The excretion of selenium in bile and urine of steers: the influence of form and amount of Se salt

BY H. W. SYMONDS, DENISE L. MATHER AND M. J. VAGG

Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, RG16 0NN

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1. The excretion of ^{75}Se and stable Se in bile and urine was measured in four steers during 6 h after intravenous injections of ^{75}Se as either selenite or selenate containing either 5 or 5000 μg carrier Se.

2. When 5000 μg Se were given, the rate of urinary excretion and plasma clearance of ^{75}Se was similar for both salts. Approximately 23% was excreted in urine and plasma clearance was triexponential, the mean half-life ($t_{\frac{1}{2}}$) of the successive components, α, β and γ, being 2·3, 15·2 and 465 min respectively. The amount of ^{75}Se excreted in bile was small; 1·94% of the $^{75}SeO_3^{2-}$ and 0·86% of the $^{75}SeO_4^{2-}$ dose.

3. When 5 μg Se were given the plasma clearance of ^{75}Se was initially biexponential but the entry of ^{75}Se-labelled protein from the liver caused an increase in plasma radioactivity after 30–40 min. The effect was most marked after 5 μg $^{75}SeO_3^{2-}$ when plasma ^{75}Se radioactivity returned to 60% of the activity present at 2 min. Values for $t_{\frac{1}{2}}$ of the two components of clearance for $^{75}SeO_3^{2-}$ and $^{75}SeO_4^{2-}$ were respectively α 2·6 and 2·5 min, and β 15·9 and 36·6 min. Similar amounts of ^{75}Se appeared in bile (0·2% of the dose) after injections of either salt but much less ^{75}Se was excreted in urine after $^{75}SeO_3^{2-}$ (6%) than after $^{75}SeO_4^{2-}$ (17%).

4. At low dosage rates (5 μg) Se is more readily incorporated into tissues from SeO_3^{2-} than from SeO_4^{2-}.

The bovine liver plays a significant role in the metabolism of selenium. When small amounts of $^{75}SeO_3^{2-}$ (5–20 μg Se) are injected intravenously the liver removes at least 40%, attaches it to protein and releases it into the plasma 30–60 min later (Symonds *et al.* 1981). Se given as selenate is metabolized less rapidly and a greater proportion is excreted in urine. Some of the Se removed by the liver is excreted in bile although there appears to be a species difference in the extent to which this occurs. In the non-ruminant (rat) approximately 5·8%/h of an intravenous dose of ^{75}Se was excreted in bile (Imbach & Sternberg, 1967) while in the sheep the maximum activity 3–4 h after dosing was approximately 0·0012%/ml bile (Dejneka *et al.* 1979). Assuming a bile flow-rate of 60 ml/h (Harrison, 1962) the total amount secreted during 96 h was less than 2%.

It is possible that, as with the trace elements copper and manganese, the concentration of Se in bile increases when the plasma concentration of the element is increased. Such an effect could explain the differences observed in the excretion rate of Se from the rat and sheep. A study was therefore made in four steers to compare the clearance of different quantities of Se (5 μg or 5000 μg), given either as $Na_2{}^{75}SeO_3$ or $Na_2{}^{75}SeO_4$, from plasma and their excretion in urine and bile. The results confirm that a much greater proportion of injected ^{75}Se is excreted in urine than in bile, that selenite is more readily metabolized than selenate at low carrier levels and that only a small proportion of injected Se is excreted in bile, although the proportion is increased when 5000 μg Se is injected.

MATERIALS AND METHODS

Animals

Four Angus × Friesian steers (A, B, C and D), weighing between 400 and 500 kg were used. Each had had its duodenum surgically altered to allow bile to be collected and its rate of flow measured. The procedure entailed isolating that section of the duodenum where the common bile duct entered and diverting the bile entering this section back to the duodenum

via a re-entrant cannula (Symonds *et al.* 1982). The steers were not used until at least 6–8 weeks after surgery.

Experimental procedure

Approximately 25 μCi ^{75}Se as either $Na_2{}^{75}SeO_3$ or $Na_2{}^{75}SeO_4$ were given as a single injection into one jugular vein. Each animal received two injections of either one or both salts. The first injection contained approximately 5 μg Se, the second given 7 d later, 5000 μg Se. Steers A and B received injections of both salts, steers C and D received $^{75}SeO_3{}^{2-}$ only, steer D receiving the 5000 μg dose first and the 5 μg dose 7 d later. Steer A also received a second series of $^{75}SeO_4{}^{2-}$ injections after an interval of several weeks. Blood was taken into bottles containing 100 IU heparin from a cannula in the jugular vein opposite to that used for dosing, at 2, 4, 6, 10, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105 and 120 min and then at 30 min intervals to 360 min. Weighed amounts of plasma were transferred to counting vials for determination of the ^{75}Se content. The rate of flow of bile was measured by collecting the bile flowing from the isolated segment during timed intervals and measuring its volume. A weighed amount was taken for ^{75}Se estimation and the remainder was returned to the duodenum by means of a peristaltic pump at a rate approximately the same as the rate of flow. Collections were made at 5 min intervals for the first 30 min, at 10 min intervals from 30 to 60 min, and subsequently when blood was sampled. To ensure that the delay due to the dead space between the sphincter of Oddi and the bile collection bag was minimal saline (9 g sodium chloride/l) was infused into the isolated section of duodenum at 9 ml/min through a small cannula. Bile flow-rate was calculated as the difference between the total volume of fluid collected and the volume of saline infused. Urine was collected in a container suspended below the steer's abdomen.

Analytical methods

The amount of ^{75}Se in urine, plasma and bile was determined by measuring the radioactivity present in weighed samples in an NE 8112 γ-spectrometer (Nuclear Enterprises) at a counting efficiency of approximately 75%. The percentage of the injected dose present was calculated using standards prepared from known dilutions of the dose.

During each infusion 1% of the volume of urine and bile passed during each collection period was pooled. The stable Se content of these pooled samples and of selected plasma samples was measured by the method used by Little *et al.* (1979). The exponential components making up the pattern of clearance of ^{75}Se from plasma were calculated by stripping off each component to obtain the best fit for up to three components (α, β and γ).

Expression of urinary ^{75}Se values

The frequency of micturition varied considerably among the steers. In order to determine the average patterns of excretion of ^{75}Se in urine after the administration of the different salts and different amounts of carrier, the cumulative excretion of ^{75}Se during the 6 h after each dose was plotted graphically for each steer from the measured values. The accumulative excretion was then calculated from the graph for each 30 min period. The average value of excretion for each 30 min period for all animals for each salt and each carrier amount was then calculated and plotted (Fig. 3).

RESULTS

The effect of the amount of carrier Se

Clearance of $^{75}SeO_3{}^{2-}$ from plasma. The values presented in Fig. 1 are the means for all steers, normalized by expressing the results for each steer as a percentage of the activity present in its plasma at 2 min after injection. The mean ($\pm$SE) percentage dose/l plasma at 2 min after injection of 5 μg and 5000 μg of Se was 3·28 ($\pm$0·22) and 2·99 ($\pm$0·13)

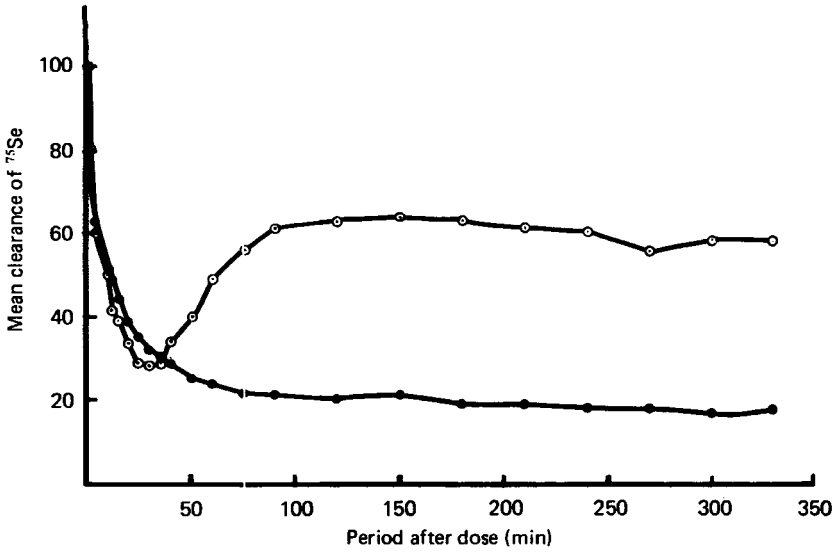

Fig. 1. The mean clearance of ^{75}Se from the plasma of four steers. ^{75}Se given as $Na_2{}^{75}SeO_3$ containing either 5 μg (⊙——⊙) or 5000 μg (●——●) carrier Se. Values are expressed as a percentage of the concentration (% dose ^{75}Se/l plasma) at 2 min.

respectively. The amount of carrier had little effect on the rate of clearance during the first 30 min, the ^{75}Se concentration decreasing to approximately 30% of the concentration at 2 min. When 5 μg Se was injected the ^{75}Se concentration in plasma then increased during the next 75 min to reach just over 60% of the concentration at 2 min and then decreased slowly with a mean $t_{\frac{1}{2}}$ of 19·3 h (Fig. 1). When 5000 μg of Se were injected there was no increase in plasma radioactivity after 30 min and by 5 h after injection the radioactivity had decreased to approximately 18% of that at 2 min.

The mean $t_{\frac{1}{2}}$ of both α and β components of clearance were similar for both carrier levels and are given in Table 1. A third (γ) component with a $t_{\frac{1}{2}}$ of 693 and 371 min respectively was present in steers C and D after 5000 μg while in steers A and B plasma radioactivity increased very slightly.

Clearance of $^{75}SeO_4{}^{2-}$ from plasma. The values presented are the means of two observations on steer A and one on steer B and normalized as previously described for $^{75}SeO_3{}^{2-}$ values. The mean (±SE) percentage dose/l plasma at 2 min after dosing with either 5 or 5000 μg was 2·65 (±0·20) and 2·52 (±0·22) respectively. During the first 45 min the rate of disappearance of ^{75}Se was similar for both carrier levels and its concentration decreased to 40% of the concentration at 2 min. Thereafter, after dosing with 5 μg there was a slow increase to 47% by 4 h. After 5000 μg, clearance continued exponentially throughout the 6 h period.

Values for $t_{\frac{1}{2}}$ of the components of clearance after 5 and 5000 μg are given in Table 1. The α component could not be measured in steer A2. The other components were similar to the components of clearance of $^{75}SeO_3{}^{2-}$ with the exception that the β component after 5 μg Se was over twice as long as that after 5 μg Se as $SeO_3{}^{2-}$.

Excretion of Se and ^{75}Se in urine. Fig. 3 shows the mean cumulative excretion of ^{75}Se in urine for both salts and carrier amounts, calculated as described on p. 488. Tables 2 and 3 give the total ^{75}Se and stable Se excreted in urine during the 6 h after dosing. The smallest loss occurred after 5 μg $^{75}SeO_3{}^{2-}$ was given. After 5 μg $^{75}SeO_4{}^{2-}$ 17% was excreted and approximately 23% was excreted after both 5000 μg doses. Urinary excretion of ^{75}Se continued for longer after both $^{75}SeO_4{}^{2-}$ doses and after the 5000 μg $^{75}SeO_3{}^{2-}$ dose. The

Table 1. *The mean half-lives (min) for the components of exponential clearance* (α, β and γ) *of* ^{75}Se *from the plasma of steers after injection of* ^{75}Se *as either* $Na_2{}^{75}SeO_3$ *or* $Na_2{}^{75}SeO_4$ *and containing either 5 or 5000 μg Se*

(Mean values with their standard errors)

Component	Selenite		Selenate	
	5 μg	5000 μg	5 μg	5000 μg
α	2·55±0·13	2·37±0·31	2·46±0·22	3·40
				1·14
β	15·93±1·95	13·65±1·54	36·63±0·98	16·76±1·00
γ		693		417·00±108·46
		371		

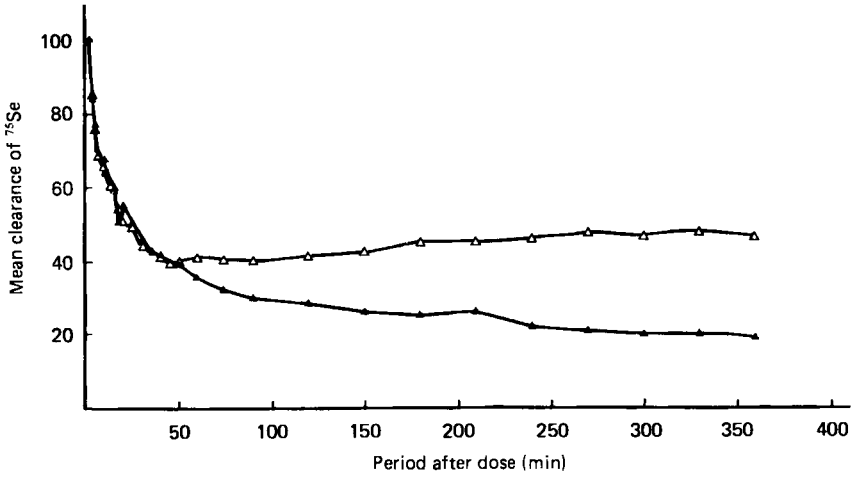

Fig. 2. The mean clearance of ^{75}Se from the plasma of two steers. ^{75}Se given as $Na^{75}SeO_4$ containing either 5 μg (△——△) or 5000 μg (▲——▲) carrier Se. Values are expressed as a percentage of the concentration (% dose ^{75}Se/l plasma) at 2 min.

principal loss of ^{75}Se after 5 μg $^{75}SeO_3{}^{2-}$ was in the first sample of urine passed. After injection of 5000 μg carrier Se, irrespective of the salt given, the percentage excreted in urine was similar to the percentage of radioactivity excreted, approximately 25% of the dose.

Excretion of Se and ^{75}Se *in bile.* When 5 μg carrier as either salt was given a similar percentage (0·2%) of the ^{75}Se was excreted in bile during the 6 h. After 5000 μg the percentage excreted increased to 1·94 and 0·86 for $^{75}SeO_3{}^{2-}$ and $^{75}SeO_4{}^{2-}$ respectively. ^{75}Se was detectable in the bile within 10 min of dosing (Fig. 4). The greatest increase in excretion rate occurred when 5000 μg Se as $SeO_3{}^{2-}$ was given.

It was not possible to calculate the absolute increase in stable Se excreted after dosing because of insufficient quantitative values for normal rates of loss before dosing. The values in parentheses in Tables 2 and 3 are for the ratio, concentration of Se in bile (and urine) after dosing: the concentration of Se immediately before dosing. An increase in concentration occurred only when 5000 μg Se as $SeO_3{}^{2-}$ was injected.

Changes in stable Se concentrations in plasma. The stable Se concentration in the plasma of four steers was determined on the predose plasma sample and on plasma samples taken at 2, 30 and 360 min after dosing. No detectable change was produced by 5 μg Se, the mean concentration at 0, 2, 30 and 360 min being 44, 49, 46 and 48 ng/ml respectively. After

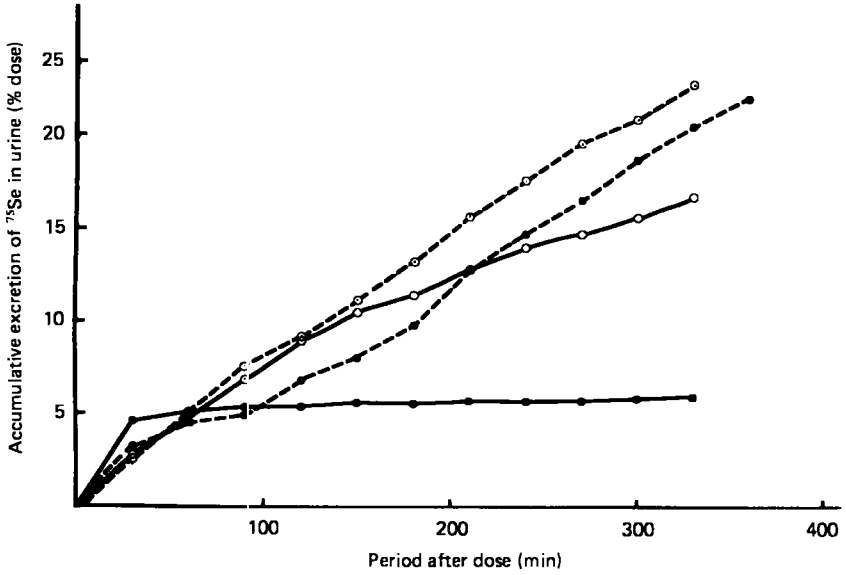

Fig. 3. Accumulative excretion of ^{75}Se in the urine during 6 h after the intravenous injection of $Na_2{}^{75}SeO_3$ containing either 5 μg (●——●) or 5000 μg (●---●) and $Na_2{}^{75}SeO_4$ containing 5 μg (○——○) or 5000 μg (⊙---⊙) carrier Se.

Table 2. *The amount of ^{75}Se (% dose) and stable Se (μg) excreted in bile and urine and the total volumes of bile and urine secreted (ml) during 6 h after the injection of $Na_2{}^{75}SeO_3$ containing either 5 or 5000 μg carrier Se*

(Values in parentheses are the ratio, mean Se concentration in the pooled sample for 6 h post dosing: Se concentration in the predose sample)

		Bile			Urine		
Steer	Carrier Se (μg)	Volume (ml)	^{75}Se (% dose)	Se (μg)	Volume (ml)	^{75}Se (% dose)	Se (μg)
A	5	1320	0·16	98 (1·0)	3070	5·9	65 (1·0)
B	1	2210	0·13	100 (1·9)	2030	5·4	203 (0·7)
C	5	4310	0·43	43 (0·9)	2310	7·4	104
D	5	2610	0·18	24 (1·0)	1700	5·4	54 (0·9)
Mean		2613	0·20	66	2277	6·0	106
A	5000	2840	2·70	221 (2·0)	2600	23·7	1012 (17·0)
B	5000	3220	1·82	197 (2·6)	2340	23·2	925 (20·8)
C	5000	5625	2·07	55 (1·0)	1095	10·3*	nd
D	5000	2370	1·15	64 (5·4)	1380	19·4	961 (13·1)
Mean		3514	1·94	127	1854	22·1	966

nd, not determined.

* 200 min collection only, values omitted from mean.

5000 μg Se the corresponding values for the mean concentrations for SeO_3^{2-} were 34, 175, 98 and 62 ng/ml and for SeO_4^{2-} 41, 153, 88 and 43 ng/ml. The mean plasma specific activity in the plasma samples taken 2 min after the 5 μg dose were 3·9 and 3·3 times greater than that present 2 min after the injection of 5000 μg SeO_3^{2-} and SeO_4^{2-} respectively.

Table 3. *The amount of ^{75}Se (% dose) and stable Se (μg) excreted in bile and urine and the total volumes of bile and urine secreted (ml) during 6 h after injection of $Na_2{}^{75}SeO_4$ containing either approximately 5 or 5000 μg carrier Se*

(Values in parentheses are the ratio, mean Se concentration in the pooled sample for 6 h post dosing: Se concentration in the predose sample)

		Bile			Urine		
Steer	Carrier Se (μg)	Volume (ml)	^{75}Se (% dose)	Se (μg)	Volume (ml)	^{75}Se (% dose)	Se (μg)
A1	7·4	3220	0·30	121 (1·1)	1550	13·2	62 (0·9)
A2	5·0	3240	0·16	nd	2270	17·8	nd
B	7·4	2988	0·12	61 (0·9)	1900	20·0	23 (1·0)
Mean		3149	0·19	91	1907	17·0	43
A1	5000	2550	0·80	111 (1·0)	1900	21·5	1040 (27·0)
A2	5000	3430	1·10	nd	2120	25·7	nd
B	5000	3870	0·67	160 (0·9)	2220	24·6	1077 (48·5)
Mean		3263	0·85	136	2080	23·9	1059

nd, not determined.

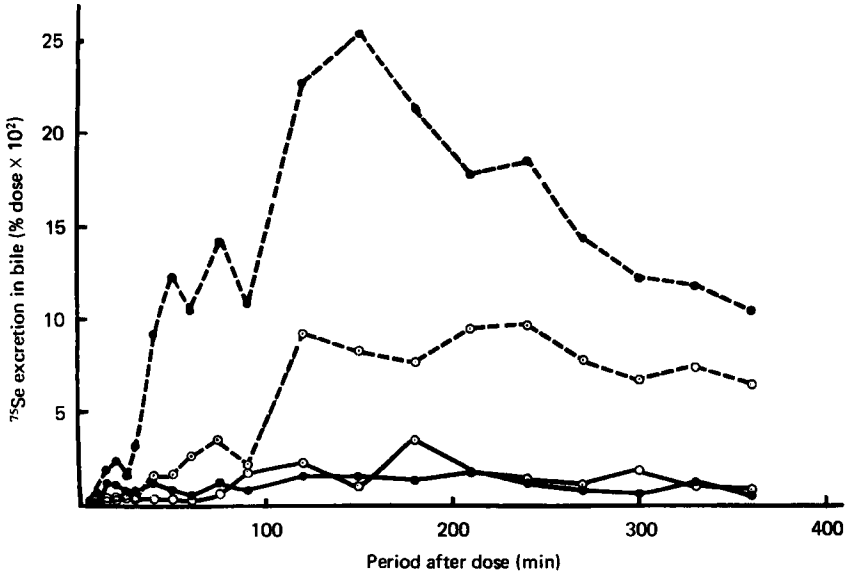

Fig. 4. Excretion of ^{75}Se in the bile (% dose) during 6 h after the intravenous injection of $Na_2{}^{75}SeO_3$ containing 5 μg (●——●) or 5000 μg (●---●) carrier Se and $Na_2{}^{75}SeO_4$ containing approximately 5 μg (○——○) or 5000 μg (⊙——⊙) carrier Se.

DISCUSSION

The results demonstrate that: (1) the biliary route is not important in the control of loss of Se from the body even when systemic plasma Se concentrations are elevated. The amounts excreted in bile represent on average the clearance of Se from less than 10 ml plasma/min at normal plasma Se concentrations; (2) the amount of carrier Se and the salt used affect the pattern of clearance of ^{75}Se from plasma; (3) the $SeO_3{}^{2-}$ form is more readily incorporated into body tissues at low carrier levels, a much greater percentage of injected

SeO_4^{2-} being excreted in urine; (4) at high carrier levels similar percentages of the two salts are excreted in urine.

The normal concentrations of Se in plasma were between 34 and 48 ng/ml. The 5 μg dose was therefore equivalent to the amount of Se contained in approximately 100 ml plasma (approximately one three-hundredth of the plasma volume) whereas the 5000 μg dose would represent that contained in 100 l plasma or approximately three to four times the plasma volume. The experiments using a dose of 5 μg Se therefore provided values more representative of the normal kinetics of Se homoeostasis. They indicated that the SeO_3^{2-} form was more readily incorporated into tissues, only 0·2% being excreted in bile and only 6% in urine. Selenate was less readily incorporated, there was a much greater and more prolonged loss in urine and the β component of plasma clearance had a much longer $t_{\frac{1}{2}}$ (36·67 min) after 5 μg was given. When 5000 μg carrier Se was used the clearance patterns from plasma and excretion in urine were similar for each salt; approximately 22 and 24% of the SeO_3^{2-} and SeO_4^{2-} doses appearing in urine during the 6 h observation period. The increased excretion in urine was prolonged beyond 6 h.

The rapid increase in plasma ^{75}Se radioactivity between 30 and 60 min after injection of 5 μg Se as $^{75}SeO_3^{2-}$ is due to the release of protein-bound ^{75}Se from the liver into the systemic circulation (Symonds *et al.* 1981). Approximately 40% of such low carrier doses of SeO_3^{2-} are removed by the liver. The absence of an increase in ^{75}Se in plasma after 30 min when 5000 μg was given was probably due to the liver removing a much smaller amount of the Se injected at a lower (approximately $\frac{1}{3}$) specific activity. These factors would result in the amount of ^{75}Se attached to the subsequently released protein being insufficient to cause any detectable increase in the declining systemic plasma radioactivity. Thus, the plasma clearance pattern when large amounts of carrier Se are given does not represent the physiological norm. In the rat, Imbach & Sternberg (1967) observed a double exponential clearance of intravenously-injected $^{75}SeO_3^{2-}$. The α component had a $t_{\frac{1}{2}}$ of 2·9 min, similar to that in the steers, while their β component ($t_{\frac{1}{2}}$ 19·5 h) was much longer than even the mean γ component for both salts (465 min) but similar to the component of clearance of the bound ^{75}Se appearing after 5 μg of Se as SeO_3^{2-} were given. Although there may be species variations in Se homoeostasis the differences observed are more likely to be due to the amount of carrier Se given. Imbach & Sternberg (1967) gave doses of 130 μg Se/kg body-weight which were approximately 13000 times greater on a body-weight basis than the 5 μg dose given to the steers. These observations emphasize the need to ensure that the amount of carrier used in radioisotope studies should be as small as possible, particularly when plasma concentrations of the element under study are normally low.

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