Metabolic studies in rats of [^{75}Se]selenomethionine and of ^{75}Se incorporated in vivo into rabbit kidney

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1. [^{75}Se]selenomethionine was administered to four rabbits and after 4 d their kidneys were removed and homogenized. The long-term fate in rats of an oral dose of this kidney homogenate (RK-^{75}Se) was compared with that of an oral dose of [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney homogenate.

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 for analysis of tissue distribution of ^{75}Se.

3. Intestinal absorption of RK-^{75}Se was 87 %; that of [^{75}Se]selenomethionine was 91 %. Urinary excretion of absorbed RK-^{75}Se was 13·3 % and that of [^{75}Se]selenomethionine was 7·6 %, in the 1st week.

4. Whole-body retention of ^{75}Se was greater for [^{75}Se]selenomethionine than for RK-^{75}Se but after the 1st week 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 in rabbit kidney is different from that of [^{75}Se]selenomethionine. However, after the 1st week ^{75}Se from these sources appears to be metabolized similarly, suggesting that Se from both is ultimately incorporated into the same metabolic pool.

Selenium is known to be associated with proteins in biological materials and is generally believed to be incorporated into proteins as seleno-amino acids. Peterson & Butler (1962) gave evidence for the incorporation of Se into plant proteins, chiefly in the form of selenomethionine and selenocystine. However, although the importance of Se for adequate nutrition and metabolic function in animals has been well established, comparatively little is known about the form in which it exists in animal tissues. Cummins & Martin (1967) and Jenkins (1968) presented evidence to suggest that Se is present in animal tissues in forms other than seleno-amino acids. Since animal products make up a high proportion of the total food intake of New Zealanders, this subject is relevant to the over-all study of Se nutrition and metabolism in the human population of this country.

Furthermore, metabolic studies with Se in animals have previously been concerned mainly with selenite, selenate and selenomethionine. Thus little is known about the metabolism of Se as it occurs in animal foods. A previous study (Thomson & Stewart, 1973) compared the metabolism of oral and intravenous doses of [^{75}Se]selenite and [^{75}Se]selenomethionine administered in solution to rats. The present investigation compares the fate in rats of an oral dose of ^{75}Se incorporated in vivo into rabbit kidney with that of an oral dose of [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney homogenate.

EXPERIMENTAL

Procedure

A dose of 150 μCi [^{75}Se]selenomethionine (Radiochemical Centre, Amersham, Bucks.), containing not more than 15 μg Se, was given to each of four rabbits by injection into an ear vein. The rabbits were killed after 4 d; all eight kidneys were homogenized together in 5 ml de-ionized water.

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 0·025 mg Se and 180 g available protein/kg. Each of the seventeen rats in one group received orally, by intragastric intubation when anaesthetized with diethyl ether, 2 ml homogenized labelled rabbit kidney (RK-^{75}Se) containing a known amount of ^{75}Se (approximately 2 μCi); a second group of seventeen rats received in the same way an equivalent amount of [^{75}Se]selenomethionine mixed with homogenized unlabelled rabbit kidney. Each dose contained 1·8 g kidney tissue and included not more than 4 μg Se.

Collection of urine and faeces

Ten 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. Radioactivity in measured portions of each sample was determined in 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. The rats were lightly anaesthetized with diethyl ether and placed in an open 2 l plastic container to be counted for 100 while rotating six times/min between two uncollimated 50 mm diameter sodium iodide scintillation crystals connected in series through a pulse-height analyser to an IDL scaler (Isotope Development Ltd, Bath Rd, Beenham, Reading, Berks.) and timer (Series No. 7000). The counting efficiency of this system is approximately 1·2 %. Radioactivity in each rat was counted in this way shortly after administration of the dose and this initial count (day 0) was used as the 100 % reference value for subsequent measurements of that animal. All whole-body radioactivity measurements were corrected for radioactive decay and for any variation in counting efficiency by reference to a ^{75}Se standard counted at the same time. The radioactivity in five rats in each group not housed in metabolism cages was counted on days 1, 2 and 4, and radioactivity in all surviving rats was counted on day 7 and then at weekly intervals for 10 weeks.

Table 1. *Absorption, excretion and retention of ^{75}Se (as a percentage of the dose) by rats during the 1st week after oral doses of RK-^{75}Se and [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney*

(Mean values with their standard errors for ten rats)

			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
RK-^{75}Se	Mean	87·3	11·6	12·8	10·8	23·6	35·2	64·8	63·5
	SE	1·7	0·8	1·7	0·3	1·4	1·5	1·5	1·2
[^{75}Se]selenomethionine with homogenized rabbit kidney	Mean	91·3	6·9*	8·6	11·6	20·2	27·1*	72·9*	72·6
	SE	1·1	0·4	1·1	0·7	1·3	1·3	1·3	0·9

RK-^{75}Se, kidney homogenate from rabbits injected with a dose of [^{75}Se]selenomethionine (see p. 46).

* Values for group receiving RK-^{75}Se were significantly different from those receiving [^{75}Se]selenomethionine ($P < 0{\cdot}001$).

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

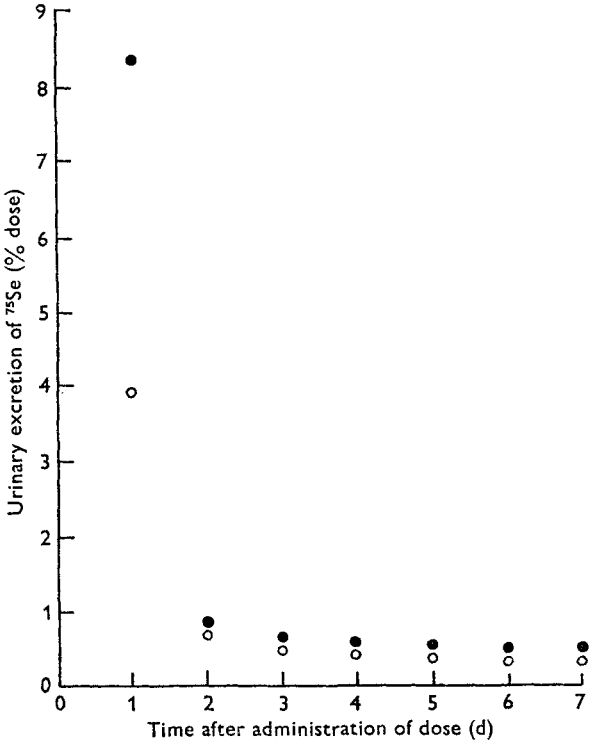

Fig. 1. Urinary excretion of ^{75}Se in two groups, each of ten rats, given oral doses of an homogenate of either ^{75}Se incorporated in vivo into rabbit kidney (see p. 46) (●) or [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney (○).

Tissue retention

Three rats from each group were bled to death from the aorta on days 7 and 14, and also two rats from each group on days 21 and 28. The heart, lungs, spleen, liver, kidneys, adrenals and ovaries and portions of thigh muscle and of the shaft of the femur were removed and weighed. Blood was allowed to clot and the serum was separated from the erythrocytes. Radioactivity in these tissues was measured in the automatic well counter with a ^{75}Se standard. Radioactivity in whole organs was expressed as a percentage of the whole-body ^{75}Se for that animal at death and radioactivity in the tissues was expressed as a percentage of whole-body ^{75}Se/g wet weight of tissue.

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 back to the zero-time intercept and this point was taken to represent the fraction of tracer not absorbed (Thomson & Stewart, 1973). Mean absorption of RK-^{75} Se was 87·3 % of the dose administered and that of [^{75}Se]selenomethionine was 91·3 % (Table 1).

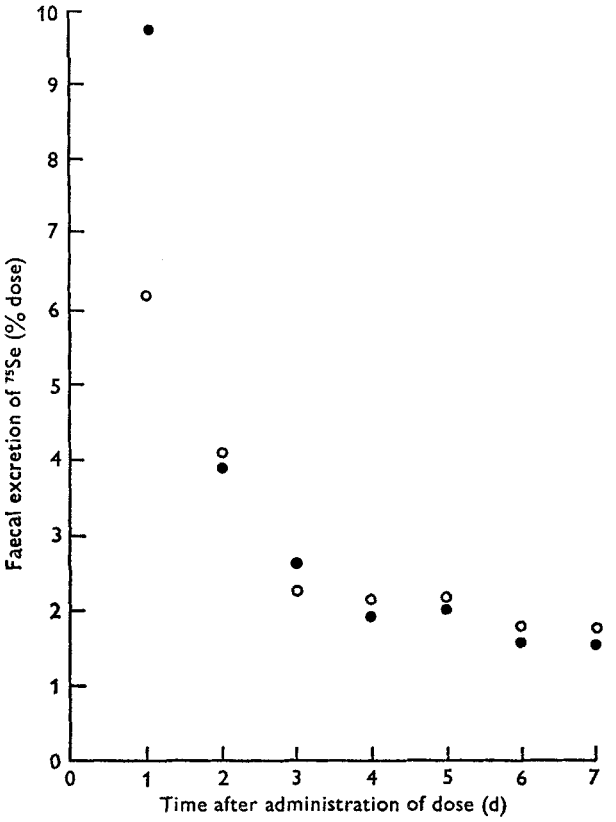

Fig. 2. Faecal excretion of ^{75}Se in two groups, each of ten rats, given oral doses of an homogenate of either ^{75}Se incorporated in vivo into rabbit kidney (see p. 46) (●) or [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney (○).

Urinary excretion of ^{75}Se during the 1st week is shown in Fig. 1. The rats given RK-^{75}Se excreted 8·4 % of the administered dose in urine during the 1st day whereas those given [^{75}Se]selenomethionine excreted only 3·9 % ($P < 0·001$). During the 2nd day urinary loss of ^{75}Se decreased to 0·7–0·8 % of the dose and on day 7 it was 0·4 % dose for both groups. There was no significant difference between total urinary excretion of ^{75}Se for days 2–7 in rats given RK-^{75}Se (3·2 % dose, SE 0·1) and in those given [^{75}Se]selenomethionine (3·0 % dose, SE 0·1). Cumulative urinary excretion at the end of the 1st week was 11·6 % of the dose for rats given RK-^{75}Se and 6·9 % of the dose for those given [^{75}Se]selenomethionine ($P < 0·001$). This accounted for 13·3 % and 7·6 % of the absorbed radioactivity, respectively.

Faecal loss of ^{75}Se during the 1st week is shown in Fig. 2. Maximum faecal excretion occurred on day 1 and was greater ($P < 0·05$) in rats given RK-^{75}Se (9·8 %) than in those given [^{75}Se]selenomethionine (6·2 %). For the remainder of the week faecal loss was similar in both groups. Cumulative faecal loss at the end of the week was slightly greater for RK-^{75}Se (23·6 %) than for [^{75}Se]selenomethionine (20·2 %) but this difference was not significant (Table 1). Endogenous faecal loss of ^{75}Se during the 1st week was determined as the difference between total faecal ^{75}Se and unabsorbed

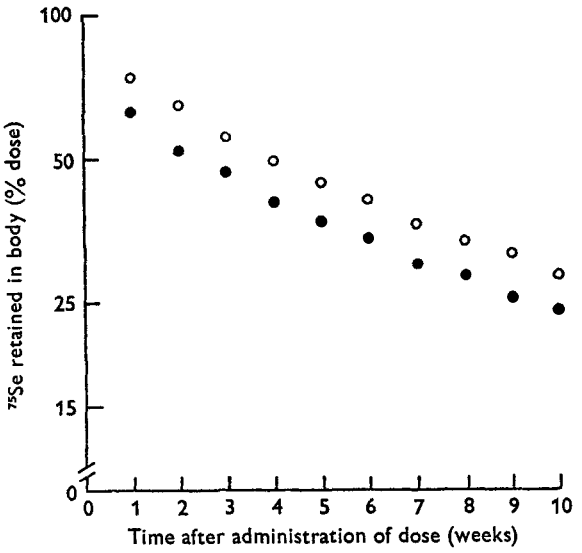

Fig. 3. Whole-body ^{75}Se in groups of rats given oral doses of an homogenate of either ^{75}Se incorporated in vivo into rabbit kidney (see p. 46) (●) or [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney (○). Each point represents the mean value for all surviving rats in that group.

^{75}Se. For the groups given RK-^{75}Se, this amounted to 10·8% of the dose or 12·4% of absorbed radioactivity; for those given [^{75}Se]selenomethionine it was 11·6% of the dose or 12·7% of absorbed ^{75}Se.

Whole-body retention and turnover

Combined urinary and faecal excretion of tracer at the end of the 1st week was 35·2% of the dose of RK-^{75}Se but only 27·1% of the [^{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 retentions calculated from urinary and faecal excretion. Retention of RK-^{75}Se calculated from excretion measurements was 64·8% of the dose; that estimated from whole-body counting was 63·5%. Retention values for [^{75}Se]selenomethionine calculated by the two methods were 72·9 and 72·6% respectively. When expressed as a percentage of the dose absorbed, retention of RK-^{75}Se calculated from excretion measurements was 74·2% and from whole-body counting was 72·7%, and retention of [^{75}Se]selenomethionine was 79·8 and 79·5% respectively. There was no evidence of respiratory loss of ^{75}Se in these animals, judging by 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.

Fig. 3 shows the mean whole-body ^{75}Se in the two groups during the 10-week period. Whole-body retention of ^{75}Se on days 1, 2 and 4 was 74, 68 and 64% of the dose respectively for five rats given RK-^{75}Se and 91, 86 and 82% for five rats given [^{75}Se]selenomethionine. Retention of RK-^{75}Se was less than that of

Table 2. *Distribution of retained ^{75}Se in tissues of rats during 4 weeks following oral doses of RK-^{75}Se and [^{75}Se]selenomethionine mixed with unlabelled rabbit kidney*

(Mean values for three rats)

Chemical form of dose ...	RK-^{75}Se				[^{75}Se]selenomethionine + rabbit kidney			
Time after dose (weeks) ...	1	2	3	4	1	2	3	4
	% Whole-body ^{75}Se/organ							
Liver	8·5	6·5	5·9	5·3	8·4	5·9	6·4	5·6
Kidney	8·7	7·4	5·6	5·9	7·5	6·5	9·4	7·8
Heart	0·55	0·60	0·63	0·65	0·46	0·52	0·59	0·60
Lung	1·1	1·4	1·2	1·2	1·0	1·1	1·2	1·2
Spleen	1·3	1·0	1·0	1·2	0·9	1·0	1·0	0·9
Adrenals	0·22	0·35	0·43	0·49	0·19	0·28	0·41	0·52
Ovaries	0·16	0·18	0·18	0·23	0·17	0·18	0·21	0·21
	% Whole-body ^{75}Se/g wet tissue							
Erythrocytes	0·74	0·72	0·76	0·90	0·69	0·70	0·77	1·00
Thigh muscle	0·37	0·34	0·33	0·33	0·44	0·46	0·46	0·48
Bone (shaft of the femur)	1·00	0·81	0·61	0·57	0·83	0·74	0·70	0·62
Serum	1·2	1·3	1·2	1·1	1·4	1·3	1·1	1·2

RK-^{75}Se, kidney homogenate from rabbits injected with a dose of [^{75}Se]selenomethionine (see p. 46).

[^{75}Se]selenomethionine at the end of the 1st week and this difference was maintained throughout the rest of the trial. Retentions of [^{75}Se]selenomethionine at the end of each week were greater ($P < 0{\cdot}001$) than those of RK-^{75}Se. The whole-body retention curve for each experimental group was calculated from the observed whole-body radioactivity by fitting the sum of the two exponents using the method of least squares. The first of these exponential components which represented urinary and faecal loss during an initial equilibration period of 3 weeks, accounts for approximately 43% of the dose in rats given RK-^{75}Se and it has a half-time of 5 d. For those given [^{75}Se]selenomethionine this exponent accounts for approximately 31% of the dose and has a half-time of 7 d. The half-times for the second exponential component, representing metabolism of retained Se were 55 d and 54 d for the two groups respectively. There was no significant difference between the slope-constants of this second exponent for each of the two groups.

Tissue distribution

^{75}Se concentrations were consistently higher in tissues of rats given [^{75}Se]selenomethionine than in those given RK-^{75}Se when expressed as a percentage of the administered dose, but as shown in Table 2, there was no consistent difference when tissue radioactivity was expressed as a percentage of ^{75}Se retained in the whole body.

Liver and kidney contained the greatest amount of radioactivity (Table 2). However, the highest concentrations of ^{75}Se were found in kidney (6·1 and 6·2% whole-body ^{75}Se/g on day 7 for RK-^{75}Se and [^{75}Se]selenomethionine respectively) and adrenals (4·5 and 5·0% whole-body ^{75}Se/g), whereas in liver the values were 1·1 and 1·3% 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 this tissue.

DISCUSSION

Kidney tissue was chosen for this study of the metabolism of dietary Se as derived from animal foods because the highest concentration of radioactivity was found in the kidney 4 d after administration of ^{75}Se to rats (Thomson & Stewart, 1973). Furthermore, Millar (1972) had shown by gel filtration that although the distribution of ^{75}Se in soluble kidney proteins changed during the first 3 d, after this time most of the radioactivity was associated with a single main protein peak.

The initial utilization of RK-^{75}Se by the rats differed from that of [^{75}Se]selenomethionine in two significant respects. First, ^{75}Se derived from RK-^{75}Se was less well absorbed from the intestine than that from [^{75}Se]selenomethionine. It appears that the kidney homogenate itself might have impeded the absorption of ^{75}Se, as in the present study absorption of [^{75}Se]selenomethionine given with homogenized kidney (91%) was less than the absorption of [^{75}Se]selenomethionine given alone (95%) under the same conditions in an earlier study (Thomson & Stewart, 1973). However, as the same amount of kidney tissue was given to all rats in the present study, this factor could not in itself account for the observed difference between the two groups

and the results suggest the effect of some chemical difference in the administered ^{75}Se doses. Thus, for example, if the radioactivity of RK-^{75}Se were present as selenomethionine taking the place of methionine in the tissue proteins, incomplete digestion of these proteins could have caused the lesser absorption of ^{75}Se. Alternatively the observed difference could have resulted if the digestion of RK-^{75}Se released not [^{75}Se]selenomethionine but another radioactive compound such as [^{75}Se]selenite. In this respect it is noteworthy that in a previous study the intestinal absorption of [^{75}Se]selenite was also 4% less than that of [^{75}Se]selenomethionine (Thomson & Stewart, 1973).

Secondly, urinary loss of absorbed ^{75}Se derived from RK-^{75}Se was greater than that from [^{75}Se]selenomethionine. In this respect also the early metabolism of RK-^{75}Se by the rats resembled that of [^{75}Se]selenite rather than that of [^{75}Se]selenomethionine. Whole-body retention of absorbed RK-^{75}Se at 7 d was 73–74% and in the previous study retention of absorbed [^{75}Se]selenite was 72–73% (Thomson & Stewart, 1973). On the other hand, whole-body retention of absorbed [^{75}Se]selenomethionine given with kidney homogenate was 80% compared with a retention of 80–84% [^{75}Se]selenomethionine administered alone in the earlier study.

However, after the initial period of equilibration, the metabolism of ^{75}Se was similar in both groups of rats studied. Thus on days 6 and 7, urinary loss of radioactivity was the same for both groups as was both endogenous faecal ^{75}Se in the 1st week and the distribution after 7 d of retained ^{75}Se between the various tissues studied. Moreover the long-term, whole-body turnover of ^{75}Se was the same in both groups with half-times of 54–55 d, and this compared with half-times of 50–59 d previously found for oral or intravenous administration of [^{75}Se]selenite or [^{75}Se]selenomethionine (Thomson & Stewart, 1973).

It appears that Se from inorganic sources is not, as thought earlier, incorporated into animal tissue proteins as the Se analogues of sulphur amino acids but might be bound between the S atoms of disulphide bonds to form –S–Se–S– bonds (Jenkins, 1968). Se released from such bonding by alkaline dialysis was present as selenite (Cummins & Martin, 1967) or selenite and elemental Se (Jenkins, 1968). The evidence from our study that the initial metabolism by rats of RK-^{75}Se differs from that of [^{75}Se]selenomethionine suggests that Se from organic sources, also, may not be incorporated in rabbit kidney protein as the Se analogue of S amino acids. Our results would be consistent with the release by digestion of [^{75}Se]selenite from the labelled kidney homogenate in the gastrointestinal tract of the rats. However after 7 d, ^{75}Se from both RK-^{75}Se and [^{75}Se]selenomethionine is metabolized similarly, suggesting that Se from both sources is ultimately incorporated into the same metabolic pool.

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