlike ferrous-iron, there was no correlation between ferritin and the intestinal uptake results, although a recent investigation carried out by Yokoi *et al.* (1994) implies a possible relation.

IV. CONCLUSION

To confirm that orally administered rare earths are not absorbed, faecal recoveries and recovery profiles of five selected rare earths (La, Sm, Eu, Tb, and Yb) have been investigated.

The suitability of rare earths for use as recovery indicators in determination of luminal disappearance of trace elements with enriched stable isotopic tracers has been considered, with particular attention to the possibility of determination of uptake from reduced faecal collection. The luminal disappearance of iron from a standard solution and from a wheat flour based farina meal, and of zinc from the meal were investigated with different rare earth markers (Sm and Yb) for each mode of intake.

Rare earth recoveries

Mean faecal recoveries of the 5 selected rare earths were found to be 93.8±6.1% for initial investigation on 2 subjects and 94.1±4.2% for 6 subjects. The insignificant difference between recoveries obtained for each of rare earths; coefficients of variation were 1% for the initial and 0.5% for the second investigation. Recoveries of Sm, Eu, Tb, and Yb were not significantly different each other ($p>0.05$), but those of La were different from the other rare earths ($p<0.05$) until account was taken of baseline levels. Concentrations of La determined in faecal samples provided at the end of the collection period were not significantly different from those determined in baseline samples. These imply that incomplete recovery is due to losses during sample collection or sample processing and that the losses due to absorption are less probable.

Quantitative recoveries of rare earths were obtained for Sm and Yb in the isotopic application, with total subject cooperation, the use of an improved faecal collection system, and improved analytical procedures. Mean recoveries were 102.8±3.0% for Sm and 100.1±3.1% for Yb. By comparing the level of the rare earths in urine samples before and after consumption, it was evident

that absorption of Sm consumed with a solution was low (<1%) whilst no absorption was found for Yb consumed with the meal.

Mean recoveries of rare earths are provided in **Table-80**. The results for recoveries of rare earths obtained by Luckey *et al.* (1977) and of Dy, which was used by Schuette *et al.* (1993) for determination of absorption of Zn, Cu, and Mg whilst the present investigation was in progress are also provided for comparison.

Table-80: Summary of faecal recoveries of rare earths

Rare earth	Number of subjects (Sex)	Recovery of RE, % of dose Mean±SDM	Range	Median	Reference
Mean of 5 RE	4 (M)	93.7±10.7	84.6-109	87.4	Luckey et al, 1977
"	3 (M)	88.4±6.2	82.7-95.0	87.4	"
"	2 (M)	93.8±6.0	89.5-98	-	This study
"	6 (5M, 1F)	94.2±4.4	88.1-101	94.5	"
Dy	6 (4M, 2F)	103±2	92-108	104	Schuette et al, 1993
"	"	105±5	96-128	103	"
Sm	7 (6M, 1F)	103±3	99.5-108	102	This study
Yb	"	100±3	96.1-105	100	"

Recoveries of 5 selected rare earths obtained in this investigation are higher than those obtained by Luckey *et. al* (1977). The coefficient of inter element variation was less than 1% (for all subjects) whilst Luckey *et. al* (1977) obtained coefficient of variation of 5%. Recoveries of Sm and Yb used in the isotopic applications were nearly quantitative for all subjects for the present investigation, those of Dy reported by Schuette *et. al* (1993) were also reported to be quantitative but some of the results, which were >100%, were explained as being due to inherent errors in administration and quantification.

From recovery profiles of rare earths for all subjects, it can be seen that the rare earths appear in the first 24 hours after consumption, and reach a maximum in 48 hours. Recovery of the major fraction is obtained with the first

two samples provided after administration; the recoveries ranged 45-82% for the initial investigation, 43-97% (median: 84%) for the extended investigation, 25-98% (median: 92%) for Sm and 30-96% (median: 86%) for Yb for the isotopic application. Rare earth recoveries with the first 3 samples collected following consumption were not significantly different from recoveries with 4-5 samples in all investigations. However, in the case of Sm which was consumed with a drink, mean of recoveries were almost quantitative by the first two samples. This suggests that the intestinal passage rate is faster after consumption of a solution than after consumption of a meal. The time of the first appearance for Sm consumed with a solution ranged 3.4-24.1 h (median: 7.2 h) compared with 7.7-28.7 h (median: 12.3 h) for Yb received with a meal.

Measurement of intestinal uptake of trace elements by the use of isotopic tracers and rare earth markers

The luminal disappearance of tracer isotopes obtained from the results of analysis of single samples, for composites of sequential output, and for total collections were evaluated for evidence of comparable behaviour of tracers and markers and to ascertain the minimum faecal output required to reliably determine uptake.

For iron consumed in solution or with a meal, ratios of tracer to marker obtained for composites of the first two and three samples were constant. For the following composites, the ratios increased due to retarded excretion of tracer initially taken up. This increase was more significant for iron consumed with a standard solution compared with that for iron consumed with the meal. For zinc, there was no significant difference between tracer to marker ratios obtained for composites of the first two to five samples indicating insignificant retarded excretion of the tracer.

The significance of differences in the kinetic behaviours of each tracer and marker were calculated as differences between their relative recoveries (fractions of total output) for all subjects, for each of one to five individual

faecal samples and are provided in **Figure-47**.

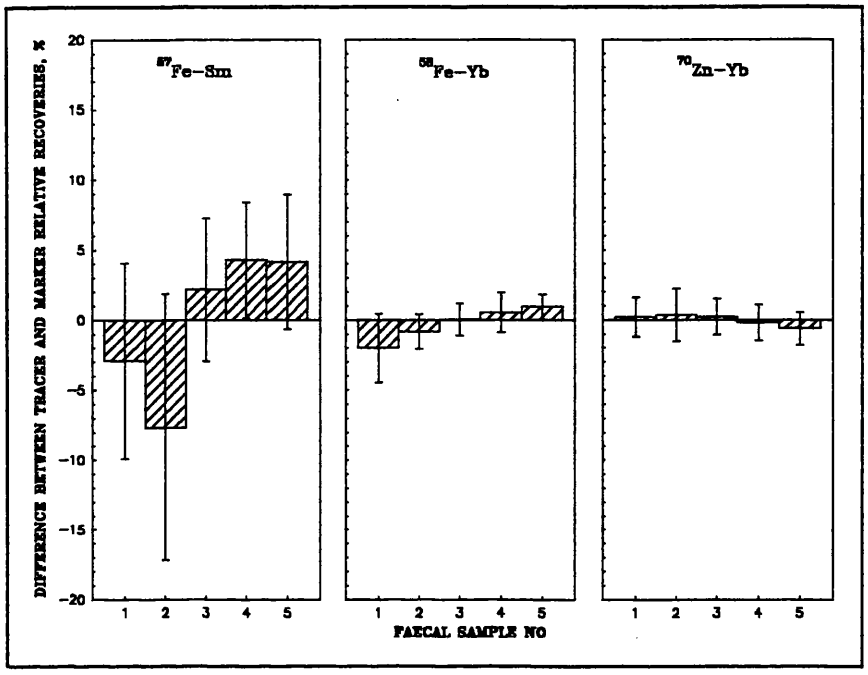


Figure-47: Means and deviations of differences between relative faecal recoveries of ^{57}Fe and Sm (with a standard solution) and ^{58}Fe, ^{70}Zn and Yb (with a meal) for 7 subjects

The mean of luminal disappearances obtained for iron and zinc for all subjects, obtained for composites of the first one to five sequential samples are provided in **Table-81**.

Table-81: Luminal disappearance and recoveries of markers for composites of 1-5 samples (mean±SDM for 7 subjects)

	Luminal disappearance for composites of 1-5 samples, %±SD				
Tracer	1 sample	2 samples	3 samples	4 samples	5 samples
^{57}Fe, with solution	24.0±16.8	30.7±16.4	28.8±15.1	24.8±16.3	22.9±17.2
^{58}Fe, with meal	23.5±7.1	23.6±6.3	23.1±6.2	22.6±5.9	21.8±6.4
^{70}Zn, with meal	40.3±5.8	38.7±7.8	38.9±7.8	39.0±7.7	39.4±7.5
	Marker recovery , %				
Sm, with solution	36±30	86±24	98±12	102±3	102±3
Yb, with meal	42±31	76±23	96±7	99±4	100±3

Luminal disappearances obtained from the first samples after consumption of the solution were not reliable. This was more obvious for the first samples in which recovery of marker was 20% or lower. For iron and zinc consumed with a meal, mean of luminal disappearances obtained for the first samples was not significantly different from those of the first two sample composites. The difference was significant for some subjects when recovery of marker with the first sample is less than 40%.

The initial intestinal uptake of iron in which contribution of retarded excretion of tracer is minimal was obtained from composites of the first two samples. The uptakes obtained for composites of the first three (or the first three or four for iron with a meal) samples were also not significantly different from that of initial uptake, $p>0.05$. Uptakes for zinc found for the first one to five samples composites were constant, $p>0.10$. This agrees with the conclusion drawn by Schuette *et. al* (1993), who also investigated applicability of dysprosium for determination absorption of Mg and Cu using enriched stable isotopic tracers. The results showed that the approach for Mg was as applicable as for Zn, but significant difference between absorption of Cu obtained from composites of initial samples and total collection was associated with re-excretion of tracer.

Conclusion

1. Investigations performed to confirm the non-absorbability of rare earths demonstrate that rare earths can be recovered quantitatively with total subject cooperation and attention to sample processing. Agreement between recoveries of the 5 selected rare earths indicates that differences of absorbability between the rare earths are negligible, confirming reproducibility of the results when any of member of this element group is used.

With attention to sample collection, recoveries were quantitative when two rare earths (Sm and Yb) were applied to measurement of intestinal uptake of iron and zinc. The quantitative recoveries obtained suggest that rare earths could be used as markers in preference to PEG, which is commonly used and for

which losses due to hydrolysis, fermentation, and absorption (Egan *et al.*, 1991) prevent reliable recovery measurement.

2. From analysis of individual faecal samples, recoveries of the rare earths for composites of the first three samples were nearly quantitative. The times of first appearance were significantly different for rare earths consumed with a solution and with a meal. This difference suggest that the rare earths could be used to determine intestinal transit times of various types of intake. Comparative studies could be carried out to investigate the effect of intestinal kinetics on absorption, such as investigation of the relation between the time available for digestion and microbic utilization and intestinal passage time by addition of a different rare earth to each substance consumed. Recovery profile and intestinal kinetic of rare earths could also provide information on time dependent biological variations in studies of intestinal physiology.

3. Results obtained from determination of the luminal disappearance of ferrous-iron consumed with a drink demonstrate that initial luminal disappearance can be determined from composites of the first two faecal sample. Composites containing samples collected beyond the second include a significant amount of retarded excretion of tracer. The coefficient of variation for the mean of results for composites of the first two to five samples, each composite being mean of 7 subject, was 13.4%.

The luminal disappearance of ferric-iron consumed with a meal was found to be more consistent when the results were obtained from the first one to seven consecutively accumulated samples. The coefficient of variation for the mean of results for composites of the first two to five samples, each composite being mean of 7 subject, was 3.3%.

The use of markers should enable investigation of the effect of oxidation state (*e.g.* Fe, Se) and/or the mode of intake of a trace element on intestinal uptake. Such investigation could be carried out for portions of a diet

administered at an interval of a few days to avoid possible isotopic exchange, by labelling with different stable isotopic tracers in different oxidation states with a rare earth marker for each.

4. The main concern of this investigation has been determination of intestinal uptake by faecal monitoring so that information for the fate of tracer taken up from the intestinal has not been provided. Significant correlation was found between serum ferritin and luminal uptake of ferrous iron (invetigated by using a tracer consumed in a solution containing ascorbic acid) but not for ferric iron (investigated by using a tracer in farina meal). This may be explained by there being two different absorption mechanism for each of the oxidation states. Determination of the amount of tracer taken up from the intestinal lumen and the fraction appearing in the blood, and their relation with ferritin levels could provide more information on the absorption mechanism.

5. For zinc, luminal disappearances derived from composites of the first one to five consecutively accumulated samples were found to be very reproducible. The coefficient of variation for the mean of results for composites of the first two to five samples, each composite being mean of 7 subject, was 0.8%.

Application of the use of multiple stable isotopic tracers for determination of intestinal uptake of zinc is limited since the amount of endogenously excreted tracer could be considerable when an enriched tracer of high natural abundance is used. Available isotopes are ^{70}Zn and ^{67}Zn with abundances of 0.6% and 4.1%, with the next lowest abundant isotope being ^{68}Zn with an abundance of 18.6%. Assuming 50% of a dose is absorbed and 75% of the unabsorbed fraction is recovered with collection of faeces for 2 days (or 2 samples), to produce a 10% enrichment over a 30 mg natural zinc content, the required doses will be 1.6 mg for ^{68}Zn, 2.2 mg for ^{66}Zn with 28% abundance, and 3.9 mg for ^{64}Zn with 47.8% abundance. Without limited collection the doses for high abundant isotopes will exceed the physiological dose, or long-term cumulative endogenous secretion could compromise

subsequent measurements and precision. This advantage obtained with reduced faecal sample collection by the use of rare earths markers could be extended to other essential trace elements where multi isotopic applications are required.

6. As used in this investigation, the required doses of ^{58}Fe (0.28% abundant) and ^{70}Zn (0.6%) were 1 mg for extrinsic labelling of a diet. By the use of rare earth markers, which enable reduced faecal collection, the required doses of tracers could be reduced to 0.1 mg, which would enable their determination with satisfactory precision in two day sample pools. For such application, 0.05-0.1 mg of rare earth marker would be sufficient for precise determination in the two day pool, where >60% of the dose is expected to be recovered. This reduction from the above used dose would provide two major advantages; 1) The cost of stable isotopic tracers for applications in mineral absorption investigations would be reduced proportionally, so that investigations could be extended to field investigations involving greater number of subjects, 2) Possible dose related effects of added stable isotopic tracers on absorption from the natural content would be reduced.

In this context, the following essential elements for which 'absorption' has been determined using stable isotopic tracers with faecal monitoring offers potential for future investigations; Copper (Johnson *et al.*, 1988, Turnlund, 1990), Calcium (Janghorbani *et al.*, 1981, Yergey *et al.*, 1990), Magnesium (Schwartz, 1982), Selenium (Patterson *et al.*, 1989), and Molybdenum (Turnlund *et al.*, 1995).

By addition of rare earths markers to different inputs, containing different elements, investigation of inter element effects (with reduced collection) should also be possible.

7. For investigations which require radioactive tracers (usually involving whole body counting) or methods based on measurement of tracer incorporated in

tissue associated with faecal monitoring (dual radio-isotopic tracers, one tracer and the other inert marker), the use of inactive rare earth would avoid the contribution of markers to radiation exposure.

In conclusion, the use of rare earths as non-absorbable markers in studies of absorption of essential elements by faecal monitoring enables determination of intestinal uptake with reduced faecal collection. In this investigation, all single faecal outputs were analyzed to establish output profiles and tracer/marker comparability. In future practical applications, analysis would be simplified by processing of pooled outputs. Errors which can arise from incomplete sample collection are accounted for by measurement of marker recovery, and the ability to determine intestinal uptake from a limited faecal output is more acceptable by subjects. This advantage makes investigations based on the use of stable isotopic tracers more economic so that field research should be possible. Applicability of the rare earths for determination of intestinal uptake of iron and zinc has been undertaken in this investigation to demonstrate their use in multi-element and -isotope applications. For wider applications to the other trace elements, further investigations of the intestinal kinetic behaviour of such element in relation to that of rare earth markers would be required. For iron and zinc further investigations of absorption in children and in unconfined situations could be performed.

APPENDICES

APPENDIX-1: Information and consent form for subjects who participated in investigation of determination of faecal recovery of rare earths

INFORMATION FOR VOLUNTEER

1.In the established method for determination of trace element absorption using stable isotopic tracers, with absorption determined by the difference between input and faecal output, measurement of complete output requires accumulation of all faecal output over a period of days. By addition of an inert marker to the tracer dose, determination of marker in faeces facilitates measurement of recovery and determination of absorption from limited faecal collection, which enables application of the method to unconfined situations.

2.The rare earths are suitable potential markers for such application, having been demonstrated to be non-toxic and not absorbed from the gastro-intestinal tract. The initial intention is to extend previous studies of the rate of throughput of such markers, with pre-irradiation separation where appropriate, to reduce radiation exposure whilst processing samples and reduce the limit of detection. On identification of the acceptable limited collection, the method will be applied to studies of the absorption of essential trace elements. The initial investigation has been approved by the Joint Ethical Committee of Grampian Health Board, since it is being carried out in association with the Department of Child Health and Nutrition, University of Aberdeen.

3. Volunteers are required to consume a marker solution with their Monday lunch,and to bring faecal collections to the laboratory for analysis over a period of a week. For this part of the investigation, it is important that no output be overlooked. The marker solution will have an acid taste, but will not be unpleasant, and sample odours should be adequately contained in the collection procedure.

4.When the marker solution is consumed, it should be followed by drinking two water rinses of the container, to ensure total consumption. Volunteers will be provided with

a ring which fits below a toilet seat, numbered biohazard bags which can be attached to the toilet-ring with an O-ring, bag sealing wires, a sealable polythene bowl, and a record card.

A bag should be securely attached to the toilet ring (check by giving slight pull) to be available when required. The ring should be located on the toilet so that the bag collects faeces but not urine. If there are significant solids on paper, these should be added to the bag. On completion, the bag should be separated from the ring, closed with the twist-wire, and further contained in the polythene bowl. The date and time of movement, and bag marker should be entered on the record card, and the sample provided to me, I will return the container for further use.

Thank you for your cooperation.

CONSENT BY VOLUNTEER

I understand that this investigation, which has been fully explained to me, is part of a research project which has been approved by the Joint Ethical Committee of Grampian Health Board, and may be no benefit to me personally.

I consent to participate in the study, and understand that I am free to withdraw if I so desire.

Signature of volunteer: Date:10.10.1992

I confirm that I have explained to the volunteer named above, the nature and purpose of the investigation.

Signature of investigator:

APPENDIX-2: Rare earth concentrations in ashed faecal samples determined by INAA

		Faecal weight, g			RE concentration in ash, μg g^{-1}±SD				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	La	Sm	Eu	Tb	Yb
SUBJECT A1									
1	18	173.9	48.4	4.90	48.36±1.04	47.92±0.48	477.95±16.04	373.48±9.64	240.75±3.80
2	41	299.2	75.9	7.88	61.50±1.40	63.09±0.58	608.66±20.02	476.79±11.29	314.62±4.89
3	48	235.4	54.5	5.02	28.09±1.73	29.58±0.55	301.60±36.28	202.80±19.07	127.49±5.85
4	67	84.3	24.9	2.51	6.16±1.07	4.76±0.18	51.22±7.04	43.10±3.73	22.24±1.90
5	100	203.8	55.3	5.41	1.16±0.15	0.66±0.03	10.58±1.99	6.10±1.31	3.56±0.57
6	124	131.4	29.4	3.25	0.81±0.14	0.13±0.02	2.44±0.92	3.41±0.72	1.66±0.35
SUBJECT A2									
1	18	86.7	28.8	3.74	16.92±0.56	11.52±0.25	108.56±3.74	81.50±2.76	56.06±0.67
2	35	95.9	24.4	2.63	156.93±3.84	139.41±2.21	1543.70±71.61	1111.20±46.30	732.80±19.88
3	60	102.4	30.7	3.13	111.67±7.45	105.06±1.65	1108.60±53.25	806.38±26.88	545.73±12.57
4	78	89.1	26.0	2.78	59.17±1.89	54.43±1.05	579.14±33.57	414.13±17.25	279.45±7.82
5	110	130.4	40.2	4.17	9.51±0.67	7.65±0.31	83.69±10.55	59.11±5.84	39.89±2.59
6	152	132.4	39.7	4.33	2.15±0.22	0.27±0.03	4.85±1.69	2.22±1.08	1.50±0.54
Administered dose, μg					980	970	10 000	7 380	4 980

APPENDIX-3: Rare earth concentrations in ashed faecal samples determined by pre-irradiation separation NAA

		Faecal weight, g			RE concentration in ash, $\mu g g^{-1} \pm SD$				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	La	Sm	Eu	Tb	Yb
SUBJECT B1									
1	20	180.3	37.0	3.38	127.55±3.09	127.92±3.08	1294.70±35.40	985.33±25.58	644.55±16.73
2	44	208.4	48.0	4.55	108.39±1.08	108.62±0.35	1096.30±22.06	868.71±10.75	555.94±5.59
3	67	228.4	47.0	5.30	8.05±0.14	3.22±0.03	26.04±1.67	32.92±0.42	19.15±0.21
4	92	165.8	3.8	4.73	2.57±0.05	0.58±0.01	4.65±0.37	4.73±0.14	3.10±0.06
5	120	106.7	34.0	3.37	2.39±0.05	0.32±0.01	1.78±0.11	1.60±0.07	1.50±0.05
6	143	263.9	62.1	5.86	2.17±0.04	0.28±0.01	1.88±0.09	2.11±0.04	1.38±0.02
SUBJECT B2									
1	11	40.9	15.6	1.43	2.87±0.18	0.27±0.02	0.70±0.09	0.54±0.07	0.35±0.04
2	30	158.3	43.6	5.69	110.44±2.30	113.05±1.26	1186.80±15.99	888.41±15.60	568.01±7.84
3	55	92.6	24.3	3.45	89.97±1.75	83.85±0.95	808.12±13.04	665.93±10.52	428.01±3.85
4	77	28.9	9.6	1.13	30.74±0.49	28.10±0.32	281.42±5.28	223.40±3.87	143.46±1.09
5	98	122.9	33.1	3.85	3.92±0.17	5.78±0.07	49.35±1.35	41.91±1.00	25.71±0.63
6	118	58.5	16.6	2.00	3.59±0.18	0.49±0.11	3.50±0.35	2.70±0.27	2.27±0.14
7	142	128.3	41.8	4.83	2.30±0.07	0.24±0.01	2.48±0.26	1.92±0.28	1.15±0.12
Administered dose, µg					980	980	10000	7720	5050

APPENDIX-3: Rare earth concentrations in ashed faecal samples determined by pre-irradiation separation NAA

		Faecal weight, g			RE concentration in ash, $\mu g g^{-1} \pm SD$				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	La	Sm	Eu	Tb	Yb
SUBJECT B3									
1	18	105.5	24.8	2.80	107.66±1.09	105.67±0.33	1079.30±19.05	815.01±10.20	551.53±5.95
2	42	223.7	43.9	5.16	89.36±1.14	84.91±0.35	920.16±23.71	697.94±10.27	440.50±6.26
3	66	259.7	50.0	5.78	20.75±0.37	21.38±0.85	213.49±4.09	161.48±2.49	103.88±1.28
4	90	192.3	42.2	5.25	5.47±0.09	3.81±0.04	37.52±1.08	34.26±0.83	22.41±0.38
5	117	302.8	64.6	8.15	2.10±0.08	0.65±0.01	6.38±0.57	5.21±0.30	3.41±0.13
6	141	289.8	65.5	7.86	1.33±0.04	0.16±0.00	1.40±0.07	1.18±0.06	0.64±0.03
7	163	350.0	70.4	9.14	1.31±0.02	0.18±0.00	0.55±0.03	0.42±0.01	0.39±0.01
SUBJECT B4									
1	19	203.2	58.9	5.84	44.05±0.64	42.57±0.51	432.36±8.73	326.91±5.64	218.69±3.29
2	42	375.4	97.1	10.74	55.80±1.06	53.65±0.27	560.61±14.40	434.66±9.08	266.56±4.36
3	67	280.3	57.4	6.34	13.97±0.28	11.45±0.16	118.61±3.47	95.83±1.83	63.24±1.06
4	90	235.3	57.0	5.82	3.97±0.09	2.32±0.03	22.68±0.92	19.10±0.53	12.76±0.26
5	116	180.7	40.0	4.35	1.58±0.02	0.20±0.00	2.07±0.14	1.77±0.09	1.16±0.02
6	140	425.6	82.5	9.26	0.76±0.02	0.12±0.00	0.32±0.07	0.25±0.06	0.27±0.02
Administered dose, µg					980	980	10000	7720	5050

APPENDIX-3: Rare earth concentrations in ashed faecal samples determined by pre-irradiation separation NAA

		Faecal weight, g			RE concentration in ash, $\mu g g^{-1} \pm SD$				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	La	Sm	Eu	Tb	Yb
SUBJECT B5									
1	14	13.6	3.5	0.51	55.73±1.09	58.03±0.90	603.92±13.07	514.67±12.61	297.06±6.27
2	39	219.6	38.9	5.50	134.69±1.54	138.45±0.45	1380.70±24.79	1051.50±13.85	691.57±2.30
3	62	148.6	23.8	2.71	30.16±0.55	29.87±0.41	331.73±8.73	253.54±6.27	161.75±3.17
4	86	71.2	11.7	1.40	4.76±0.10	3.57±0.03	40.71±1.21	26.47±0.81	18.04±0.36
5	96	110.9	22.4	2.52	2.68±0.04	0.86±0.01	10.71±0.22	7.05±0.16	4.81±0.09
6	113	126.7	14.8	1.82	1.08±0.02	0.11±0.00	0.55±0.02	0.42±0.01	0.28±0.01
7	135	50.4	9.2	1.02	1.25±0.01	0.10±0.01	0.39±0.07	0.30±0.05	0.20±0.03
SUBJECT B6									
1	7	51.5	14.7	1.52	2.39±0.07	0.13±0.02	0.66±0.09	0.51±0.02	0.33±0.02
2	28	123.0	28.8	2.85	144.97±1.68	135.93±0.54	1436.10±33.57	1114.90±16.16	729.33±10.04
3	54	50.4	16.2	1.48	196.73±2.19	184.15±0.77	1923.60±44.14	1500.70±21.04	954.04±13.31
4	73	176.3	40.5	4.35	46.57±0.74	45.94±0.22	475.17±8.81	350.86±4.79	233.46±2.79
5	99	39.0	12.4	1.22	24.18±0.48	16.31±0.24	147.54±4.92	154.40±0.67	78.23±2.48
6	124	109.9	28.4	3.06	9.16±0.15	4.42±0.05	42.81±1.20	41.12±0.84	24.92±0.55
7	144	211.4	52.7	5.38	3.83±0.06	1.55±0.02	14.50±0.32	15.50±0.29	11.73±0.03
Administered dose, µg					980	980	10000	7720	5050

APPENDIX-4: Information and consent form for subjects who participated in investigation of determination of intestinal uptake of trace elements using stable isotopic tracers and rare earth markers

INFORMATION FOR VOLUNTEERS

In the established method for determination of the absorption of trace element using stable isotopic tracers, with absorption determined by the difference between input and output, measurement of complete output requires accumulation of all faeces over a period of days. By addition of an inert marker to the tracer dose, determination of marker in faeces facilitates measurement of recovery and determination of absorption from limited faecal collection. The rare earths are suitable potential markers for such application, having been demonstrated to be non-toxic and not absorbed from the gastro intestinal tract.

The principal of determination of absorption with limited sample collection will be validated by application to measurement of the absorption of Fe and Zn by healthy volunteers.

Requirements are for consumption of a standard solution and a standard meal on subsequent mornings, and the provision of faecal output over a period of 10 days, and urine sample collection for 24 h, following each of the inputs for analysis. A blood sample is also required for determination of haematologic characteristics. The required procedure is based on the objectives of the study mentioned above. It is requested that the following principles of the procedure should be taken into account;

1. Because of the effects of sample losses during the collection period on results, it is important that no output be overlooked, and toilet papers used should also be collected.

2. The determination of isotopic enrichment above baseline level of a particular isotope is the principal of measurement of absorption. Excessive consumption of Fe and Zn effects the measurement of enrichment resulting in less precision. For this reason participants are requested to avoid excessive consumption of meat and meat

products (particularly heart, kidney, and liver), and some of cereals *e.g.* All-Bran and Special K.

Procedure

1. On day 1, after an overnight fast, 50 mL of standard drink containing 3 mg of Fe, 20 mg of ascorbic acid (Vitamin-C), and 1 mg of Sm as marker will be provided. This should be consumed with two rinses of water to ensure total consumption. No food or drink should be taken for 3 h after the standard drink.

2. On day 4, after overnight fast a wheat flour based standard meal (served with milk) containing 3 mg of iron, 3 mg of Zn, and 1 mg of Yb as marker will be provided. This should be eaten slowly and to ensure complete consumption with two rinses of milk. No food or drink should be taken for 3 h after the consumption.

3.The following samples will be required;

a) Stools should be collected from the day before the input and for 10 days thereafter,

b) Urine samples should be collected for 24 h before the first input and for 24 h following each of the inputs.

c)A 5 mL of blood samples will be taken to establish haematological characteristics at the end of study period, at Royal Infirmary, Glasgow by an authorized person.

4. Volunteers will be provided with commode specimen collection systems, and plastic containers containing 20 mL of 2 M HCl for urine collection.

5. For stool sample collection, the ring should be located on the toilet and the specimen collection system should be securely attached to the toilet ring so that the bowl collects faeces nut not urine. On completion, the lid should be snapped on tightly and the frame of system should be removed (see the instruction given on lids). The bowl should then be contained one of the numbered bags and closed with a twist wire. Sample need to be stored in a cool place until it can be brought to a freezer at the SURRC. The date and time of movement and bag number should be entered on the record sheet enclosed.

CONSENT BY VOLUNTEER

I understand that this investigation, which has been fully explained to me, is part of a research project which has been approved by The Research Ethics Committee of Greater Glasgow Health Board, Royal Infirmary Unit, and may be no benefit to me personally.

I consent to participate in the study, and understand that I am free to withdraw if I so desire.

Signature of volunteer: Date: 15.10.1993

I confirm that I have explained to the volunteer named above, the nature and purpose of the investigation.

Signature of investigator:

APPENDIX-5: Concentrations of Sm marker, ^{57}Fe tracer, and Fe (determined by CNAA, ICP-MS, and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, μg g^{-1}±SD		
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Sm (CNAA)	^{57}Fe (ICP-MS)	Fe (AAS)
SUBJECT C1							
1	25	193.5	56.8	5.03	157.59±2.84	201.61±8.06	2912.7±43.7
2	49	161.7	36.7	3.96	65.46±1.18	142.92±4.00	2656.9±39.9
3	73	114.5	24.6	2.11	5.19±0.17	88.33±1.86	3101.5±46.5
4	97	110.0	31.5	2.99	0.87±0.04	92.50±0.56	3538.6±53.1
5	121	190.3	45.8	5.44	0.34±0.02	71.29±2.07	3091.8±46.4
6	145	225.6	52.0	6.26	0.20±0.02		
7	169.5	221.6	51.2	5.89	0.15±0.01		
8	193.5	249.6	54.5	6.26	0.14±0.01		
SUBJECT C2							
1	13.3	134.2	42.9	5.45	15.60±0.28	110.71±2.77	3798.3±57.0
2	39	64.2	20.6	2.54	73.54±1.32	189.78±4.18	3097.3±46.5
3	63.3	56.7	23.1	3.05	128.99±2.45	261.63±4.45	3546.3±53.2
4	98.4	171.7	56.0	7.24	29.22±0.56	137.81±3.58	3101.8±46.5
5	135.6	73.8	26.3	3.18	1.67±0.04	115.75±3.59	3237.7±48.6
6	159.4	317.0	80.3	8.42	0.58±0.01		
7	183.7	69.1	19.8	2.2	0.27±0.01		
8	218.1	90.5	29.9	3.38	0.16±0.00		
SUBJECT C3							
1	10.3	280.3	54.4	7.25	83.31±1.83	149.17±2.09	1920.9±28.8
2	24.1	234.0	49.6	5.72	60.73±1.52	112.88±2.48	1924.5±28.9
3	48.6	277.4	66.8	7.73	8.49±0.20	57.11±1.31	1652.4±24.8
4	71.6	279.4	78.0	8.98	0.94±0.07	54.07±0.76	1354.1±20.3
5	83.3	100.2	31.2	4.67	0.25±0.06	43.70±0.79	1510.3±22.7
6	95	56.6	17.5	2.55	0.25±0.03		
7	103.9	165.9	48.3	9.01	0.21±0.02		
8	121.4	194.6	40.7	7.27	0.23±0.02		
9	144.8	387.4	107.0	14.66	0.14±0.01		
10	154.2	203.9	50.2	9.25	0.12±0.01		
Administered dose, μg					1017	2023	

APPENDIX-5: Concentrations of Sm marker, ^{57}Fe tracer, and Fe (determined by CNAA, ICP-MS, and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, μg g^{-1}±SD		
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Sm (CNAA)	^{57}Fe (ICP-MS)	Fe (AAS)
SUBJECT C4							
1	21.9	235.8	55.9	5.69	114.11±2.05	244.50±5.13	2499.1±37.5
2	45.9	197.0	51.1	4.98	80.59±1.45	166.83±6.67	2317.8±34.8
3	69.5	137.4	39.4	4.18	13.25±0.28	72.92±1.53	2071.7±31.1
4	96.2	180.8	47.6	4.95	0.29±0.02	85.50±1.37	2160.2±32.4
5	120.1	106.7	20.0	2.16	0.11±0.01	78.64±1.73	2769.0±41.5
6	142.9	113.7	34.6	3.4	0.11±0.01		
7	166.3	250.4	75.0	7.05	0.13±0.01		
8	190.2	194.9	38.1	4.39	0.15±0.00		
SUBJECT C5							
1	15.3	170.6	55.4	5.19	39.01±0.90	164.60±5.10	4625.0±69.4
2	30.6	198.3	49.0	5.24	144.05±3.46	224.47±1.80	4518.6±67.8
3	60.6	303.1	65.1	6.14	9.51±0.24	109.00±2.40	3471.8±52.1
4	81.2	125.9	55.5	4.9	0.94±0.06	108.04±6.05	4457.4±66.9
5	106.4	138.2	34.7	3.96	0.44±0.05	85.96±1.03	3352.9±50.3
6	133	267.0	71.2	8.04	0.30±0.03		
7	158.7	54.8	13.9	1.48	0.31±0.05		
8	170.3	470.0	83.3	1.54	0.17±0.00		
SUBJECT C6							
1	3.5	46.7	5.7	0.76	144.60±0.43	237.29±6.88	1534.7±23.0
2	22.5	127.1	37.0	4.73	165.40±0.33	337.38±5.40	1781.3±26.7
3	48.3	170.5	21.8	2.64	43.63±0.17	127.14±3.18	2372.0±35.6
4	53.5	138.4	24.6	3.37	9.01±0.07	68.57±0.75	2226.8±33.4
5	71.4	247.9	29.9	4.35	2.39±0.04	55.83±0.67	2068.0±31.0
6	97.7	144.0	32.4	2.73	0.45±0.03	76.45±1.22	2386.2±35.8
7	121.5	303.2	60.5	7.53	0.21±0.02		
8	143	49.4	14.7	1.69	0.22±0.01		
9	153	176.9	34.7	4.3	0.26±0.01		
10	167.3	48.1	12.7	1.61	0.28±0.01		
Administered dose, μg					1017	2023	

APPENDIX-5: Concentrations of Sm marker, ^{57}Fe tracer, and Fe (determined by CNAA, ICP-MS, and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, µg g^{-1}±SD		
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Sm (CNAA)	^{57}Fe (ICP-MS)	Fe (AAS)
SUBJECT C7							
1	8.5	36.2	9.9	1.03	89.76±1.89	270.66±7.85	4042.0±60.6
2	23.4	240.4	39.5	4.89	141.67±2.27	231.52±9.26	3339.3±50.1
3	47.8	160.2	23.9	2.92	52.20±0.94	109.33±4.15	1924.8±28.9
4	73.3	70.9	16.3	1.58	9.10±0.25	81.18±2.27	2164.1±32.5
5	95.8	145.9	30.7	3.62	1.10±0.03	123.15±3.45	2697.9±40.5
6	109.6	98.1	19.8	1.88	0.53±0.04		
7	120.6	82.1	23.0	2.3	0.31±0.04		
8	145.1	143.3	27.7	3.31	0.24±0.01		
9	154.4	40.3	10.2	0.95	0.29±0.01		
10	167.1	233.1	43.4	4.6	0.19±0.00		
Administered dose, µg					1017	2023	

APPENDIX-6: Concentrations of Yb marker, ^{58}Fe and ^{70}Zn tracers, and Fe and Zn (determined by CNAA and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, μg g^{-1}±SD				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Yb (CNAA)	^{58}Fe (CNAA)	Fe (AAS)	^{70}Zn (CNAA)	Zn (AAS)
SUBJECT C1									
1	22.3	110.0	31.5	2.99	40.09±0.56	44.34±0.36	3538.6±53.1	37.33±1.86	2137.5±32.1
2	46.3	190.3	45.8	5.44	94.48±1.03	89.28±0.53	3091.8±46.4	72.63±1.93	1783.9±26.8
3	70.3	225.6	52.0	6.26	54.10±0.41	53.33±0.32	3178.9±47.7	45.56±1.30	1634.6±24.5
4	94.8	221.6	51.2	5.89	4.96±0.06	15.11±0.15	3392.0±50.9	10.69±1.22	1804.5±27.1
5	118.8	249.6	54.5	6.26	0.46±0.04	11.62±0.12	3670.5±55.1	13.65±2.31	2166.1±32.5
6	142.8	206.6	55.0	5.49	0.50±0.07	8.80±0.30	2755.8±41.3		
7	168.5	73.5	21.7	2.91	1.37±0.09	10.34±0.31	3057.1±45.9		
SUBJECT C2									
1	25.7	171.7	56.0	7.24	30.51±0.08	31.82±0.29	3101.8±46.5	30.86±1.12	1905.6±28.6
2	62.9	73.8	26.3	3.18	176.29±0.87	135.78±1.00	3237.7±48.6	130.73±3.38	2333.0±35.0
3	86.7	317.0	80.3	8.42	32.22±0.07	32.67±0.26	2908.6±43.6	34.34±1.05	2247.1±33.7
4	111.0	69.1	19.8	2.20	1.55±0.04	10.48±0.13	2716.5±40.7	18.04±1.50	2286.8±34.3
5	145.4	90.5	29.9	3.38	0.26±0.02	8.80±0.09	2687.3±40.3	17.81±0.83	2898.3±43.5
6	167.0	36.1	12.6	1.57	0.25±0.03	11.55±0.31	3306.0±49.6		
7	195.8	111.9	34.9	4.87	0.21±0.02	8.90±0.13	2874.8±43.1		
Administered dose, μg					1040	1010		1008	

APPENDIX-6: Concentrations of Yb marker, ^{58}Fe and ^{70}Zn tracers, and Fe and Zn (determined by CNAA and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, µg g^{-1}±SD				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Yb (CNAA)	^{58}Fe (CNAA)	Fe (AAS)	^{70}Zn (CNAA)	Zn (AAS)
SUBJECT C3									
1	11.4	100.2	31.2	4.67	14.95±0.12	16.64±0.21	1510.3±22.7	16.33±2.10	1152.8±17.3
2	23.1	56.6	17.5	2.55	90.32±0.40	75.82±0.88	1460.6±21.9	69.20±2.27	1053.6±15.8
3	30.0	165.9	48.3	9.01	57.81±0.25	52.02±0.63	1547.6±23.2	45.76±1.73	1094.9±16.4
4	47.5	194.6	40.7	7.27	18.12±0.16	18.37±0.21	2047.2±30.7	18.36±0.73	1101.8±16.5
5	70.9	387.4	107.0	14.66	2.19±0.04	9.25±0.12	2085.1±31.3	6.85±1.02	1149.8±17.2
6	80.3	203.9	50.2	9.25	0.11±0.02	5.01±0.07	1424.4±21.4	7.72±1.01	1162.9±17.4
7	96.9	108.0	28.1	3.83	0.34±0.07	4.17±0.10	1250.6±18.8		
8	124.8	87.9	23.8	3.41	0.42±0.08	5.89±0.11	1603.8±24.1		
9	142.6	247.0	83.8	11.66	0.26±0.05	6.23±0.08	2175.8±32.6		
10	154.6	155.6	55.8	7.84	0.31±0.04	9.46±0.13	3103.1±46.5		
Administered dose, µg					1040	1010		1008	

APPENDIX-6: Concentrations of Yb marker, ^{58}Fe and ^{70}Zn tracers, and Fe and Zn (determined by CNAA and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, µg g^{-1}±SD				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Yb (CNAA)	^{58}Fe (CNAA)	Fe (AAS)	^{70}Zn (CNAA)	Zn (AAS)
SUBJECT C4									
1	24.0	180.8	47.6	4.95	184.47±1.30	140.25±1.82	2160.2±32.4	118.45±2.55	2289.7±34.3
2	47.9	106.7	20.0	2.16	32.13±0.24	34.86±0.95	2769.0±41.5	28.52±0.81	1669.8±25.0
3	70.7	113.7	34.6	3.40	7.75±0.13	16.61±0.18	2939.0±44.1	14.35±0.83	1872.8±28.1
4	94.1	250.4	75.0	7.05	1.32±0.03	9.57±0.10	2144.7±32.2	12.27±0.56	1833.6±27.5
5	118.0	194.9	38.1	4.39	0.24±0.02	7.81±0.29	2063.1±30.9	10.08±0.73	1664.9±25.0
6	142.3	128.8	28.7	3.22	0.17±0.01	9.98±0.39	2629.5±39.4		
7	165.5	324.3	58.4	5.87	0.25±0.02	7.40±0.21	2560.9±38.4		
SUBJECT C5									
1	8.2	125.9	55.5	4.90	89.37±0.45	82.50±0.72	4457.4±66.9	59.80±1.33	2448.5±36.7
2	33.3	138.2	34.7	3.96	108.17±0.74	98.56±1.18	3352.9±50.3	63.40±1.12	2306.0±34.6
3	59.9	267.0	71.2	8.04	14.96±0.22	22.79±0.28	3059.3±45.9	20.75±0.82	2185.5±32.8
4	85.6	54.8	13.9	1.48	5.80±0.35	15.94±0.21	3019.1±45.3	19.65±1.52	2711.0±40.7
5	97.2	470.0	83.3	9.41	0.34±0.02	9.76±0.10	3000.3±45.0	13.44±0.81	2236.1±33.5
6	124.2	54.6	15.5	1.54	0.28±0.05	11.43±0.16	3260.7±48.9		
7	149.5	155.0	51.4	4.94	0.18±0.02	11.56±0.13	3395.5±50.9		
Administered dose, µg					1040	1010		1008	

APPENDIX-6: Concentrations of Yb marker, ^{58}Fe and ^{70}Zn tracers, and Fe and Zn (determined by CNAA and AAS) in ashed faecal samples

		Faecal weight, g			Concentrations in ash, μg g^{-1}±SD				
Faecal sample No	Time post-dosing, h	Wet	Dry	Ash	Yb (CNAA)	^{58}Fe (CNAA)	Fe (AAS)	^{70}Zn (CNAA)	Zn (AAS)
SUBJECT C6									
1	25.0	144.0	32.4	2.73	180.80±0.63	125.56±1.14	2386.2±35.8	115.89±3.20	2392.0±35.9
2	48.8	303.2	60.5	7.53	57.65±0.30	45.88±0.49	2243.5±33.7	51.31±1.42	2808.7±42.1
3	70.3	49.4	14.7	1.69	20.71±0.18	20.58±0.28	2523.9±37.9	28.37±1.89	3159.3±47.4
4	80.3	48.1	12.7	1.61	0.76±0.06	8.24±0.14	2257.4±33.9	19.45±2.30	3139.3±47.1
5	94.8	176.9	34.7	4.30	0.35±0.02	7.64±0.15	2693.1±40.4	15.23±1.02	2541.7±38.1
6	118.8	295.4	49.6	6.30	0.33±0.02	9.29±0.12	2750.0±41.3		
7	142.1	309.7	52.1	6.50	0.45±0.03	6.11±0.09	1788.1±26.8		
SUBJECT C7									
1	23.0	145.9	30.7	3.62	193.01±1.06	142.07±2.56	2697.9±40.5	143.07±4.24	1875.4±28.1
2	36.8	98.1	19.8	1.88	100.05±0.69	82.35±1.56	2339.9±35.1	84.01±2.97	2825.6±42.4
3	47.8	82.1	23.0	2.30	28.18±0.51	27.01±0.62	2014.9±30.2	31.74±2.01	2087.9±31.3
4	72.3	143.3	27.7	3.31	5.23±0.18	15.25±0.11	2724.7±40.9	14.82±1.23	1534.9±23.0
5	81.6	40.3	10.2	0.95	3.33±0.03	14.55±0.16	2728.3±40.9	12.12±1.48	1762.8±26.4
6	94.3	233.1	43.4	4.60	0.49±0.03	6.49±0.09	1608.5±24.1		
7	118.3	168.1	25.8	3.05	0.75±0.02	6.89±0.06	1731.6±26.0		
8	142.3	172.4	29.7	3.30	0.75±0.10	7.87±0.22	2089.7±31.3		
9	166.7	82.0	24.7	1.82	1.31±0.16	7.02±0.13	1916.1±28.7		
Administered dose, μg					1040	1010		1008	

APPENDIX-7.A: Natural levels of rare earths in ashed faecal samples determined by ICP-MS

RE CONCENTRATIONS IN FAECAL SAMPLES, μg g^{-1} ASH									
Subj.→ RE	C1	C2	C3	C4	C5	C6	C7	Mean±SDM	Mean±SDM[1]
La	0.82±0.02	2.12±0.05	0.91±0.02	0.85±0.02	1.68±0.05	0.81±0.01	1.13±0.03	1.19±0.52	1.43±0.51
Ce	1.15±0.03	2.38±0.04	1.16±0.02	1.49±0.05	2.07±0.05	1.30±0.03	1.32±0.01	1.55±0.48	1.92±0.84
Pr	0.19±0.01	0.47±0.01	0.22±0.01	0.21±0.02	0.35±0.02	0.19±0.01	0.27±0.01	0.27±0.10	0.32±0.12
Nd	0.69±0.03	1.47±0.04	0.80±0.03	0.77±0.03	1.35±0.16	0.66±0.02	0.92±0.03	0.95±0.33	1.06±0.35
Sm	0.16±0.02	0.19±0.02	0.15±0.03	0.16±0.02	0.19±0.03	0.15±0.01	0.18±0.05	0.17±0.02	0.19±0.03
Eu	0.05±0.001	0.05±0.01	0.04±0.001	0.07±0.02	0.07±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.14±0.08
Gd	0.13±0.002	0.20±0.005	0.12±0.06	0.11±0.02	0.23±0.06	0.12±0.01	0.17±0.01	0.16±0.05	0.21±0.05
Tb	0.02±0.005	0.03±0.002	0.02±0.001	0.04±0.003	0.04±0.006	0.02±0.001	0.03±0.001	0.03±0.01	0.09±0.06
Dy	0.11±0.01	0.18±0.02	0.12±0.02	0.13±0.01	0.19±0.01	0.09±0.006	0.14±0.01	0.14±0.04	0.18±0.04
Ho	0.03±0.001	0.03±0.004	0.02±0.003	0.02±0.004	0.04±0.006	0.02±0.002	0.03±0.003	0.03±0.006	0.04±0.01
Er	0.07±0.01	0.01±0.002	0.06±0.006	0.07±0.005	0.11±0.02	0.06±0.003	0.08±0.006	0.06±0.03	0.10±0.02
Tm	0.02±0.001	0.02±0.001	0.01±0.002	0.01±0.005	0.02±0.004	0.02±0.004	0.02±0.004	0.02±0.003	0.02±0.002
Yb	0.26±0.01	0.27±0.02	0.12±0.01	0.35±0.04	1.013±0.04	0.75±0.03	0.26±0.003	0.43±0.32	0.14±0.03
Lu	0.02±0.001	0.02±0.002	0.01±0.001	0.01±0.002	0.02±0.002	0.02±0.001	0.02±0.002	0.02±0.004	0.02±0.002

1.Results obtained in rare earth recovery investigation for 4 subjects (Table-30, page-94)

APPENDIX-7.B: Rare earth concentrations in urine samples provided before (baseline), and after the administrations of the standard drink and meal

	RARE EARTH IN URINE SAMPLES, ng L^{-1}										
	BASELINE			AFTER STANDARD DRINK				AFTER STANDARD MEAL			
Subject→ RE	C2	C6	C7	C1	C2	C6	C7	C1	C2	C6	C7
La	133±20	87±11	223±33	79±17	69±11	77±11	71±8	80±15	60±2	50±14	30±10
Ce	240±34	137±41	564±17	147±23	153±39	130±54	167±41	168±19	145±32	105±12	109±40
Pr	165±37	127±11	172±11	156±24	172±51	141±5	111±6	169±26	108±11	124±28	122±45
Nd	ND	95±34	213±66	112±49	ND	109±33	111±9	153±50	91±14	ND	ND
Sm	147±34	120±17	193±14	1824±171	853±256	1342±184	976±318	262±57	190±56	121±33	73±34
Eu	107±20	79±31	100±22	123±7	108±29	58±10	55±8	75±20	50±9	72±28	59±13
Gd	71±12	ND	ND	137±71	ND	79±40	ND	87±48	ND	ND	44±7
Tb	72±5	48±1	89±9	41±5	106±4	50±14	89±9	31±11	78±4	52±3	65±6
Dy	44±6	ND	ND	61±13	40±11	ND	ND	ND	ND	ND	ND
Ho	15±3	25±14	ND	33±3	23±11	ND	ND	32±4	17±4	16±3	10±6
Er	ND	ND	ND	44±25	35±19	7±3	25±2	60±3	21±9	41±18	ND
Tm	6±2	ND	5±2	22±8	ND	ND	ND	20±5	7±1	8±4	ND
Yb	14115	87±30	287±20	80±8	119±29	103±13	143±25	72±22	86±15	104±12	108±30
Lu	46±3	32±4	49±14	29±4	61±11	30±7	39±17	22±3	40±7	28±4	37±12

APPENDIX-8: List of suppliers

Materials	Supplier
Hydrochloric acid (Aristar grade)	BDH or Merck/ UK
Nitric acid (Aristar grade)	"
Hydrogen peroxide (Aristar grade)	"
AAS standards (specpure)	Johnson Matthey, Material
Rare earth oxides (specpure)	Technology/UK
Isotope enriched iron and zinc (metallic form)	Europa Scientific Ltd/UK
Farina, wheat based flour	Cook, J.D., Deparment of Medicine, Kansas University Medical Centre/USA

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