Metabolic studies in rats of ^{75}Se incorporated in vivo into fish muscle

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1. [^{75}Se]selenite or [^{75}Se]selenomethionine was injected into the coelomic cavity of fish. After 2 d or 14 d the muscle portion of the fish was removed and homogenized. The long-term fate in rats of an oral dose of each labelled homogenate was compared with that of an oral dose of [^{75}Se]selenite or [^{75}Se]selenomethionine mixed with unlabelled fish homogenate.

2. Urinary and faecal radioactivity 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 ^{75}Se given as labelled fish homogenate was less complete than that of ^{75}Se mixed with unlabelled homogenate, and the absorption of ^{75}Se from the 14 d-labelled fish homogenate derived from [^{75}Se]selenite was less complete than that of ^{75}Se from the other labelled homogenates.

4. Urinary excretion of absorbed ^{75}Se in the first 7 d was in the range 5–8 % absorbed dose and was slightly greater in the rats given ^{75}Se as selenite or derived from selenite than in those given ^{75}Se as selenomethionine or derived from selenomethionine. Endogenous faecal excretion of absorbed Se was similar in all groups, as also were tissue distribution of retained ^{75}Se and long-term whole-body turnover rate.

5. The results of these studies are compared with those of earlier studies of the metabolism in rats of [^{75}Se]selenomethionine, [^{75}Se]selenite, [^{75}Se]selenocystine and ^{75}Se incorporated in vivo into rabbit kidney. There were differences in the initial utilization of ^{75}Se from these various sources but after the 1st week ^{75}Se from all sources appeared to be metabolized similarly, suggesting that for rats dietary Se of all forms is ultimately incorporated into the same metabolic pool.

In New Zealand, where soil and food Se levels are low, fish, liver and kidney are relatively rich sources of dietary Se (Robinson, 1975). However, Se in these different foods is not equally available (Miller, Soares, Bauersfield & Cuppett, 1972; Cantor, Scott & Noguchi, 1975) and it is not known whether it is metabolized in the same manner. We have previously reported studies of the metabolism in rats of ^{75}Se administered as sodium selenite, or selenomethionine (Thomson & Stewart, 1973), as selenocystine (Thomson, Robinson, Stewart & Robinson, 1975) or as ^{75}Se incorporated in vivo into rabbit kidney (Thomson, Stewart & Robinson, 1975). This report presents the results of a study of the intestinal absorption, endogenous excretion, tissue distribution and whole-body turnover in rats of ^{75}Se incorporated in vivo into fish flesh.

EXPERIMENTAL

Procedure

A dose of 500 μCi [^{75}Se]selenomethionine or [^{75}Se]selenite containing not more than 500 μg Se (Radiochemical Centre, Amersham, Bucks.) was injected into the coelomic cavity of *Pseudolabrus celidotus*, a common and hardy marine fish found in Otago harbour, New Zealand. Six fish were dosed with [^{75}Se]selenomethionine in February and six fish were dosed with [^{75}Se]selenite in July. They were kept at the harbour edge in a tank which was flushed continuously with sea-water. Three fish were killed 2 d after injection with ^{75}Se and three fish were killed at 14 d. The muscular portions of the three fish in each group were

pooled and homogenized in 75 ml distilled water using a combination of Silverson (Gallenkamp, Technico House, London) and Waring blenders (Waring Products Division, Dynamics Corporation of America, New Hartford, Conn., USA). The homogenate was stored at 4° until used.

Each experimental group comprised eighteen female Wistar rats from the same colony and initially weighing 130–150 g. These rats were housed individually in stainless-steel mesh cages and were given distilled water and a pelleted stock diet containing (/kg) 0·033 mg Se and 180 g available protein. Food and water were available *ad lib.* except for the 18 h immediately preceding administration of the dose of fish homogenate. Each of the rats received orally, by intragastric intubation when anaesthetized with diethyl ether, 6 ml labelled fish homogenate containing a known amount of ^{75}Se (4·3–5·0 μCi). Details of the homogenate fed to each group are summarized in Table 1. In addition a control group of rats received a mixture of unlabelled fish homogenate with either [^{75}Se]selenomethionine or [^{75}Se]selenite. Each dose comprised approximately 3 g fish and contained not more than 2 μg Se. The selenomethionine doses were given in three portions in a 6 h period and the selenite doses were given in two portions in a 5 h period.

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 a large-volume counter (constructed in the Department of Medical Physics, Wakari Hospital, Dunedin; Thomson, Stewart & Robinson 1975), with a ^{75}Se standard. 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 the large-volume counter and were made for each rat shortly after administration of the dose. 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 were made on all rats not in metabolism cages on days 1, 2, 3 and 4, and on all surviving rats on day 7 and then weekly for 9 weeks.

Tissue retention

Three rats from each group were killed on day 7, two each on days 14, 21, 28, 35 and 56, and the remaining animals on day 70. Rats were anaesthetized with diethyl ether, 4 ml blood was removed from the jugular vein and the animals were killed by replacing them in the anaesthetizing jar. Radioactivity was measured in whole blood, plasma and erythrocytes, and in the liver, kidneys, adrenals, pancreas, heart, spleen, intestines, head, skin and carcass using the large-volume counter. 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 other tissues was expressed as a percentage of 'whole-body' ^{75}Se/g tissue wet weight.

RESULTS

^{75}*Se balance during the* 1*st week*

The intestinal absorption of the ^{75}Se tracer was calculated by plotting cumulative faecal excretion of ^{75}Se *v.* time (Lutwak, 1969) as previously described (Thomson & Stewart, 1973).

Table 1. *Details of ^{75}Se-labelled and unlabelled fish homogenates administered orally to six groups of rats*

(Each group contained eighteen rats)

Expt	Group of rats	Form of dose	Preparations of fish homogenate: Tracer administered to fish	Preparations of fish homogenate: period after administration (d) when fish were killed	Preparations of fish homogenate: Season	Code for chemical form of dose
1. Selenomethionine	1a	^{75}Se-labelled fish muscle	[^{75}Se]selenomethionine	14	Summer	[^{75}Se]FM-Semet-14d
	1b	^{75}Se-labelled fish muscle	[^{75}Se]selenomethionine	2	Summer	[^{75}Se]FM-Semet-2d
	1c	[^{75}Se]selenomethionine with fish-muscle homogenate	—	—	Summer	[^{75}Se]Semet + FM
2. Selenite	2a	^{75}Se-labelled fish muscle	[^{75}Se]selenite	14	Winter	[^{75}Se]FM-selenite-14d
	2b	^{75}Se-labelled fish muscle	[^{75}Se]selenite	2	Winter	[^{75}Se]FM-selenite-2d
	2c	[^{75}Se]selenite with fish-muscle homogenate	—	—	Winter	[^{75}Se]selenite + FM

FM, fish muscle; Semet, selenomethionine; 14d, 2d, labelled for 14d and 2d respectively.

Table 2. *Absorption, excretion and retention of ^{75}Se (% administered dose) by rats during the 1st week after oral doses of ^{75}Se-labelled fish homogenates and ^{75}Se mixed with unlabelled fish homogenate**

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

Group of rats	Chemical form of dose†		Intestinal absorption of ^{75}Se	Excretion					Retention	
				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
1a	[^{75}Se]FM-Semet-14d	Mean	72·4	4·4	27·6	10·6	38·2	42·6	57·4	64·0
		SE	0·6	0·2	0·6	0·8	0·5	0·5	0·5	0·5
1b	[^{75}Se]FM-Semet-2d	Mean	74·0	4·1	26·0	10·5	36·5	40·6	59·4	61·8
		SE	0·5	0·2	0·5	1·0	0·9	0·9	0·9	1·0
1c	[^{75}Se]Semet + FM	Mean	95·9	5·2	4·1	13·1	17·2	22·4	77·6	75·8
		SE	0·1	0·3	0·1	0·4	0·4	0·5	0·5	1·4
2a	[^{75}Se]FM-selenite-14d	Mean	64·2	5·3	35·8	9·4	45·2	50·5	49·5	50·3
		SE	0·3	0·4	0·3	1·2	1·2	1·2	1·2	0·8
2b	[^{75}Se]FM-selenite-2d	Mean	77·3	5·6	22·7	10·2	32·9	38·5	61·5	60·6
		SE	0·1	0·3	0·1	1·2	1·2	1·2	1·2	0·9
2c	[^{75}Se]selenite + FM	Mean	84·0	5·7	16·0	14·0	30·0	35·7	64·3	70·6
		SE	0·8	0·4	0·8	1·5	1·3	1·3	1·3	1·1

FM, fish muscle; Semet, selenomethionine; 14d, 2d, labelled for 14 d and 2 d respectively.

* For details of procedures, see p. 19.

† For details, see Table 1.

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

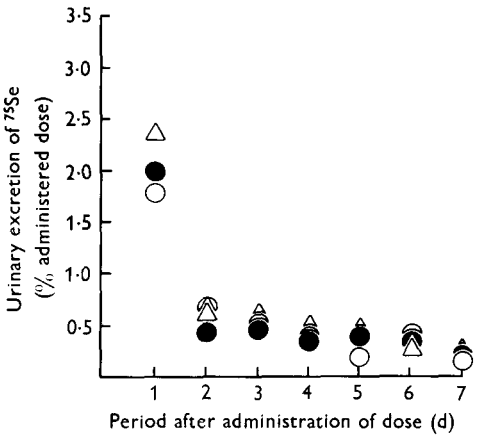

Fig. 1

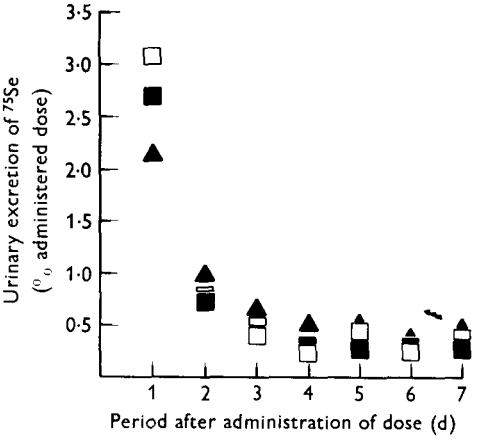

Fig. 2

Fig. 1. Urinary excretion of the ^{75}Se (% administered dose) in three groups, each of twelve rats, given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle, 14 d (group 1a) (●) or 2 d (group 1b) (○) after an injection of [^{75}Se]selenomethionine (see p. 19), or [^{75}Se]selenomethionine mixed with unlabelled fish muscle (group 1c) (△).

Fig. 2. Urinary excretion of ^{75}Se (% administered dose) in three groups, each of twelve rats, given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle, 14 d (group 2a) (■) or 2 d (group 2b) (□) after an injection of [^{75}Se]selenite (see p. 19), or [^{75}Se]selenite mixed with unlabelled fish muscle (group 2c) (▲).

Mean absorption was 72, 74 and 96 % administered dose for the rats in groups 1a, 1b and 1c given the selenomethionine series of homogenates (Table 2) respectively, and 64, 77 and 84 % for those in groups 2a, 2b and 2c given the selenite series of homogenates respectively (Table 2).

Urinary excretion of ^{75}Se during the 1st week is shown in Figs. 1 and 2. Mean excretion in the 1st day was 1·6–2·4 % administered dose for the three groups given ^{75}Se derived from selenomethionine (Fig. 1), with the rats given the unlabelled homogenate (group 1c) excreting more than those in group 1b ($P < 0·001$). For the groups given ^{75}Se derived from selenite (Fig. 2) the mean excretion on the 1st day was 2·2–3·1 % administered dose, with those given unlabelled homogenate excreting less than the other two groups ($P < 0·01$). There was no difference between the two groups given unlabelled homogenate, but groups 1a and 1b given fish labelled with ^{75}Se derived from [^{75}Se]selenomethionine excreted less than the corresponding groups 2a and 2b given fish labelled with ^{75}Se derived from [^{75}Se]-selenite ($P < 0·001$).

Urinary loss of ^{75}Se decreased progressively and for all groups was less than 0·5 % administered dose/d on day 7. Cumulative urinary excretion of ^{75}Se in the 1st week was 4·4, 4·1 and 5·2 % administered dose for the rats in groups 1a, 1b and 1c respectively, and it was 5·3, 5·6 and 5·7 % administered dose for those in groups 2a, 2b and 2c respectively (Table 2). This accounted for 5·4–6·1 % absorbed dose for the groups given ^{75}Se derived from selenomethionine (Table 3), which was just less than the excretion of 6·8–8·3 % absorbed dose for the corresponding groups given ^{75}Se derived from selenite ($P < 0·05$ for groups 1c and 2c, $P < 0·01$ for groups 1b and 2b, $P > 0·001$ for groups 1a and 2a). Cumulative urinary losses of the absorbed dose did not differ for the selenomethionine series of homogenates, whereas in the selenite series of homogenates, the loss was 6·8 % absorbed dose for group 2c compared with 8·3 % absorbed dose for group 2a ($P > 0·02$).

Faecal loss of ^{75}Se during the 1st week is shown in Figs. 3 and 4. Most of the unabsorbed radioactivity was passed on day 1 or day 2 and on day 7 faecal ^{75}Se was less than 2 %

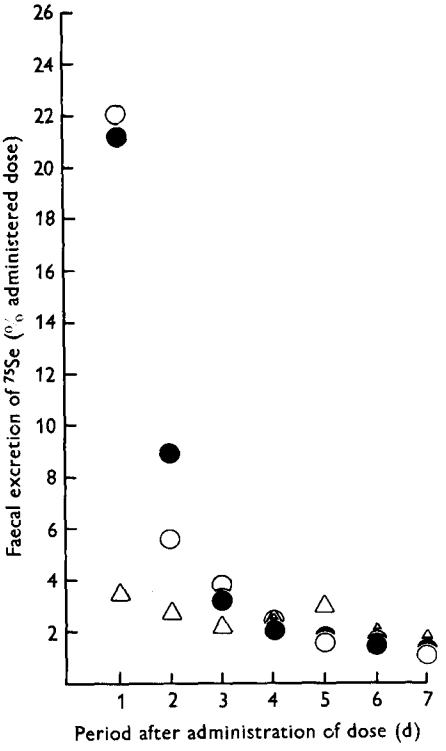

Fig. 3

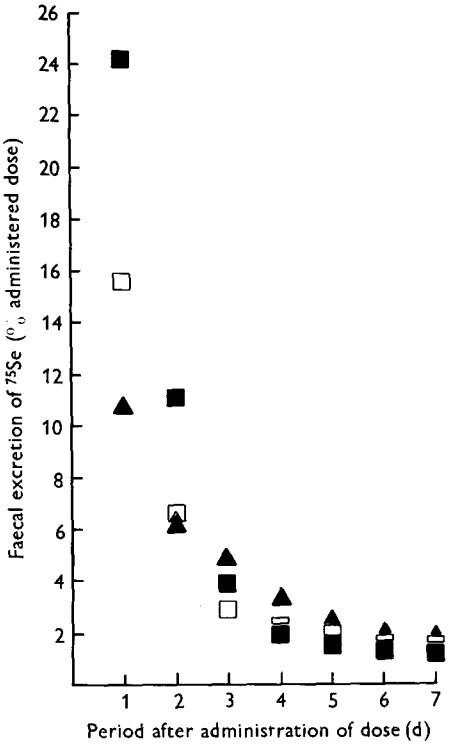

Fig. 4

Fig. 3. Faecal excretion of ^{75}Se (% administered dose) in three groups, each of twelve rats, given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle, 14 d (group 1a) (●) or 2 d (group 1b) (○) after an injection of [^{75}Se]selenomethionine (see p. 19), or [^{75}Se]selenomethionine mixed with unlabelled fish muscle (group 1c) (△).

Fig. 4. Faecal excretion of ^{75}Se (% administered dose) in three groups, each of twelve rats, given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle, 14 d (group 2a) (■) or 2 d (group 2b) (□) after an injection of [^{75}Se]selenite (see p. 19), or [^{75}Se]selenite mixed with unlabelled fish muscle (group 2c) (▲).

administered dose/d for all groups. Cumulative faecal losses of ^{75}Se in the 1st week were 38, 37 and 17% administered dose for groups 1a, 1b and 1c respectively; and were 45, 33 and 30% administered dose for groups 2a, 2b and 2c respectively (Table 2). 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 9–14% administered dose for the six groups (Table 2) or 13–17% absorbed ^{75}Se (Table 3).

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

Whole-body retentions of ^{75}Se on day 7 were calculated from combined urinary and faecal losses of radioactivity and by whole-body counting (Table 2). There was close agreement between the values obtained by the two methods for four of the groups, but the value obtained by whole-body counting was 6–7% higher for groups 1a and 2c (Table 2). Whole-body retention at day 7 calculated from excretion measurements was 57, 59 and 78% administered dose for groups 1a, 1b and 1c respectively, and 50, 62 and 64% administered dose for groups 2a, 2b and 2c respectively (Table 2). When expressed as a percentage of absorbed dose the values were in range 77–81% absorbed dose (Table 3). Retention of ^{75}Se

Table 3. *Comparison of results obtained for absorption (% administered dose) excretion and retention (% absorbed dose) of ^{75}Se by rats during the 1st week after dose of ^{75}Se-labelled fish homogenates and ^{75}Se mixed with unlabelled fish homogenates**

(Mean values for twelve rats/treatment)

Group of rats	Chemical form of dose†	Intestinal absorption of ^{75}Se	Excretion		Retention	
			Urine	Endogenous faecal ^{75}Se‡	From excretion by differences	By whole-body counting
1a	[^{75}Se]FM-Semet-14d	72	6·1	14·6	79·3	88·4
1b	[^{75}Se]FM-Semet-2d	74	5·5	14·2	80·3	83·5
1c	[^{75}Se]Semet+FM	96	5·4	13·7	80·9	79·0
2a	[^{75}Se]FM-selenite-14d	64	8·3	14·6	77·1	78·3
2b	[^{75}Se]FM-selenite-2d	77	7·2	13·2	79·6	78·4
2c	[^{75}Se]selenite+FM	84	6·8	16·7	76·5	84·0

FM, fish muscle; Semet, selenomethionine; 14d, 2d, labelled for 14 d and 2 d respectively.

* For details of procedures, see p. 19.

† For details, see Table 1.

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

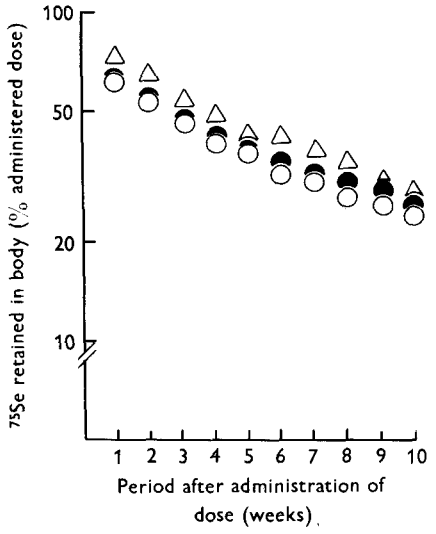

Fig. 5

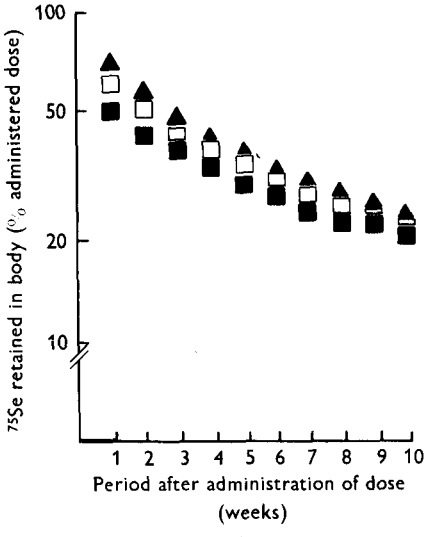

Fig. 6

Fig. 5. Whole-body ^{75}Se (% administered dose) in groups of rats given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle, 14 d (group 1a) (●) or 2 d (group 1b) (○) after an injection of [^{75}Se]selenomethionine (see p. 19), or [^{75}Se]selenomethionine mixed with unlabelled fish muscle (group 1c) (△). Each point represents the mean value for all surviving rats in that group (see p. 20).

Fig. 6. Whole-body ^{75}Se (% administered dose) in groups of rats given oral doses of an homogenate of ^{75}Se incorporated in vivo into fish muscle 14 d (group 2a) (■) or 2 d (group 2b) (□) after an injection of [^{75}Se]selenite (see p. 19), or [^{75}Se]selenite mixed with unlabelled fish muscle (group 2c) (▲). Each point represents the mean value for all surviving rats in that group (see p. 20).

at 7 d measured by whole-body counting (% administered dose) was 64, 62 and 76 for groups 1a, 1b and 1c respectively, and 50, 61 and 71 for groups 2a, 2b and 2c respectively, or 78–88 % absorbed dose (Table 3).

Mean whole-body ^{75}Se in the surviving rats of the six groups for the 10-week period is shown in Figs. 5 and 6. For all six 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 of these exponential components represents the phase of urinary and faecal loss of ^{75}Se during an initial equilibration period (phase 1) and the second exponential component represents long-term whole-body turnover of retained ^{75}Se (phase 2). The half-times for phase 1 were 8·5 and 8·1 and 14·1 d for groups 1a, 1b and 1c respectively, and 8·0, 10·5 and 14·3 d for groups 2a, 2b and 2c respectively. The half-times for phase 2 were 45·9–49·8 d and there was no significant difference among the six groups.

Tissue distribution of ^{75}Se

The pattern of distribution of ^{75}Se between the tissues examined was similar for all groups. The highest concentration of ^{75}Se was found in kidney and liver, but the greatest amount of ^{75}Se was present in the carcass comprising the skeleton and skeletal muscle.

DISCUSSION

This study, the fourth in a series of studies on the metabolism in rats of various sources of Se, has revealed some differences in the initial metabolism of 'fish' Se compared with that of Se ingested in the other forms (Table 4). Further, the metabolism of the labelled fish muscle may have been influenced by the form in which ^{75}Se was administered and by the period which elapsed after the Se was taken up by the fish.

Millar (1972) had shown that injected ^{75}Se from [^{75}Se]selenite and [^{75}Se]selenomethionine was rapidly incorporated into rat tissues within 2–3 d. Because it was not known whether this would also occur in fish muscle, the fish were left for 2 d and 14 d before killing, and the fish homogenate was then fed to rats.

The metabolism of 'fish' Se derived from selenomethionine was not different from that of ^{75}Se given as [^{75}Se]selenomethionine together with the non-radioactive fish homogenate, except for a greatly reduced intestinal absorption. In addition there was no difference between the metabolism of the 2 d- and 14 d-labelled fish homogenates. This suggests that after digestion the greater part of the ^{75}Se in these homogenates was released as selenomethionine or a similarly metabolized compound. However, the lesser intestinal absorption of the tracer from the fish muscle shows that either digestion of the homogenate was incomplete or a proportion of the ^{75}Se had been converted into a non-absorbable or non-digestible form. If the latter were the situation existing, then the change would have occurred within 2 d of the administration of the [^{75}Se]selenomethionine to the fish. There was no evidence of any effect upon the intestinal absorption or initial metabolism of [^{75}Se]selenomethionine by unlabelled fish homogenate (Table 4) despite the bulk of the dose and the necessity of administering it in three divided doses in a period of 6 h.

It is of interest to compare the metabolism of 'fish' Se derived in vivo from [^{75}Se]selenomethionine with that of rabbit kidney similarly derived (Thomson, Stewart & Robinson, 1975). 'Rabbit-kidney' Se was more completely absorbed than 'fish' Se (87 *v.* 72–74 % administered dose), but in the 1st week after absorption more of the absorbed radioactivity was excreted in the urine from the labelled rabbit kidney than from the fish (13·3 *v.* 5·5–6·1 % administered dose), the difference occurring principally on the 1st day (10 *v.* 3 % administered dose). It is likely that the Se had been incorporated into different compounds in the rabbit kidney and the fish muscle, because of metabolic differences between species

Table 4. *Comparison of results obtained for absorption (% administered dose), excretion and retention (% absorbed or intravenous dose) of ^{75}Se during the 1st week after doses of [^{75}Se]selenite, [^{75}Se]selenomethionine, [^{75}Se]selenocystine, RK-^{75}Se and ^{75}Se-labelled fish homogenate**

(Mean values for ten or twelve rats/treatment)

Chemical form of dose	Method of administration	Source of results	Intestinal absorption of ^{75}Se	Excretion: Urine: Day 1	Excretion: Urine: Day 2–7	Excretion: Endogenous faecal ^{75}Se‡	Retention from excretion by difference
[^{75}Se]selenite	Intravenous	Thomson & Stewart (1973)	—	16	3	13	68
[^{75}Se]selenomethionine	Intravenous		—	2	3	12	83
[^{75}Se]selenite	Oral	Thomson & Stewart (1973)	91	11	3	13	73
[^{75}Se]selenomethionine	Oral		95	2	2	11	84
[^{75}Se]selenocystine	Oral	Thomson, Robinson, Stewart & Robinson (1975)	81	10	4	10	76
[^{75}Se]selenomethionine	Oral		86	3	3	10	84
RK-^{75}Se	Oral	Thomson, Stewart & Robinson (1975)	87	10	4	12	74
[^{75}Se]selenomethionine + rabbit kidney homogenate	Oral		91	4	3	13	80
[^{75}Se]FM-Semet-14d†	Oral	Present study	72	3	3	15	79
[^{75}Se]FM-Semet-2d†	Oral		74	2	3	14	80
[^{75}Se]Semet + FM†	Oral		96	2	3	14	81
[^{75}Se]FM-selenite-14d†	Oral	Present study	64	5	3	15	77
[^{75}Se]FM-selenite-2d†	Oral		77	3	4	13	80
[^{75}Se]selenite + FM†	Oral		84	2	4	17	77

RK-^{75}Se, kidney homogenate from rabbits given [^{75}Se]selenomethionine (Thomson, Stewart & Robinson, 1975).
FM, fish muscle; Semet, selenomethionine; 14d, 2d, labelled for 14 d and 2 d respectively.
* For details of procedure, see p. 19.
† For details, see Table 1.
‡ Calculated as the difference between total faecal ^{75}Se and unabsorbed faecal ^{75}Se.

or between tissues. It is noteworthy that the metabolism of [^{75}Se]selenomethionine mixed with unlabelled fish muscle or rabbit kidney homogenates was similar.

The ^{75}Se in homogenates from fish given [^{75}Se]selenite was also less well absorbed by the rats than ^{75}Se administered as selenite with unlabelled fish homogenate, but in this experiment there was a difference between the 2 d- and 14 d-labelled homogenates. Absorption of the 14 d-labelled homogenates was less than the 2 d-labelled homogenate which indicates that in the 14 d after dosing there was a continuing coversion in the fish of ^{75}Se derived from [^{75}Se]selenite to a less digestible or less absorbable form. However, the selenite experiment was undertaken in July when the mean sea-water temperature was 7°, whereas the selenomethionine experiment was done in February when the mean sea-water temperature was 16°. This difference in environmental temperature for the poikilothermic fish could have made an almost twofold difference in their metabolic rate (Bullock, 1955), which may have accounted in part at least for the differences between the [^{75}Se]selenite homogenates of 2 d- and 14 d-labelled fish.

Urinary output of ^{75}Se where [^{75}Se]selenite was mixed with unlabelled homogenate was much lower than that previously found for [^{75}Se]selenite given orally in solution or by intravenous injection to rats (Table 4). In the earlier studies 16 % injected dose and 11 % absorbed dose were excreted during the 1st day, and most probably within a few hours of the dose being given, if one can extrapolate from the studies of [^{75}Se]selenite in young women (Thomson & Stewart, 1974). In the present study only 2 % absorbed dose was excreted which suggests that there was some in vitro interaction between the selenite and the fish homogenate during the storage for 18 h in the refrigerator before administration. According to Levander (1976), most of the selenite would have been reduced to selenide, or complexed in some other way. This might also account for the slightly reduced intestinal absorption of [^{75}Se]selenite given with the unlabelled fish homogenate compared with that of [^{75}Se]selenite given alone (84 *v.* 91 % administered dose) (Table 4).

Little is known about the chemical nature of Se in seafoods. Se is known to become associated in fish with heavy metals, particularly mercury, with which it forms complexes, possibly metal selenides (Ganther, Goudie, Sunde, Kopecky, Wagner, Oh & Hoekstra, 1972; Pařízek, Kalousková, Babický, Beneš & Pavlik, 1974). It is also known that Se in fish meal has a low biological availability (Ganther, Wagner, Sunde & Hoekstra, 1972; Cantor *et al.* 1975) but this does not appear to depend directly upon the concentration of Hg in the fish as availability of Se is low, even from fish meals low in Hg (Ganther & Sunde, 1974). The fish muscle used in the present study contained 0·6 μg Se/g and 0·06 μg Hg/g (J. V. Dunckley, private communication) giving a value for the molar ratio, Hg:Se of 0·04, which was less than the value of 0·07 found in low-Hg tuna meal (Ganther & Sunde, 1974). It seems unlikely that more than a small proportion of the Se in our fish homogenate was complexed with Hg or other heavy metals, the remainder probably being incorporated into tissue proteins or other compounds (Lunde, 1973*a*, *b*; Levander, 1976; Moffit & Clary, 1974; Potter & Matrone, 1974).

The present study gives no clear information on the actual forms of ^{75}Se in the labelled fish, but it would seem unlikely that they would be identical judging from the different chemical and metabolic behaviour of selenite and selenomethionine. The differences in intestinal absorption by the rat of the labelled fish homogenates and in the urinary excretion during the 1st day suggest that ^{75}Se in fish muscle differed chemically according to the form of the tracer injected into the fish. But from the 1st day onwards there was close agreement with all groups of rats given fish in their handling of absorbed tracer.

From our comparisons of the results from all our studies of ^{75}Se in rats (Table 4; see also Table 3 of Thomson, Robinson *et al.* 1975), it seems that 'fish' Se is less well absorbed than all the other forms studied. But once absorbed, the differences which existed in the urinary

excretion of the tracer during the 1st week, occurred almost entirely during the 1st day; 'fish' Se resembled selenomethionine whereas 'rabbit-kidney' Se resembled selenocystine and selenite. It is not clear whether the endogenous faecal output during the 1st week differed according to the dose given. Nevertheless on all occasions in this study and in our earlier studies (Thomson & Stewart, 1973; Thomson *et al.* 1975; Thomson, Stewart & Robinson, 1975), whole-body turnover rates for ^{75}Se after the 1st week were closely similar. This supports our previous claim that the long-term metabolism of retained Se in rats appears to be independent of the chemical form from which it was derived. In this respect the metabolism of Se in rats is different from that in man for whom the long-term turnover rate for ^{75}Se given as selenite is much more rapid than that of ^{75}Se given as selenomethionine (Thomson & Stewart, 1974; Griffiths, Stewart & Robinson, 1976).

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