The metabolism of [^{75}Se]selenite in young women

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1. The long-term fate of an oral dose of [^{75}Se]selenite was studied in three young women.

2. Urinary and faecal excretion, respiratory and dermal losses and whole-body retention of ^{75}Se were measured, and also ^{75}Se turnover in whole body, blood and tissues during a period of 16–20 weeks.

3. Intestinal absorption of [^{75}Se]selenite by the three subjects was 70, 64 and 44 % of the dose.

4. Urinary excretion accounted for 14–20 % of absorbed ^{75}Se in the 1st week. There were only trace amounts of radioactivity in expired air and no dermal loss was detected.

5. After an initial phase in which radioactivity decreased rapidly, whole-body retention of ^{75}Se diminished exponentially with a half-time of 96–144 d. Radioactivity in the liver, heart and plasma decreased more rapidly than that in the whole body, but radioactivity in skeletal muscle and bone decreased more slowly.

Decreased fertility, increased perinatal mortality and a type of muscular dystrophy called white muscle disease are well recognized consequences in New Zealand of a dietary deficiency of selenium in cattle and sheep (Andrews, Hartley & Grant, 1968). Laboratory animals also need dietary supplements of Se for growth and good health (Schwarz & Foltz, 1957) but it is still uncertain whether Se is an essential dietary constituent for man.

When developing methods for assessing the nutritional status of Se in New Zealand people, information was required about the metabolism of this element in man. The long-term fate of ^{75}Se administered orally or intravenously to rats as selenite or selenomethionine had been studied earlier (Thomson & Stewart, 1973) and the results provided the basis for this investigation of the fate of an oral dose of [^{75}Se]selenite in three women.

EXPERIMENTAL

Procedure

The subjects G, W and R were three young women aged 33, 20 and 25 years respectively, with a mean height of 1·60 m and a mean weight of 57 kg. While fasting, each received a measured oral dose of approximately 10 μCi [^{75}Se]selenite (Radiochemical Centre, Amersham) containing not more than 10 μg Se.

Collection of urine

In the first 24 h after the dose of ^{75}Se, urine was collected every h for 10 h, then every 2 h for a further 6 h and finally for the remaining 8 h. Subsequently, 24 h collections were made daily for the next 13 d and then once each week for 14–19 weeks.

Urine samples were collected in plastic bottles and the volume made up to 1·7 l with deionized water before radioactivity was measured in a large volume counter together with a ^{75}Se reference standard.

The large volume counter was constructed in the Department of Medical Physics, Wakari Hospital, Dunedin. Radioactivity in the urine samples or reference standard was determined while the container was rotated at 0·1 Hz between two uncollimated 50 mm diameter sodium iodide scintillation crystals connected in series through a pulse height analyser to an I.D.L. scaler and timer (Series No. 7000). The counting efficiency of this system is approximately 1·2%. Tests have shown that the counting rate recorded from a ^{75}Se source in this system is not dependent upon the volume of distribution of the source or its shape or position within the counting system, provided that the whole source is contained within the space occupied by the 1·7 l container.

Stable Se in each 24 h urine collection was determined fluorimetrically (Watkinson, 1966). Urinary excretion of radioactivity after the 2nd week was calculated as: specific activity of the weekly urine sample × mean daily excretion of stable Se for the same subject determined from the samples obtained during the first 14 d.

Collection of faeces

A gelatin capsule containing 50 mg brilliant blue marker (FD & C No. 1, Bates Chemical Division, Crompton & Knowles Corporation, Landsdowne, Pa) and 200 mg methyl cellulose (Kempthorne & Prosser, Dunedin, New Zealand) was swallowed immediately after the ^{75}Se dose. All individual stools passed by subjects G, W and R until day 45, day 14 and day 20 respectively, were collected separately. Thereafter a single faecal sample was obtained each week on the day of the urine collection.

Faeces were collected in weighed, waxed-cardboard containers. Radioactivity was measured in the large volume counter and the faeces were then dried to constant weight (60°–80°), ground to a fine powder and the stable Se content determined fluorimetrically (Watkinson, 1966). Faecal loss of ^{75}Se after the 2nd week was calculated by the method used for urinary excretion.

Collection of blood samples

Blood samples (10–15 ml) were collected in heparinized tubes from each subject at hourly intervals for 10 h after administration of the dose. Samples were then taken daily for the first week, on days 10 and 14 and then weekly for 19 weeks.

Plasma and erythrocytes were separated by centrifugation and the cells washed with normal saline. Radioactivity in 2 ml portions of plasma and erythrocytes was measured in an automatic sample counter (Autogamma, Nuclear Chicago Corporation) with a ^{75}Se standard.

Measurement of respiratory excretion

Expired air from each subject was collected in Douglas bags for 8–10 min periods at regular intervals during the first 9 h after administration of the ^{75}Se dose and also from subject R for one period of 10 min on day 2. Radioactivity in each filled bag was measured by the method used for whole-body counting (see below). The bag was then

emptied by bubbling the air serially through two bottles containing saturated mercuric chloride solution and a third bottle containing concentrated HNO_3. Radioactivity in these bottles was measured in the large volume counter.

Measurement of dermal loss

Two methods were used: (*a*) absorption of dermal losses in cotton underwear, long underpants and T-shirts, worn continuously for 2 d by subject G (days 1 and 2) and subject W (days 2 and 3) and (*b*) collection of 'arm-bag sweat' in plastic bags enclosing the hand and arm up to the axilla worn by subjects G and R for periods of 30–50 min at intervals on day 1. Radioactivity in clothing and plastic bags was measured in the large volume counter.

Whole-body counting

Whole-body radioactivity was measured for 200 s with the subject seated 1·8 m from an uncollimated NaI crystal, 120 mm in diameter. The crystal was linked to an automatic scaler–timer with pulse height analyser (Radiax, Deltronic Nuclear, Delft, Holland). The efficiency of this whole-body counting system was approximately 0·1 %. Radioactivity was measured immediately after ingestion of the dose, at hourly intervals for 9–11 h, daily for 1 week, on days 10 and 14 and then once each week for 14–19 weeks.

Measurement of organ radioactivity

An estimate of radioactivity in the liver, heart, knee (chosen to represent bone) and thigh (subject G) or calf muscle (subjects W and R) was made using the 120 mm NaI crystal with a flat field collimator, the face of which was applied to the body. On day 1 the position of maximum counts over each organ was marked on the body to ensure identical positioning for the subsequent measurements. When counting over the liver or heart the collimator was directed in such a way as not to accept gamma-rays originating from the region of the kidney. These measurements were made each day for 1 week, on days 10 and 14 and thereafter weekly for 14–19 weeks.

RESULTS

Urinary excretion of ^{75}Se

^{75}Se was excreted rapidly in urine during day 1, and the peak excretion rates of 0·4–1·4 % ^{75}Se dose/h occurred within 2 h. The excretion rate then decreased to less than 0·1 % dose/h at 12 h.

Urinary losses on day 1 were 6·1, 4·2 and 2·8 % dose for subjects G, W and R, respectively. By day 2 they had decreased to 1·7, 0·7 and 0·6 % dose/d and by the end of the 2nd week were 0·2–0·4 % dose/d. Total urinary excretion in these 14 d was 14 % for subject G, 9 % for subject W and 7 % for subject R (Table 1). This accounted for 20, 14 and 16 % of absorbed tracer, respectively. Urinary excretion of ^{75}Se continued to decrease gradually throughout the investigation and at 16–20 weeks the value was 0·06–0·08 % dose/d.

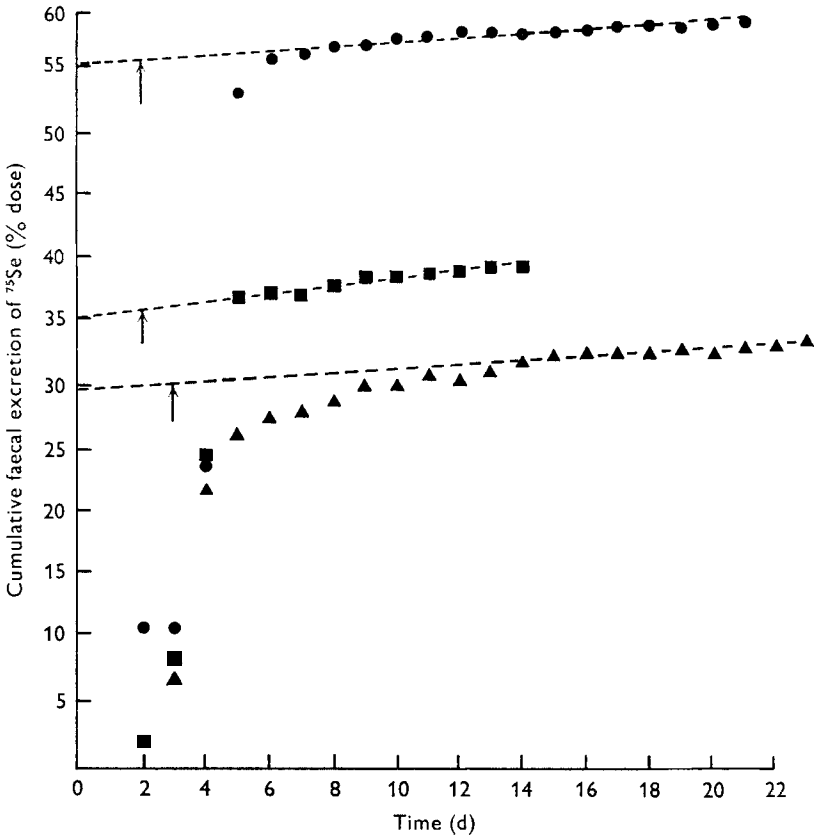

Fig. 1. Cumulative faecal excretion of ^{75}Se in subjects G (▲), W (■), and R (●) given oral doses of [^{75}Se]selenite. The arrows indicate the first appearance of brilliant blue faecal marker which was given with the ^{75}Se.

Urinary excretion (mean ± SD) of stable Se was 16·7 ± 1·8, 8·5 ± 1·0 and 12·7 ± 1·7 μg/24 h respectively for subjects G, W and R.

Faecal loss and intestinal absorption of ^{75}Se

The cumulative loss of ^{75}Se in faeces is illustrated in Fig. 1. Faecal radioactivity and the coloured marker both appeared together on day 2 or 3 and peak excretion of ^{75}Se occurred on day 4 or 5. The marker persisted in faeces until radioactivity in individual samples had decreased to less than 1 % of the dose on day 6 or 7.

Intestinal absorption of ^{75}Se was estimated by plotting cumulative faecal excretion of ^{75}Se during the first 2–3 weeks against time (Lutwak, 1969). The straight line joining the last points on the curve was extrapolated back to the time of first appearance of brilliant blue faecal marker (day 2 for subjects W and R and day 3 for subject G). This zero point on the extrapolated line represents the fraction of tracer not absorbed (Fig. 1). By this method it was calculated that 70, 64 and 44 % dose was absorbed by subjects G, W and R, respectively (Table 1).

Cumulative faecal excretion at day 14 was 33, 40 and 58 % dose for subjects G, W

Table 1. *Absorption, excretion and retention of ^{75}Se by three women during the first 2 weeks after receiving oral doses of [^{75}Se]selenite*

(Values expressed as a percentage of the dose)

Subject	Absorption	Excretion			Retention at day 14	
		Urine	Faeces	Total	From excretion measurements	From whole-body counting
G	70	14	33	47	53	46
W	64	9	40	49	51	44
R	44	7	58	65	35	34

and R (Table 1). Endogenous faecal loss, estimated as cumulative faecal loss at day 14 minus non-absorbed tracer, was 3–4% dose and this accounted for 4–6% of absorbed tracer.

Faecal excretion of ^{75}Se continued throughout the study and had decreased to 0·02–0·03% dose/faecal sample by weeks 16–20.

Mean faecal stable Se during the initial collection period was 9·7, 9·8 and 10·2 μg/24 h for subjects G, W and R, respectively.

Respiratory and dermal losses of ^{75}Se

Measurements with the whole-body counter indicated that there was no radioactivity in the Douglas bags containing expired air. However on day 1, traces of ^{75}Se of less than 0·02% of the dose were found in the HNO_3 and mercuric chloride solutions through which expired air had been passed, but there was no ^{75}Se in the air collected on day 2.

No radioactivity was detected in 'arm-bag sweat' or in cotton underwear worn during the first 2 or 3 d.

Whole-body retention and turnover of ^{75}Se

Total body retention of ^{75}Se was estimated direct from whole-body radioactivity measurements and indirect from estimates of urinary and faecal losses.

Whole-body radioactivity measurements during day 1 varied from hour to hour by as much as 15% of the dose. This may have resulted from changes in the distribution of radioactivity in the body. For example the whole-body counts of subjects G and W increased considerably after a meal, possibly because of increased blood flow to the digestive organs or the effect of peristalsis upon intestinal ^{75}Se. Small changes of body position in the chair used for the measurement had no effect on counting rates.

Body retention of ^{75}Se is illustrated in Fig. 2. At the end of the 2nd week, values for retention of ^{75}Se estimated by whole-body counting were less than those calculated from urinary and faecal excretion by 7% of the dose for both subjects G and W (Table 1). After this period the curves for body retention *v.* time for each method of estimation were parallel. The retention curves for subject R showed no consistent pattern in the first 6 d but after this period the values obtained by the two methods were similar.

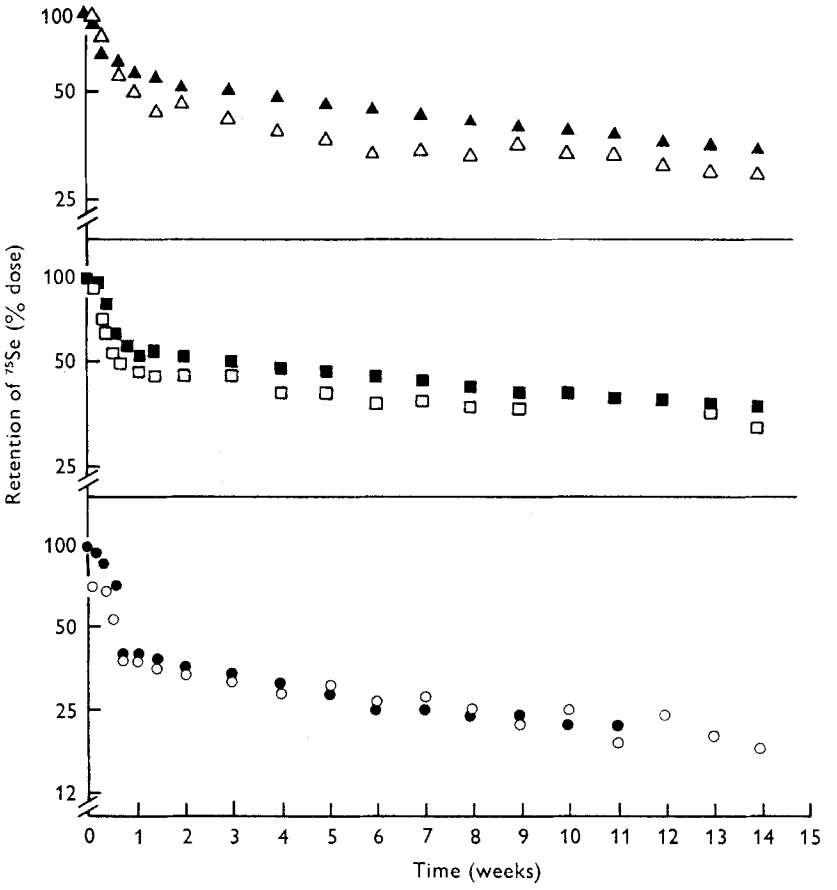

Fig. 2. Total body retention of ^{75}Se in subjects G (△, ▲), W (□, ■) and R (○, ●) given oral doses of [^{75}Se]selenite; △, □, ○ represent values estimated from whole-body radioactivity measurements and ▲, ■, ● represent values estimated from urinary and faecal excretion.

Values for total body retention of ^{75}Se, estimated from measured urinary and faecal losses, were 60, 56, and 38% of the dose at day 7 and 53, 51 and 35% at day 14 for subjects G, W and R, respectively. Values for the retention of absorbed tracer for the three subjects were 82, 87 and 84% at day 7 and 76, 80 and 82% at day 14 respectively.

The whole-body retention curves could each be resolved into three exponential components. The first two of these described an initial phase of rapid decrease corresponding with the elimination of unabsorbed faecal tracer and urinary excretion of absorbed but non-utilized ^{75}Se. Half-times for retention obtained using whole-body counting measurements were 1·4, 0·8 and 0·8 d for phase 1, and 14, 7 and 4 d for phase 2 in subjects G, W and R, respectively; corresponding values obtained using urinary and faecal excretion measurements were 0·8, 0·7 and 1·2 d and 7, 11 and 8 d. These two phases were followed by a more gradual decrease in radioactivity which also approximated to an exponential decline with half-times of 109, 143 and 92 d (from whole-body counting) or 109, 144 and 96 d (from measured excretion) for subjects G, W and R, respectively.

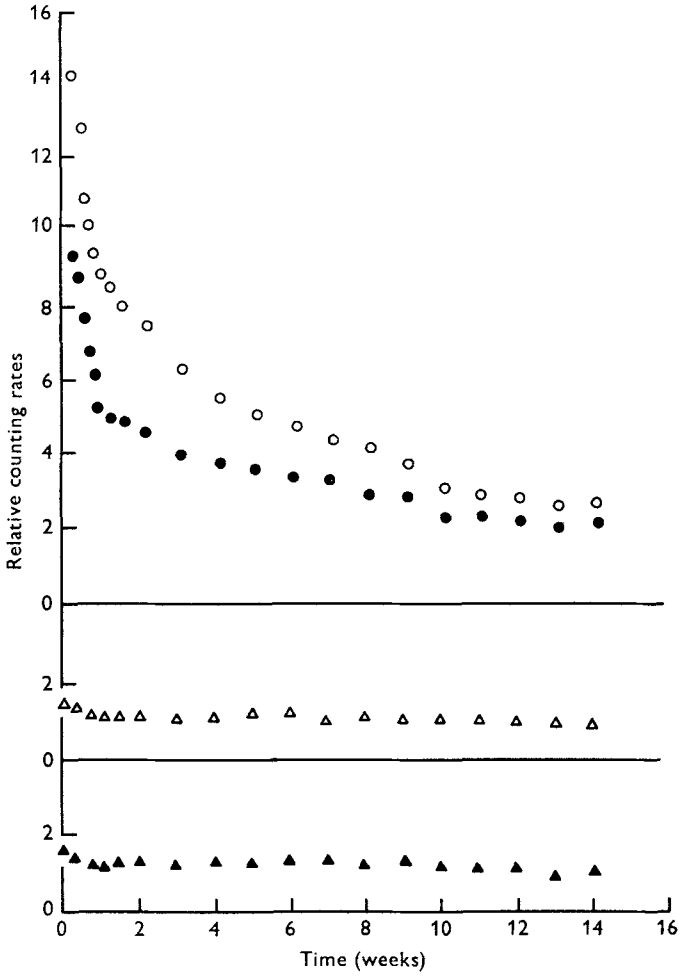

Fig. 3. ^{75}Se in the liver (○), heart (●), skeletal muscle (△) and bone (▲) for subjects G, W and R (individual results pooled) given oral doses of [^{75}Se]selenite.

Tissue retention and turnover of ^{75}Se

Estimates of radioactivity in the liver, heart, knee and calf or thigh muscle were combined for the three subjects (Fig. 3). ^{75}Se retention curves obtained for the liver and heart could be resolved into three exponential components (cf. whole-body curves). There was a more rapid decline of radioactivity in the liver than in the heart. Half-times for phases 1, 2 and 3 were 0·65, 6·7 and 73 d respectively for the liver, and 1·3, 15 and 89 d for the heart. Only small amounts of radioactivity were found in muscle and knee and these decreased during the first 5 d. After this period they remained relatively constant.

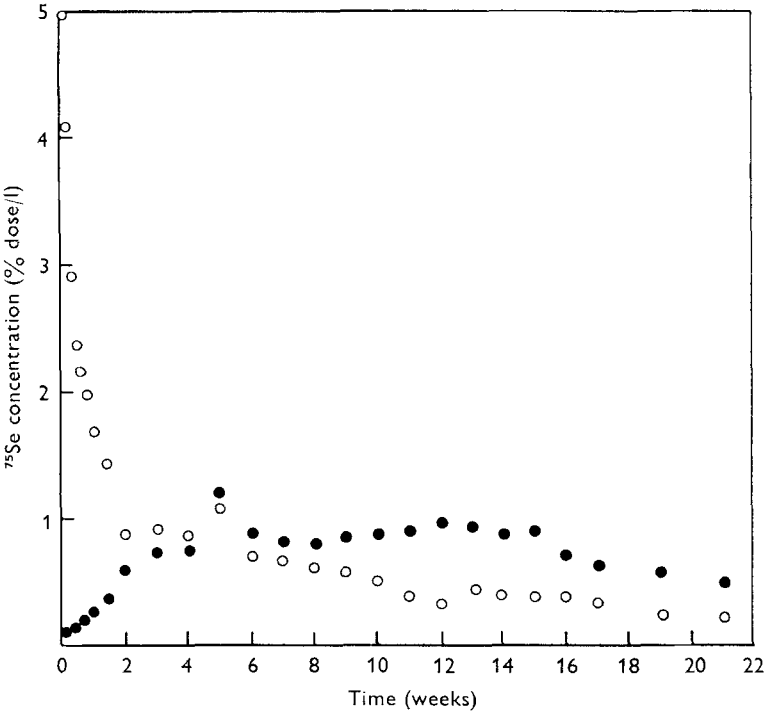

Fig. 4. ^{75}Se concentrations in plasma (○) and erythrocytes (●) after day 1 for subject R given an oral dose of [^{75}Se]selenite.

^{75}Se turnover in plasma and erythrocytes

^{75}Se concentrations in plasma during day 1 increased after the first hour to reach a peak at 7, 10 and 12 h for subjects G, W and R respectively. Only trace amounts of ^{75}Se were found in erythrocytes on day 1. Highest concentrations of ^{75}Se were found in the plasma of subject G, while the lowest concentrations were found in subject R; these values corresponded to the relative completeness of absorption of ^{75}Se by these subjects.

Concentrations of ^{75}Se in plasma and erythrocytes of subject R after day 1 are illustrated in Fig. 4. The curves for plasma ^{75}Se concentrations *v.* time for all three subjects were resolved into three exponential components with respective half-times of 0·7–1·4 d, 5·2–9·1 d and 69–77 d. ^{75}Se concentrations in erythrocytes increased in the first 2 weeks to reach a plateau which was maintained for about 13 weeks. After this period erythrocyte ^{75}Se concentration decreased.

DISCUSSION

Intestinal absorption of [^{75}Se]selenite

The validity of using this method for the measurement of intestinal absorption of ^{75}Se selenite was established with rats by Thomson & Stewart (1973). In rats, absorption was also estimated by two other independent methods which utilized (*a*) the

difference in total faecal excretion of ^{75}Se between rats given an oral dose of tracer and those given an intravenous dose, and (*b*) the ratio of whole-body radioactivities of the same two groups of rats 7 d after administration of the dose. If either of these methods were to be employed in human subjects it would be necessary to administer selenite orally and intravenously using two different radionuclides of Se. This was not possible as there is no other nuclide suitable for biological studies.

Absorption of [^{75}Se]selenite varied considerably among the three subjects and it was considerably less than the mean absorption, 92% of the dose, in rats.

Respiratory excretion of ^{75}Se

As the capacity of the Douglas bag restricted a single collection of expired air to a period of 8–10 min, total respiratory loss of ^{75}Se could not be estimated accurately. However, values obtained were less than 0·14% dose/h in the first 9 h and ^{75}Se was not detected in expired air collected on day 2.

Other workers have shown that in animals there is a respiratory loss of Se as dimethyl selenide only during the first few hours after administration of an oral or intravenous dose of selenite or selenomethionine (Hirooka & Galambos, 1966; McConnell & Roth, 1966; Lopez, Preston & Pfander, 1969; Handreck & Godwin, 1970). Lathrop, Harper & Malkinson (1968) found that less than 2% of an intravenous dose of [^{75}Se]selenomethionine was eliminated in the breath of their two patients, mainly in the first few hours. No radioactivity was detected after 60 h. Thus respiratory loss of tracer doses of ^{75}Se does not appear to be significant in man.

Dermal losses of ^{75}Se

There were no detectable skin losses of ^{75}Se in the first 3 d. Loss by desquamation of skin was not measured specifically but it would have been demonstrated by the detection of radioactivity in the clothing. Lathrop *et al.* (1968) estimated that only 2·5% of a dose of [^{75}Se]selenomethionine was lost in psoriatic scales, mainly after day 40.

Whole-body retention of ^{75}Se

Measurement of whole-body retention. There was a large difference in values for whole-body retention of ^{75}Se obtained by whole-body counting and from combined urinary and faecal excretion in subjects G and W. It is unlikely that this difference was the result of underestimating losses by excretion, as respiratory and dermal losses of ^{75}Se in the first few days when the discrepancy became apparent were negligible. The considerable variation in apparent whole-body radioactivity on day 1 suggested that this measurement was affected by a change in the distribution of the ^{75}Se in the body. Furthermore, plots of whole-body retention *v.* time for the two methods were parallel from 4 to 7 d after administration of the dose; after this period regional distribution of ^{75}Se in rats is fairly constant (Thomson & Stewart, 1973).

Initial retention of ^{75}Se. Retention of ^{75}Se by the three human subjects given an oral dose of [^{75}Se]selenite was less than that by rats because intestinal absorption was greater in rats (Thomson & Stewart, 1973). However, retention of absorbed tracer

was greater in the human subjects. Calculated values for the amount of absorbed [^{75}Se]selenite retained by rats indicated that 73 and 62% was retained at 7 and 14 d, respectively. The lower retention in rats was associated with a greater urinary loss and may have resulted from the larger carrier doses of stable Se relative to body-weight given to the rats. It has been shown that the amount of ^{75}Se retained by rats at the end of the initial period of rapid elimination is inversely related to the amount of carrier Se (Ewan, Pope & Baumann, 1967; Burk, Brown, Seely & Scaief, 1972).

Tissue turnover of ^{75}Se

The turnover rates for the liver were the highest of the four tissues studied, particularly during the first few days, suggesting that this organ has a special role in the early metabolism of Se.

The similarity between the ^{75}Se turnover curve for the heart and that for whole blood, and the higher concentrations of ^{75}Se in blood than in the heart muscle of rats (Thomson & Stewart, 1973), suggested that it was the ^{75}Se content of the cardiac blood pool which was estimated.

Turnover rates of ^{75}Se in skeletal muscle and bone were lower than those of liver and blood, indicating relatively slow metabolism of Se in these tissues. The decrease in radioactivity of muscle and bone during the first few days might have resulted from a decrease in plasma ^{75}Se content.

^{75}Se turnover in plasma

The continuing increase in plasma ^{75}Se content during the first 7–12 h was not the result of delayed absorption from the gastrointestinal tract. The maximum rate of excretion of radioactivity in urine occurred within 2 h of administration of the dose, indicating that absorption had been rapid. Awwad, Potchen, Adelstein & Dealy (1966) have suggested that after intravenous injection, ^{75}Se is rapidly cleared from the blood by the liver and is then returned to the blood in a protein-bound form. This 'post-hepatic' form of ^{75}Se is not excreted in urine as readily as selenite, as the amount of ^{75}Se in urine was decreasing during the period in which plasma radioactivity was increasing.

The initial rapid phases in the decrease of plasma ^{75}Se content after day 1 could represent utilization of this 'post-hepatic' form of Se by the tissues or turnover of ^{75}Se incorporated into other rapidly metabolized serum proteins. The final phase might represent long-term metabolism and re-utilization of ^{75}Se incorporated into serum proteins with a slow turnover rate.

Whole-body turnover of ^{75}Se

The initial phases of whole-body turnover were followed by a gradual disappearance of radioactivity which appeared to represent metabolic turnover and excretion in urine and faeces of ^{75}Se which had become incorporated into a long-term Se pool.

There have been considerable differences in reported turnover rates of this Se pool in human subjects. Cavalieri, Scott & Sairenji (1966) found a mean biological half-time of 65 d after an intravenous dose of [^{75}Se]selenite, and after intravenous doses of

[^{75}Se]selenomethionine a half-time of 91 d was observed by Ben-Porath, Case & Kaplan (1968), whereas Lathrop, Johnston, Blau & Rothschild (1972) found one of 220 d. These workers may have given larger carrier doses of Se than those used in our experiment, but in rats neither carrier dose size (Ewan *et al.* 1967) nor the chemical form in which it is administered (Thomson & Stewart, 1973) affects long-term ^{75}Se turnover. However, the long-term turnover rate of ^{75}Se in rats is directly related to the dietary Se intake before and during the study (Ewan *et al.* 1967; Burk *et al.* 1972).

Although whole-body retention of ^{75}Se during this long-term phase could be fitted by a single exponent, it is apparent that the concept of one main long-term Se pool is an over-simplification. The differing biological half-lives of ^{75}Se in the various tissues examined suggest that the Se turnover rate is largely determined by metabolic factors in the individual tissues and that net whole-body turnover is the sum of a large number of individual exponents which only approximates to a single turnover rate.

Se metabolism in man

This study was designed to obtain results for estimating quantitative Se metabolism in man. However, in the absence of information about the chemical nature of man's dietary Se, a similar study of the absorption, utilization and long-term turnover of ^{75}Se given as selenomethionine must be done. Information is also needed about the variations in stable Se content of urine and faeces in relation to dietary intake and further studies are in progress.

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