Metabolic studies of [75Se]selenomethionine and [75Se]selenite in the rat

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1. Information was sought concerning the long-term fate of orally and intravenously administered [75Se]selenomethionine and [75Se]selenite in rats.

2. Urinary and faecal radioactivity was assayed during the 1st week and whole-body radio-activity was determined weekly for 16 weeks. Rats were killed at intervals for analysis of 75Se tissue distribution.

3. Intestinal absorption after oral administration was estimated to be 91–93 % for selenite and 95–97 % for selenomethionine.

4. Urinary excretion of absorbed [75Se]selenite was greater than that of [75Se]seleno-methionine during the 1st week.

5. After the 1st week, whole-body retention diminished exponentially at a similar rate in rats given either selenomethionine or selenite. Except for the erythrocytes, 75Se content of individual tissues also decreased exponentially.

6. It appears that, after an initial period, 75Se from either selenomethionine or selenite is metabolized similarly, suggesting that Se from both potential dietary sources is ultimately incorporated into the same metabolic pool.

Selenium deficiency in cattle and sheep has had serious economic consequences in New Zealand because the soil in many areas contains less than adequate amounts of this trace element (Cousins & Cairney, 1961; Hartley & Grant, 1961). For this reason, the attention of research workers has hitherto been centred upon the role of Se in animal nutrition. Interest in its possible role in human nutrition in New Zealand has been stimulated by reports from residents in low-Se areas of alleged benefit and relief from muscular complaints by self-medication with sodium selenite (Hickey, 1968).

The present study was undertaken as a preliminary to an investigation of Se metabolism in human subjects. This was considered necessary because, although much is known about short-term Se turnover in rats, details of its long-term disposal are uncertain. Moreover, it is not known whether the metabolic fate of ingested Se depends upon its chemical form.

Se metabolism has accordingly been studied in rats for periods of up to 16 weeks after the administration of [75Se]selenomethionine or [75Se]selenite. After the 1st week the tissue distribution and turnover of 75Se from sodium selenite was similar to that from selenomethionine suggesting that Se from both potential dietary sources had been incorporated into the same metabolic pool.

EXPERIMENTAL

Procedure

Four groups of rats were studied. Each group comprised twenty female Wistar rats bred from the same colony and initially weighing 140–160 g. The rats were maintained on tap-water and a stock pellet diet containing 0·05 mg Se and 180 g available protein/kg. In two groups, each of the rats received a measured dose of approximately 5 μCi [^{75}Se]selenomethionine containing not more than 5 μg Se, one group by intravenous injection and the other by intragastric intubation. The remaining two groups similarly received intravenous or oral doses of [^{75}Se]selenite, again containing not more than 5 μg Se.

For all procedures the rats were anaesthetized by intraperitoneal injection of 5–10 mg sodium pentobarbitone.

Collection of urine and faeces

Twelve rats from each group were placed in metabolic cages for the separate collection of urine and faeces. These collections were completed at 24 h intervals for 7 d. Portions of each sample were counted in an automatic well-counter together with an appropriate ^{75}Se standard. Radioactivity in each 24 h urine or faecal sample was expressed as a percentage of the administered dose.

Whole-body counting

Measurements of whole-body radioactivity were made with a large-volume counter. For this purpose the lightly anaesthetized rat was placed in an open 2 l plastic container and counted for 100 s while rotating six times/min between two uncollimated sodium iodide scintillation crystals. Shortly after the administration of the dose each rat was counted in this way and this initial count (day 0) was used as the 100% reference value for all subsequent measurements. All whole-body counts were corrected for radioactive decay by reference to a ^{75}Se standard counted at the same time. Surviving rats were counted daily during the 1st week, twice weekly for a further 3 weeks and then weekly to the 16th week.

Tissue retention

Two rats from each group were bled to death from the aorta on days 1, 2, 3, 5, 7, 14, 28, 42, 56 and 112. The heart, lungs, spleen, liver, kidneys, adrenals and ovaries were removed from each rat and weighed, as were portions of the shaft of the femur and thigh muscle. Blood was allowed to clot and the serum was separated from the erythrocytes. These tissues were counted together with a ^{75}Se standard in the automatic well-counter. Radioactivity was expressed as a percentage of the dose contained in the whole organ and as a percentage of the dose per g wet weight of tissue.
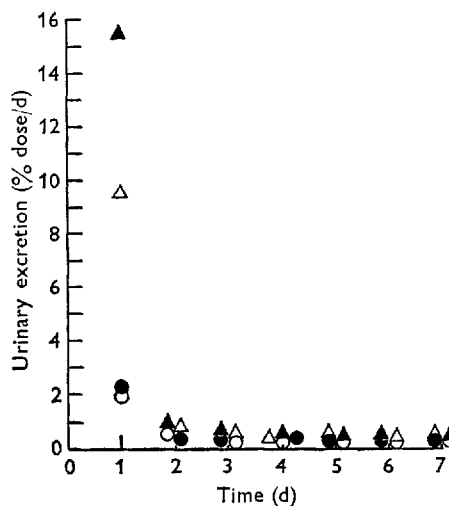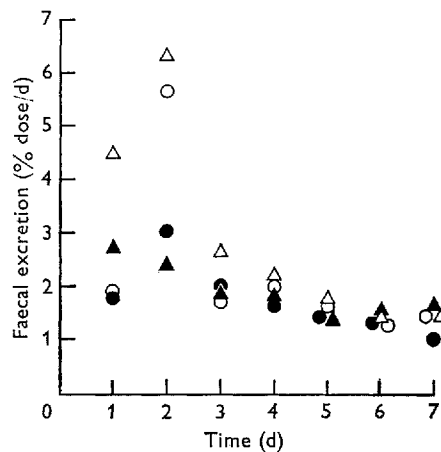
Fig. 1. Urinary excretion of ^{75}Se in four groups each of twelve rats given [^{75}Se]selenomethionine intravenously (●), [^{75}Se]selenomethionine orally (○), [^{75}Se]selenite intravenously (▲) and [^{75}Se]selenite orally (△).

Fig. 2. Faecal excretion of ^{75}Se in four groups each of twelve rats given [^{75}Se]selenomethionine intravenously (●), [^{75}Se]selenomethionine orally (○), [^{75}Se]selenite intravenously (▲) and [^{75}Se]selenite orally (△).

RESULTS

Se balance during the 1st week

Urinary excretion of ^{75}Se during the 1st week is shown in Fig. 1. The animals given [^{75}Se]selenite intravenously excreted 16% of the dose in the urine during the 1st day and those given [^{75}Se]selenite orally excreted 10%, whereas the urinary loss of [^{75}Se]selenomethionine was 2% for both oral and intravenous groups. During the 2nd day urinary loss of ^{75}Se fell to 0·9% in those given selenite and to 0·4% in those given selenomethionine. For the rest of the week urinary excretion of ^{75}Se was 0·2–0·4% of the dose/d for all groups.

Faecal loss of ^{75}Se during the 1st week is illustrated in Fig. 2. Peak faecal excretion occurred on the 2nd day and was about twice as great for the oral doses of ^{75}Se as for the intravenous doses. On days 6 and 7 the faecal loss of ^{75}Se was between 1·0 and 1·7% of the dose/d and was no greater in the groups given the oral dose than in the groups given the intravenous dose, suggesting that at this time the loss was of endogenous origin. Cumulative faecal losses at the end of the week were respectively 21 and 16% of the oral dose of [^{75}Se]selenite and [^{75}Se]selenomethionine, and 13 and 12% of the intravenous dose (Table 1).

The intestinal absorption of the selenite and selenomethionine tracers was estimated by three independent methods. The first method was applied to the results for rats given the oral tracer. Cumulative faecal excretion of ^{75}Se during the first 7 d was plotted against time and the straight line joining the last four points on the curve was extrapolated back to the zero-time intercept, which was taken to represent the
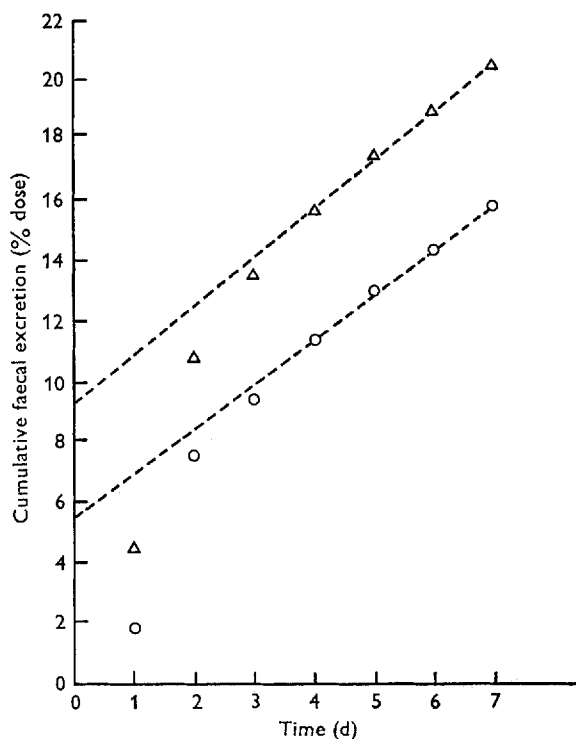
Fig. 3. Cumulative faecal excretion of ^{75}Se in two groups each of twelve rats given [^{75}Se]selenomethionine orally (○) and [^{75}Se]selenite orally (△).

fraction of non-absorbed tracer (Lutwak, 1969). By this method it was calculated that 91% of the selenite and 95% of the selenomethionine were absorbed (Fig. 3).

Absorption was also estimated from the difference in total faecal excretion of ^{75}Se at 7 d between the corresponding groups given the oral and the intravenous dose. This difference was assumed to represent non-absorbed tracer and amounted to 7% for selenite and 3% for selenomethionine. Thirdly, absorption was calculated from the ratio at 7 d of whole-body retention of radioactivity between the oral group and the corresponding intravenous group. By this method intestinal absorption was found to be 92% for selenite and 95% for selenomethionine. These results are summarized in Table 1.

Combined urinary and faecal excretion of tracer during the 1st week was 33% of the oral dose of selenite and 32% of the intravenous dose, and for rats given selenomethionine it was 20% of the oral dose and 17% of the intravenous dose (Table 1). Whole-body retentions measured by whole-body counting on day 7 agreed closely with those obtained from these excretion values. Retentions of [^{75}Se]selenite calculated from excretion values were 67% of the oral dose and 68% of the intravenous; those estimated from whole-body counting were respectively 66 and 71%. Retentions of [^{75}Se]selenomethionine estimated from excretion values were 80% of the oral dose and 83% of the intravenous dose and those calculated from whole-body counting were 76 and 80% respectively.

Table 1. *Absorption, excretion and retention of ^{75}Se by rats during the week after oral or intravenous doses of [^{75}Se]sodium selenite and [^{75}Se]selenomethionine*

(All results are expressed as a percentage of the dose given; mean values and standard errors for twelve rats)

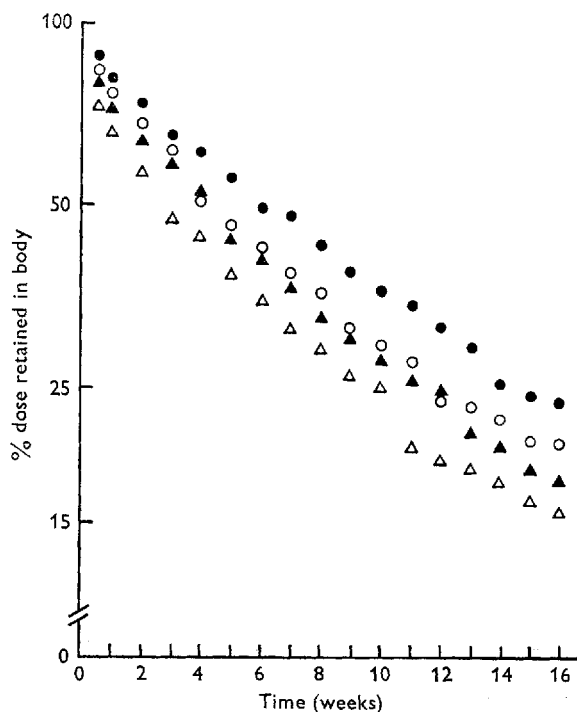| Chemical form of dose	Method of administration		Excretion			Retention		Absorption		
			Urine	Faeces	Total	From excretion by difference	By whole-body counting	From cumulative faecal excretion	From total faecal excretion	From whole-body counting
[^{75}Se]sodium selenite	Oral	Mean	12·7	20·6	33·3	66·7	65·8	91	93	92
		SE	0·5	1·4	1·4	1·4	1·0			
	Intravenous	Mean	18·9	13·5	32·4	67·6	71·2	—	—	—
		SE	1·0	0·5	1·0	1·0	1·0			
[^{75}Se]seleno-methionine	Oral	Mean	4·2	15·6	19·8	80·2	75·7	95	97	95
		SE	0·2	0·9	1·1	1·1	2·3			
	Intravenous	Mean	4·6	12·3	17·0	83·0	79·5	—	—	—
		SE	0·1	0·3	0·5	0·5	0·7			

Fig. 4. Whole-body radioactivity curves of four groups of rats given [^{75}Se]selenomethionine intravenously (●), [^{75}Se]selenomethionine orally (○), [^{75}Se]selenite intravenously (▲) and [^{75}Se]selenite orally (△). Each point represents the mean value for all surviving rats in that group.

Whole-body retention

Fig. 4 shows the average whole-body radioactivity of the surviving rats in the four groups during the 16-week period. For all groups the amount of radioactivity in the body could be fitted by the sum of two exponential curves. The first exponent had a half-time of 3–4 d and appears to represent urinary and faecal loss during an initial equilibration period. The second exponent had a half-time of 50–59 d and seems to represent metabolic turnover of tracer after equilibration in the body tissues. There was no significant difference between the slope-constants of the second exponents for each of the four groups, suggesting that the fate of ^{75}Se after the 1st week was independent of the method of administration or the chemical form given.

Tissue distribution

No significant difference was observed between the four groups of rats with respect to initial uptake, initial retention or content of ^{75}Se tracer in the individual tissues examined at any time during the 16 weeks of observation. Accordingly the results from the four groups have been combined for analysis.

The initial uptake is defined as the maximum amount and concentration of tracer observed in the tissue during the 1st week. The initial uptake was highest in the liver and kidneys and the greatest concentrations were achieved in the kidneys and adrenals

Table 2. *Uptake, retention and turnover of ^{75}Se in tissues of rats given oral or intravenous doses of [^{75}Se]sodium selenite and [^{75}Se]selenomethionine*

	Initial uptake*		Initial retention†		Turnover rate	
Tissue	% dose	% dose/g	% dose	% dose/g	Fraction/d	Half-time (d)
Serum	—	1·8	—	1·3	0·019	37
Kidneys	5·9	4·4	5·1	3·5	0·018	38
Lungs	1·1	1·2	1·0	1·0	0·017	40
Ovaries	0·2	1·5	0·2	1·1	0·017	41
Adrenals	0·2	2·8	0·2	3·7	0·017	41
Spleen	0·8	1·6	0·7	1·5	0·016	44
Bone	—	0·5	—	0·5	0·016	44
Liver	12·9	1·7	6·1	0·9	0·014	49
Heart	0·4	0·7	0·4	0·6	0·011	61
Skeletal muscle	—	0·3	—	0·3	0·009	74
Erythrocytes	—	0·7	—	0·4	—	—

* See p. 144.
† See below.

(Table 2). Although the initial concentration of ^{75}Se in skeletal muscle was only 0·3 % dose/g, because this tissue comprises 43 % of body-weight (Donaldson, 1924), approximately 20 % of the administered dose was taken up in it.

The initial retention, on the other hand, was calculated as the zero-time intercept of the regression line representing tissue content or concentration of the tracer between weeks 1 and 16. For the most part, initial retention corresponded closely to initial uptake except for the liver in which the initial retention was about half the initial uptake. This organ contained 12·9 % of the dose on day 1, but this value fell rapidly to 6·7 % on day 7. This pattern contrasted with that observed in other tissues such as the adrenals and spleen, in which there was a progressive increase in ^{75}Se content during the 1st week, and the kidneys, ovaries, heart, skeletal muscle and erythrocytes, in which the levels remained relatively constant.

The radioactive Se content of each individual tissue except the erythrocytes decreased exponentially after the 1st week. Serum, kidneys, lungs, ovaries, adrenals, spleen and bone all displayed a more rapid rate of turnover than that of the whole body. The turnover in the liver was intermediate and that of cardiac and skeletal muscle was slower (Table 2).

Erythrocytes, on the other hand, had a constant ^{75}Se content from 1 to 6 weeks. It then diminished to low values between 8 and 16 weeks.

DISCUSSION

The closely similar values for the intestinal absorption of the ^{75}Se tracer calculated by three independent methods confirms the validity of the assumptions made and demonstrates the accuracy of the methods used. The slightly higher apparent absorption obtained by the second method might have been caused by a greater endogenous faecal loss of intravenous tracer than of absorbed oral tracer. Intestinal absorption of the oral dose of both chemical forms was greater than 90 %, but selenite was some-

what less well absorbed than selenomethionine. Moreover, urinary loss of selenite was greater, so that initial retention of this chemical form was 13–15 % less than that of selenomethionine. Respiratory excretion of dimethyl selenide has been shown by McConnell & Roth (1966) to occur in normal animals given selenite and to a rather lesser extent in those given selenomethionine. However, the amount of dimethyl selenide excreted seems to be dependent upon the quantity of Se administered, and these workers demonstrated that less than 1 % of tracer quantities similar to those used in the present study appeared in this form. Although small losses of ^{75}Se as dimethyl selenide would not have been detected by our methods, the results indicated that significant quantities were not excreted in this manner. Thus there was good agreement between values for whole-body retention of ^{75}Se at the end of the 1st week obtained by whole-body counting and those derived by measurement of urinary and faecal loss. Any considerable loss of ^{75}Se as dimethyl selenide should have been revealed as a loss by the whole-body counting not accounted for by measured excretory losses.

On days 6 and 7, faecal and urinary loss of tracer was the same for all groups irrespective of chemical form or route of administration. It therefore seems that, after the initial period of equilibration, retention of tracer is independent of the chemical form in which it had been administered.

Whole-body turnover of Se after the 1st week was also independent of the chemical form and method of administration, as were rates of tissue retention and tissue turnover. The first exponent of the whole-body ^{75}Se turnover curve represents faecal excretion of tracer not absorbed from the gastro-intestinal tract and the initial utilization and excretion of absorbed Se that has not become incorporated into the ultimate Se pool. On the other hand, the second exponent represents metabolic turnover of a long-term Se pool in which the Se from either chemical source would seem to have the same fate. The whole-body turnover of ^{75}Se during this phase was governed for the most part by turnover in the skeletal muscle, liver and kidneys, tissues which contained the greatest proportion of the administered dose. The slow turnover rate observed in these rats probably reflects the marginal Se level in the stock pellet diet. Ewan, Pope & Baumann (1967) had found that whole-body turnover of ^{75}Se in the 'fixed pool' of the rat varied from 27 to 77 d depending upon the dietary content of Se. Moreover, initial retention of ^{75}Se in the present study was high compared with those obtained by other workers (Blincoe, 1960; Yousef, Coffman & Johnson, 1968). This probably reflects the smaller dose of carrier Se administered in our study. Ewan et al. (1967) observed a much larger retention of smaller carrier doses of Se in the body at the end of the initial phase of ^{75}Se turnover than of larger doses.

Turnover of ^{75}Se in the liver during the 1st week was more rapid than in other tissues. Awwad, Potchen, Adelstein & Dealy (1966) observed that in human subjects given [^{75}Se]selenomethionine, non-protein-bound ^{75}Se was cleared from the plasma by the liver and subsequently returned bound to protein. Although at present the chemical nature of this protein-bound Se remains uncertain (Awwad et al. 1966; Jenkins, 1968), our findings support the view that the liver has a special role in the early metabolism of this element.

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