Metabolic studies of [^{75}Se]selenocystine and [^{75}Se]selenomethionine in the rat

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1. The long-term fate in rats of an oral dose of [^{75}Se]selenocystine was compared with that of an oral dose of [^{75}Se]selenomethionine.

2. Urinary and faecal radioactivities were measured during the 1st week and whole-body radioactivity was determined for 10 weeks. Rats were killed at weekly intervals for 4 weeks and at weeks 6 and 10 for analysis of tissue distribution of ^{75}Se.

3. Intestinal absorption of [^{75}Se]selenocystine was 81 % of the administered dose; that of [^{75}Se]selenomethionine was 86 %. Urinary excretion of absorbed [^{75}Se]selenocystine was 13·9 % and that of [^{75}Se]selenomethionine was 5·8 %, in the 1st week.

4. Whole-body retention of ^{75}Se was greater for [^{75}Se]selenomethionine than for [^{75}Se]-selenocystine but after the 1st week it decreased at a similar rate in both groups. Tissue distribution of retained ^{75}Se was also similar in both groups.

5. The initial utilization of [^{75}Se]selenocystine was different from that of [^{75}Se]seleno-methionine. However, after the 1st week ^{75}Se from both sources appeared to be metabolized similarly, suggesting that dietary Se of both forms is ultimately incorporated into the same metabolic pool.

6. When these findings were compared with those of earlier studies with [^{75}Se]selenite and ^{75}Se incorporated in vivo into rabbit kidney (RK-^{75}Se) (Thomson, Stewart & Robinson, 1975) the metabolism of [^{75}Se]selenocystine resembled that of [^{75}Se]selenite and RK-^{75}Se, rather than that of [^{75}Se]selenomethionine.

Selenocystine is one of the main forms in which selenium exists in plant proteins (Peterson & Butler, 1962). However, little is known about the fate of selenocystine in the animal body, as reported metabolic studies of Se in animals have been concerned mainly with the metabolism of selenite, selenate and selenomethionine.

The results of a previous study (Thomson & Stewart, 1973) in rats indicated that after an initial equilibration period of rapid metabolism, the fates of oral and intravenous doses of [^{75}Se]selenite and [^{75}Se]selenomethionine were similar. A more recent study in rats (Thomson, Stewart & Robinson, 1975) indicated that the long-term fate of an oral dose of ^{75}Se incorporated in vivo from [^{75}Se]selenomethionine into rabbit kidney (RK-^{75}Se) was also similar to that of [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney homogenate. However, the initial metabolism of RK-^{75}Se resembled that previously found for [^{75}Se]selenite rather than that of [^{75}Se]selenomethionine. The present study describes the initial and long-term metabolism of oral doses of [^{75}Se]selenocystine and [^{75}Se]selenomethionine, and compares them to the findings from the earlier studies with [^{75}Se]selenite and RK-^{75}Se.

EXPERIMENTAL

Procedures

The procedures used in the present metabolic study were almost identical to those described previously (Thomson & Stewart, 1973; Thomson *et al.* 1975).

Female Wistar rats bred from the same colony and initially weighing 90–120 g were used for the metabolic study. They were maintained on tap-water and a pelleted stock diet containing 180 g available protein and 0·025 mg Se/kg. Each of the twenty-five rats in one group were anaesthetized with 5 mg sodium pentobarbitone and given by gastric intubation a known amount (approximately 5 μCi) of [^{75}Se]selenocystine (Radiochemical Centre, Amersham, Bucks.); a second group of twenty-five rats received by the same method a known amount (approximately 2 μCi) of [^{75}Se]-selenomethionine (Radiochemical Centre, Amersham, Bucks.). Each dose contained not more than 5 μg Se.

Collection of urine and faeces

Twelve rats from each group were placed in metabolism cages for the separate collection of urine and faeces. These collections were completed at 24 h intervals for 7 d. The amount of radioactivity in measured portions of each sample was determined using an automatic sample counting system (Autogamma; Searle Analytic Inc., 2000 Nuclear Drive, Des Plaines, Illinois, USA) with a ^{75}Se standard and the total amount 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 constructed in the Department of Medical Physics, Wakari Hospital, Dunedin (Thomson *et al.* 1975). Measurements were made for each rat shortly after administration of the dose and this initial count (day 0) was used as the 100% reference value for subsequent measurements for that animal. All whole-body radioactivity measurements were corrected for radioactive decay and any variation in counting efficiency by reference to a ^{75}Se standard counted at the same time. Measurements for surviving rats were made once per week for 10 weeks.

Tissue retention

Once per week for 4 weeks and at weeks 6 and 10, three rats from each group were bled to death from the aorta. The heart, lungs, spleen, liver, kidneys, adrenals, ovaries and portions of the shaft of the femur and thigh muscle were removed and weighed. Blood was allowed to clot and the serum was separated from the erythrocytes. The amount of radioactivity in these tissues was measured using the automatic well-counter with a ^{75}Se standard. The amount of radioactivity in whole organs was expressed as a percentage of the whole-body ^{75}Se for that animal at death and for the tissues was expressed as a percentage of whole-body ^{75}Se/g wet weight of tissue.

Table 1. *Absorption, excretion and retention of ^{75}Se (as a percentage of the administered dose) by rats during the 1st week after oral doses of [^{75}Se]selenocystine and [^{75}Se]selenomethionine**

(Mean values with their standard errors for twelve rats/treatment)

			Excretion					Retention	
Chemical form of dose		Intestinal absorption of ^{75}Se	Urine	Unabsorbed faecal ^{75}Se	Endogenous faecal ^{75}Se†	Total faecal ^{75}Se	Total excretion of ^{75}Se	From excretion by difference	By whole-body counting
[^{75}Se]selenocystine	Mean	81·1	11·4	18·9	8·2	27·1	38·5	61·5	62·1
	SE	0·5	0·6	0·5	0·4	0·5	0·9	0·9	1·3
[^{75}Se]selenomethionine	Mean	86·4	5·0	13·6	8·6	22·2	27·2	72·8	77·0
	SE	0·8	0·2	0·8	0·4	0·7	0·8	0·8	1·3

Values for the group receiving [^{75}Se]selenocystine were significantly different from those for the group receiving [^{75}Se]selenomethionine ($P < 0{\cdot}001$), except values for endogenous faecal ^{75}Se.

* For details of procedures, see p. 502.

† Calculated as the difference between total faecal ^{75}Se and unabsorbed faecal ^{75}Se.

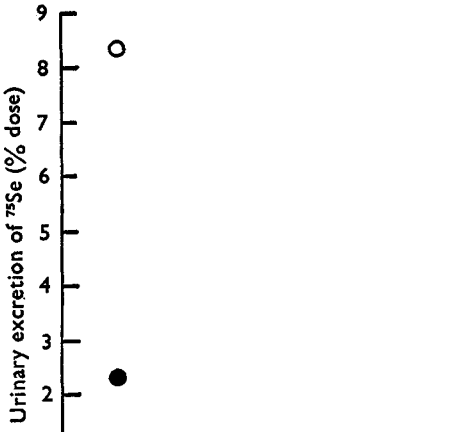

Fig. 1

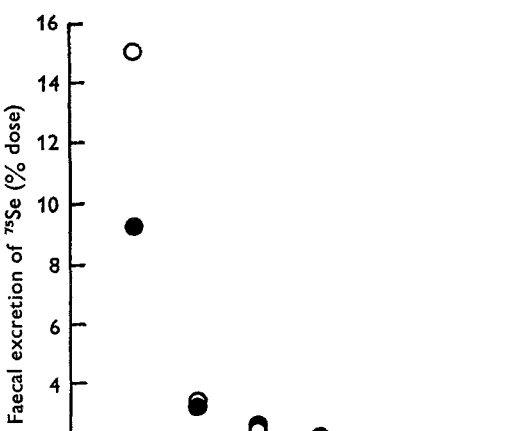

Fig. 2

Fig. 1. Urinary excretion of ^{75}Se in two groups, each of twelve rats, given oral doses of either [^{75}Se]selenocystine (○) or [^{75}Se]selenomethionine (●). For details of procedures, see p. 502.
Fig. 2. Faecal excretion of ^{75}Se in two groups, each of twelve rats, given oral doses of either [^{75}Se]selenocystine (○) or [^{75}Se]selenomethionine (●). For details of procedures, see p. 502.

RESULTS

Se balance during the 1st week

The intestinal absorption of the ^{75}Se tracer was calculated by plotting cumulative faecal excretion of ^{75}Se *v.* time (Lutwak, 1969). The straight line joining the last three points on the curve was extrapolated to the zero-time intercept and this point was taken to represent the proportion of tracer not absorbed (Thomson & Stewart, 1973). Mean absorption of [^{75}Se]selenocystine was 81·1% of the administered dose and that of [^{75}Se]selenomethionine was 86·4% of the dose (Table 1).

Urinary excretion of ^{75}Se during the 1st week is shown in Fig. 1. The rats given [^{75}Se]selenocystine excreted 8·3% of the administered dose in the urine during the 1st day but those given [^{75}Se]selenomethionine excreted only 2·3% ($P < 0.001$). During the 2nd day urinary loss of ^{75}Se decreased to 0·8 (SE 0·02) and 0·6 (SE 0·02)% of the dose respectively and on day 7 it was 0·4% of the dose/d for both groups. There was no significant difference between daily urinary excretion of ^{75}Se by the two groups for days 5–7. Cumulative urinary excretion at the end of the 1st week was 11·4% of the dose for rats given [^{75}Se]selenocystine and 5·0% of the dose for those given [^{75}Se]selenomethionine ($P < 0.001$) (Table 1). This accounted for 13·9 and 5·8% of the absorbed radioactivity respectively.

Faecal loss of ^{75}Se during the 1st week is shown in Fig. 2. The rats given [^{75}Se]-selenocystine excreted 15·0% of the administered dose in the faeces during the 1st day but those given [^{75}Se]selenomethionine excreted only 9·2% ($P < 0.001$). For the

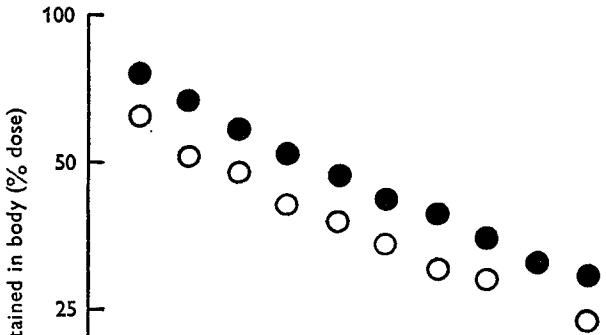

Fig. 3. Whole-body ^{75}Se in groups of rats given oral doses of either [^{75}Se]selenocystine (○) or [^{75}Se]selenomethionine (●). Each point represents the mean value for all surviving rats in that group. For details of procedures, see p. 502.

remainder of the week faecal loss was similar in both groups. Cumulative faecal loss at the end of the week was 27·1% of the dose for rats given [^{75}Se]selenocystine and 22·2% of the dose for those given [^{75}Se]selenomethionine ($P < 0{\cdot}001$; Table 1). Endogenous faecal loss of ^{75}Se during the 1st week was determined as the difference between total faecal ^{75}Se and unabsorbed ^{75}Se. This amounted to 8·2% of the dose, or 10·0% of the absorbed radioactivity, for the rats given [^{75}Se]selenocystine, and 8·6% of the dose, or 9·9% of the absorbed radioactivity, for those given [^{75}Se]selenomethionine.

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

Combined urinary and faecal excretion of ^{75}Se at the end of the 1st week was 38·5% of the dose of [^{75}Se]selenocystine but only 27·2% of the dose of [^{75}Se]selenomethionine ($P < 0{\cdot}001$; Table 1). Whole-body retentions of ^{75}Se measured by whole-body counting on day 7 agreed closely with retention values calculated from urinary and faecal excretion. Retention of [^{75}Se]selenocystine calculated from excretion measurements was 61·5% of the dose; that estimated from whole-body counting was 62·1%. Retentions of [^{75}Se]selenomethionine calculated by these two methods were 72·8 and 77·0% of the dose respectively. When expressed as a percentage of the dose absorbed, retention of [^{75}Se]selenocystine calculated from excretion measurements was 75·8 and from whole-body counting was 76·5, while retentions of [^{75}Se]selenomethionine were 84·2 and 89·1 respectively. The close 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 indicated that there was negligible respiratory loss of ^{75}Se in these animals.

Mean whole-body ^{75}Se in the surviving rats of the two groups during the 10-week

Table 2. *Distribution of retained whole-body ^{75}Se in tissues of rats during 10 weeks after oral doses of [^{75}Se]selenocystine and [^{75}Se]selenomethionine**

(Mean values for three rats)

Chemical form of dose ...	[^{75}Se]selenocystine						[^{75}Se]selenomethionine					
Time after dose (weeks) ...	1	2	3	4	6	10	1	2	3	4	6	10
	% Whole-body ^{75}Se retained/organ											
Liver	7·0	6·5	5·5	5·5	5·3	4·6	5·9	4·5	4·2	4·1	4·2	4·2
Kidney	6·4	6·9	6·3	6·7	5·9	4·5	7·7	7·5	5·3	5·4	4·3	4·2
Heart	0·58	0·53	0·52	0·57	0·54	0·53	0·42	0·41	0·47	0·45	0·45	0·47
Lung	1·2	1·2	1·1	0·9	1·1	1·0	1·0	0·9	0·9	1·0	1·4	0·9
Spleen	1·2	1·1	1·0	1·1	0·9	0·8	0·9	0·9	0·8	0·7	0·7	0·5
Adrenals	0·21	0·30	0·39	0·39	0·41	0·37	0·23	0·29	0·36	0·31	0·37	0·25
Ovaries	0·23	0·22	0·19	0·17	0·16	0·13	0·22	0·18	0·15	0·15	0·15	0·11
	% Whole-body ^{75}Se retained/g wet wt tissue											
Erythrocytes	0·57	0·69	0·73	0·88	0·77	0·50	0·66	0·59	0·62	0·74	0·86	0·53
Serum	1·2	1·6	1·2	1·1	1·0	1·1	1·0	1·0	1·0	0·9	0·8	0·7
Thigh muscle	0·24	0·26	0·32	0·29	0·27	0·28	0·49	0·36	0·38	0·35	0·32	0·35
Bone (shaft of the femur)	0·68	0·74	0·57	0·49	0·38	0·33	0·40	0·64	0·54	0·41	0·41	0·29

* For details of procedures, see p. 502.

period is shown in Fig. 3. Whole-body retention of [^{75}Se]selenocystine was less than that of [^{75}Se]selenomethionine at the end of the 1st week ($P > 0.001$) and this difference was maintained throughout the study. For both groups the whole-body retention curve could be resolved into two exponential components and regressions for each were calculated by the method of least squares. The first phase, which represented urinary and faecal loss during an initial equilibration period of 3 weeks, had a half-time of 5 d for the rats given [^{75}Se]selenocystine and 6 d for those given [^{75}Se]selenomethionine. The second phase from weeks 4 to 10, followed a single exponential curve with half-times of 54 and 49 d respectively for the two groups. There was no significant difference between the slope-constants of this second exponent for each of the two groups.

Tissue distribution of ^{75}Se

There was no consistent difference between concentrations of whole-body ^{75}Se in tissues of rats given [^{75}Se]selenocystine and in those of rats given [^{75}Se]selenomethionine (Table 2). Liver and kidney contained the greatest total amount of radioactivity with lesser amounts in the other organs studied. The highest concentrations of ^{75}Se were found in kidney and adrenals (4·4–6·2 and 3·7–5·8% whole-body ^{75}Se/g respectively on day 7), whereas the concentration in liver was 0·9–1·0% whole-body ^{75}Se/g.

Although the concentration of ^{75}Se in skeletal muscle was low (0·4% whole-body ^{75}Se/g on day 7), because this tissue comprises 43% of the total body-weight (Donaldson, 1924) approximately 25% of the retained ^{75}Se was contained within it.

DISCUSSION

The striking finding of the present study was that the initial utilization by rats of Se derived from selenocystine resembled that of Se derived from selenite or from RK-^{75}Se (rabbit kidney labelled in vivo with ^{75}Se) rather than that of Se derived from selenomethionine (Thomson & Stewart, 1973; Thomson *et al.* 1975). The relevant findings from the three experiments are compared in Table 3. Intestinal absorption of ^{75}Se administered as selenocystine was 5% less than that of ^{75}Se given as selenomethionine, a difference similar to that found in the previous studies between the absorption of (*a*) [^{75}Se]selenite and [^{75}Se]selenomethionine and (*b*) RK-^{75}Se and [^{75}Se]selenomethionine. Urinary loss of absorbed ^{75}Se derived from selenocystine was also greater than that from selenomethionine. In this respect too, the early metabolism of [^{75}Se]selenocystine resembled that of [^{75}Se]selenite and RK-^{75}Se and differed from that of [^{75}Se]selenomethionine. There is no obvious explanation for the lower absorption of [^{75}Se]selenomethionine in the present study compared with that found previously, but it might be related to the quantity of food remaining in the gut of the rat at the time of administration of the dose.

After the initial period of equilibration, the metabolism of ^{75}Se was similar in both groups of rats studied. Thus on days 5–7 urinary loss of radioactivity was the same for both groups, as were both endogenous faecal ^{75}Se during the 1st week and the distribution of whole-body ^{75}Se between the various tissues studied after 7 d. Moreover the long-term whole-body turnover of ^{75}Se was similar in both groups with half-times

Table 3. *Comparison of results obtained for absorption (as a percentage of the administered dose), excretion and retention (as a percentage of absorbed radioactivity) of ^{75}Se by rats during the 1st week after oral doses of [^{75}Se]selenite, RK-^{75}Se, [^{75}Se]-selenocystine and [^{75}Se]selenomethionine*

(Mean values for ten or twelve rats/treatment)

Chemical form of dose	Source of results	Intestinal absorption of ^{75}Se	Excretion		Retention	
			Urine	Endogenous faecal ^{75}Se*	From excretion by difference	By whole-body counting
[^{75}Se]selenite	Thomson & Stewart (1973)	91	13·9	12·7	73·3	72·3
RK-^{75}Se	Thomson *et al.* (1975)	87	13·3	12·4	74·2	72·7
[^{75}Se]selenocystine	Present study	81	13·9	10·0	75·8	76·5
[^{75}Se]selenomethionine	Thomson & Stewart (1973)	95	4·4	11·2	84·4	79·7
[^{75}Se]selenomethionine + rabbit kidney homogenate	Thomson *et al.* (1975)	91	7·6	12·7	79·8	79·5
[^{75}Se]selenomethionine	Present study	86	5·8	9·9	84·2	89·1

RK-^{75}Se, kidney homogenate from rabbits given [^{75}Se]selenomethionine (Thomson, Stewart & Robinson, 1975).
* Calculated as the difference between total faecal ^{75}Se and unabsorbed faecal ^{75}Se.

of 49 and 54 d. This compared with half-times of 50–59 d found previously after oral and intravenous administration of [^{75}Se]selenite and [^{75}Se]selenomethionine (Thomson & Stewart, 1973) and 54–55 d following oral administration of RK-^{75}Se and [^{75}Se]selenomethionine mixed with rabbit kidney homogenate (Thomson *et al.* 1975).

Therefore, although there were differences in initial utilization, long-term metabolism of retained Se in the rat appears to be independent of the chemical form from which it is derived.

New Zealand is a low-Se country and the stock pellet diet used in all three experiments contained only 0·025 mg Se/kg. This happens to have been the level used by other workers in their low-Se basal diets for studies in rats of the effect of adding different amounts and forms of dietary Se. Cary, Allaway & Miller (1973) found that below 0·1 mg Se/kg diet, differences in the dietary form of Se, for example selenite, selenomethionine or seleniferous grain, did not produce measurable differences in tissue Se concentrations. Our studies using the four different forms of Se support and extend this view. Further, Burk, Seely & Kiker (1973) found that with the basal diet (0·024 mg Se/kg) the urinary output accounted for about 10% of an intraperitoneal injection of [^{75}Se]selenite; our values for urinary output from oral dosing, when expressed as a percentage of absorbed radioactivity, are of the same order with selenite, but with selenocystine and RK-^{75}Se are consistently slightly higher, and with selenomethionine are slightly lower.

Results obtained in the previous study with RK-^{75}Se (Thomson *et al.* 1975) suggest that Se administered to rabbits as selenomethionine is not incorporated in vivo into kidney protein in this same chemical form, but are consistent with the release by digestion of [^{75}Se]selenite from the labelled kidney homogenate in the gastrointestinal tract of the rats. Results of the present study might suggest that oxidation of [^{75}Se]selenocystine during digestion could release [^{75}Se]selenite. It is not yet understood why the metabolism of [^{75}Se]selenocystine resembles that of [^{75}Se]-selenite and of RK-^{75}Se but not that of [^{75}Se]selenomethionine until after the 1st week, or whether this was in any way associated with the very low dietary intake of Se by the rats (Stadtman, 1974). The results are however in general agreement with Frost's (1972) hypothesis of the central role of selenite in the metabolism of Se.

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