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UPTAKE OF Se^{75} IN TISSUES OF SHEEP AFTER ADMINISTRATION OF A SINGLE DOSE OF Se^{75}-SODIUM SELENITE, Se^{75}-SELENOMETHIONINE, OR Se^{75}-SELENOCYSTINE *)

By

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The distribution of toxic doses of selenium in sheep has been described by several authors (*Dudley* 1936; *Rosenfeld & Beath* 1945; *Kuttler et al.* 1961; *Glenn et al.* 1964). The results indicate that the highest concentration is present in liver, with relatively decreasing concentration in kidneys, lungs, spleen, and myocardium.

In sheep the distribution of subtoxic doses of inorganic selenium was studied by *Cousins & Cairney* (1961), *Kuttler et al.*, and *Wright & Bell* (1964). They found the highest concentration in kidneys, followed in most cases by liver, spleen, blood, and lungs.

Dietary selenium is believed to be largely in organic form, probably as selenium analogues of sulphur-containing amino acids (*Cousins & Cairney*). *Smith et al.* (1938) demonstrated that organic selenium is retained by the tissues to a greater extent than is the inorganic form, and that such retention is cumulative. On the other hand, they found that tissue levels are less increased by prolonged administration of inorganic selenium salts. Organic selenium has been shown to accumulate not only in liver and

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kidneys but also in pancreas, stomach wall, and intestinal wall (*Jones & Godwin* 1963; *Hansson* 1963; *Hansson & Blau* 1963; *Anghileri & Marqués* 1965).

In mammalian organisms selenium has been found to have an ability to apparent fixation or binding in various proteins (*Smith et al.; McConnell & van Loon* 1955; *Rosenfeld & Eppson* 1964).

The present work was undertaken to compare the distribution of selenium administered orally and parenterally in therapeutic and tracer doses as Se^{75}-sodium selenite, Se^{75}-selenomethionine, and Se^{75}-selenocystine.

MATERIAL AND METHODS

Se^{75}-sodium selenite and Se^{75}(L)-selenomethionine were obtained in aqueous solution from The Radiochemical Centre, Amersham, England. Non-radioactive sodium selenite was added to the Se^{75}-sodium-selenite solution in those cases in which the highest doses were given. Non-radioactive (DL)-selenomethionine from Calbiochem, Los Angeles, USA, was added to the Se^{75}(L)-selenomethionine.

Se^{75}(L)-selenocystine was obtained as powder from Farbwerke Hoechst AG, Germany. The powder was dissolved in 0.1 N-HCl.

The sheep were of Swedish "lantras" type. In order to produce autoradiographic pictures two lambs, females, 6 weeks old and weighing 9.4 and 12.9 kg were used. The other distribution studies comprised 32 sheep of both sexes, 7 months to 10 years old and with body-weights between 22 and 62 kg. In the account of Se^{75}-concentration in the tissues, whole blood, and plasma under "Results" the values obtained for each animal have been recalculated so as to correspond with a standard weight of 40 kg. During the experiments the animals received conventional fodder of hay, straw, grain, and wheat bran.

The selenium doses per kg calculated on the standard weight of 40 kg, routes of administration, type of selenium compound administered, and number of animals are shown in Tables 1 to 4. The total dose of selenium was equally high for all animals in each series, irrespective of body-weight. The radioactive dose of selenium varied between 30 μC and 540 μC of Se^{75} per animal.

After injection of the respective solutions heparinized blood samples were taken from the jugular veins at intervals as indicated in Figs. 1 and 2. At times as set out in Tables 1 and 3, the sheep were anaesthetized with pentobarbital sodium and bled to death. The weights of the organs in Tables 2 and 4 were determined. Approximately 1 or 2 g of the tissues were weighed out for measurement of the amount of radioactive selenium. The weight of skeletal muscle was calculated to be 29 per cent*) of the body-weight and the volume of

*) Information from S.G.S., the Stockholm-Gävle Slaughterhouse Association.

whole blood 80 ml per kg (*Dukes* 1947). The packed cell volume (PCV) of blood was estimated by the Department of Clinical Biochemistry, Royal Veterinary College, Stockholm, at 33.2 ± 2.4 ($\bar{x} \pm s$) on blood samples from 31 healthy animals. This value was used for calculation of the amount of radioactivity in the blood cells and in the plasma.

In the autoradiographic studies 0.1 mg of selenium per kg body-weight as Se^{75}-sodium selenite was injected subcutaneously in two lambs. The dose of radioactivity was 1.4 mC for one and 1.8 mC for the other lamb. The animals were killed as described in the foregoing 1 hour and 2 days, respectively, after the injections. Kidneys and adrenals were taken out immediately and frozen at —20°C. The autoradiographic procedure described by *Ullberg* (1954, 1958) was used. 20-μ and 80-μ sections through the organs were taken at —10°C. Autoradiographic exposure was made by apposition against Industrex X-ray film.

Liver, kidney, myocardium, intestine, and pancreas from some animals (Table 5) were homogenized in an equal volume of water. The proteins of the homogenized tissues and some plasma samples (Fig. 3) were precipitated with an equal volume of 20 % trichloracetic acid and then washed twice with 10 % trichloracetic acid and once with ethanol, ethanol-ether (1:1 v/v), and ether, respectively. The radioactivity in the trichloracetic-acid-soluble fraction and the protein fraction was determined.

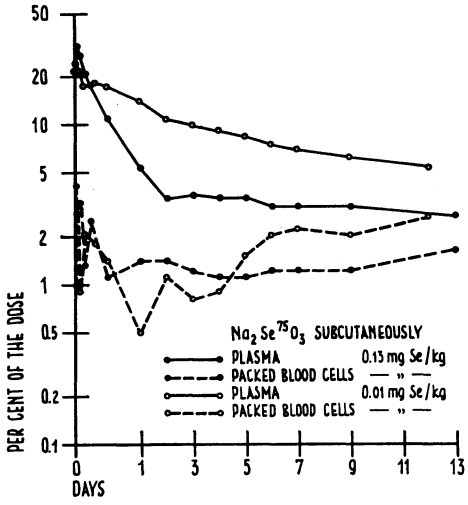
The radioactivity was measured with a well-type scintillation detector connected to a single-channel analyzer and a scaler or in an auto-gamma spectrometer. The measurements were made on specimens of about 1 or 2 g of tissues and 1.0 or 2.0 ml of plasma, whole blood, trichloracetic acid, and washing solutions.

Some retention data were analyzed statistically, using Student's t-test.

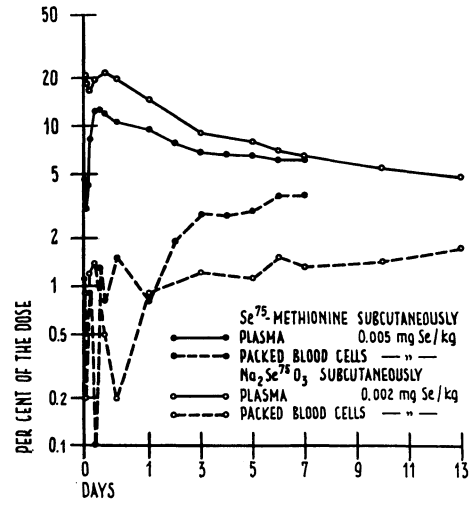
RESULTS

The Se^{75}-concentration in blood plasma rose very quickly after subcutaneous injection of Se^{75}-sodium selenite and Se^{75}-selenomethionine. The plasma contained the largest amount of the isotope within 1 hour of the injection of the two highest doses (Fig. 1 a). When the Se^{75}-selenomethionine and the lowest dose of Se^{75}-sodium selenite were injected subcutaneously, the amount of Se^{75} in the plasma decreased in the first hour and the first 2 hours, respectively. Thereafter it increased to a maximum 6 and 8 hours, respectively, after the injection (Fig. 1 b). During the following 48 hours after injection the amount of Se^{75} decreased quickly. During the rest of the observation period it decreased more slowly (Figs. 1 a and b).

The Se^{75}-content in the packed blood cells varied greatly during the first 24 hours after injection of Se^{75}-selenomethionine and



1 a.



1 b.

Figure 1 a. Se^{75}-amount in blood plasma and blood cells of sheep after subcutaneous injection of 0.01 or 0.13 mg Se/kg as Se^{75}-sodium selenite. The curves represent the mean values for 8 and 5 animals, respectively. The values correspond with a standard body-weight of 40 kg.

Figure 1 b. Se^{75}-amount in blood plasma and blood cells of sheep after subcutaneous injection of 0.002 mg Se/kg as Se^{75}-sodium selenite or 0.005 mg Se/kg as Se^{75}-methionine. Each curve represents the mean values for 2 animals. The values correspond with a standard body-weight of 40 kg.

Se^{75}-sodium selenite (Figs. 1 a and b). In contrast to that in the blood plasma, the radioactivity in the blood cells increased after the first day and throughout the observation period.

When Se^{75}-sodium selenite and Se^{75}-selenomethionine were injected intraruminally, the amount of Se^{75} in the plasma increased to a maximum after 36 hours. Then it decreased during the observation period (Figs. 2 a and b). The Se^{75}-content in the packed blood cells varied during 36 hours after the injection and then increased during the observation period, as will be seen from Figs. 2 a and b.

Tables 1 and 3 show the Se^{75}-concentration in various tissues after subcutaneous and intraruminal injection of Se^{75}-sodium selenite. High values were found in kidneys, liver, and adrenals and, after the lower selenium doses, in the testes as well. It will be seen from the tables and Figs. 4 and 5 that in the kidneys

Table 1. Se^{75}-concentration in tissues of sheep after injection of Se^{75}-sodium selenite subcutaneously. All values are recalculated to a standard body-weight of 40 kg.

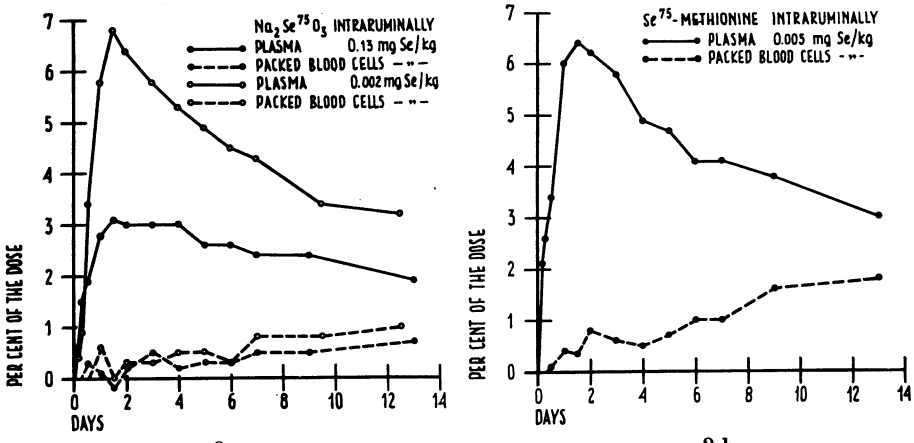
Results expressed in per cent of dose per gram wet tissue × 10^3

Dose, mg Se/kg	0.002	0.01	0.13					
Days after injection	13	13	2		8	13		17-22
Number of animals (sex)	2(1♀,1♂)	2(2♂)	5(3♀,2♂)		3(2♀,1♂)	5(3♀,2♂)		2(2♀)
	$\bar{x}$	$\bar{x}$	$\bar{x}$	s	$\bar{x}$	$\bar{x}$	s	$\bar{x}$
Kidney	51.3	38.0	11.0	1.9	13.1	7.9	0.3	10.0[1]
„ cortex	—	—	17.8	2.4	19.0	12.2	1.7	13.3
„ medulla	—	—	3.3	0.4	4.4	2.7	0.7	3.0
Liver	6.8	7.7	20.4	8.8	19.1	12.2	2.6	13.2
Pancreas	6.4	9.8	2.6	0.3	3.4	2.2	0.5	3.0
Parotis	6.6	6.1	2.6	0.7	1.4[1]	1.9	0.1	—
Abomasum wall	3.8	2.4	1.6	0.4	1.4	0.9	0.1	0.9[1]
Intestinal wall	4.4	4.2[1]	1.7	0.4	1.2[1]	1.2	0.2	—
Rumen wall	1.6	2.2	1.1	0.3	0.5[1]	0.5	0.1	—
Rumen content	0.02	0.04	0.3	0.07	0.03	0.002	—	—
Lung	4.6	5.1	3.7	0.7	3.3	1.7	0.2	2.0
Myocardium	4.4	5.4	2.1	0.6	1.8	1.4	0.2	1.9
Skeletal muscle	0.8	1.1	0.34	0.05	0.36	0.22	0.03	0.26
Spleen	7.3	7.8	2.6	0.7	2.9	2.0	0.4	2.6
Thymus	—	3.2[1]	1.4	0.2	1.6	1.2	0.2	1.3[1]
Mesenteric lymph node	9.8	9.2[1]	2.7	0.8	2.5	2.0	0.2	2.8[1]
Lymph node	8.8	8.4	1.9	0.5	2.3	1.9	0.2	2.7
Adrenal	13.0	13.2	2.9	0.5	3.2	2.4	0.6	3.0
Thyroid	10.0	7.0	4.0	2.4	2.4	1.7	0.2	2.2
Skin + wool	0.7[1]	2.2[1]	0.8	0.2	0.6[1]	0.6	0.1	—
Testis	23.7[1]	17.2	1.2[1]	—	—	2.0[1]	—	—
Epididymis	21.7[1]	6.4	0.6[1]	—	—	0.8[1]	—	—
Ovary	3.3[1]	—	2.0	—	2.5[1]	1.5	0.3	2.7[1]
Uterus	2.8[1]	—	1.6	—	—	1.0	0.0	—
Whole blood[2]	1.9	2.3	1.8	0.5	1.7	1.3	0.2	1.6
Plasma[2]	2.0	2.5	2.0	0.7	1.9	1.2	0.2	1.2

[1]) Only one sample examined.
[2]) Expressed per ml.

and adrenals Se^{75} was accumulated in the cortex. The Se^{75}-concentration in skeletal muscle was low, irrespective of the size of the Se^{75}-dose and the route of administration.

When Se^{75}-sodium selenite was injected subcutaneously, an approximately equally high percentage of the two lowest doses had been taken up after 13 days. The tissues absorbed a greater



2 a. 2 b.

Figure 2 a. Se^{75}-amount in blood plasma and blood cells of sheep after intraruminal injection of 0.002 or 0.13 mg Se/kg as Se^{75}-sodium selenite. The curves represent the mean values for 4 and 5 animals, respectively. The values correspond with a standard body-weight of 40 kg.

Figure 2 b. Se^{75}-amount in blood plasma and blood cells of sheep after intraruminal injection of 0.005 mg Se/kg as Se^{75}-methionine. Each curve represents the mean value for 2 animals. The values correspond with a standard body-weight of 40 kg.

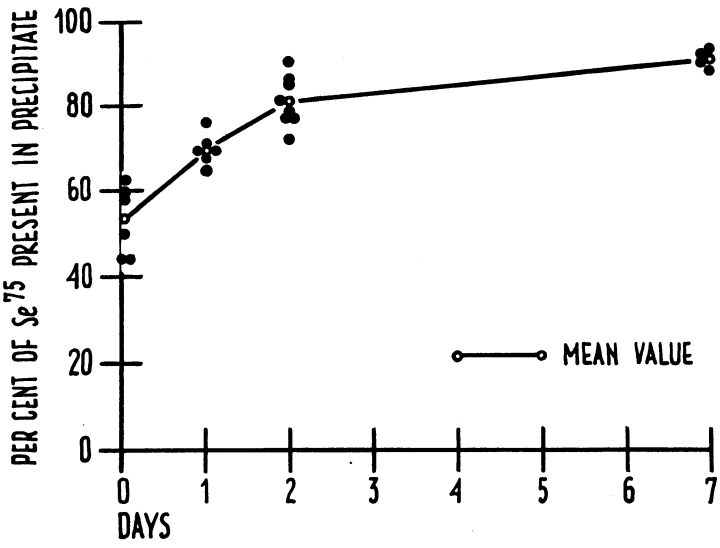


Figure 3. The binding of Se^{75} to protein in blood plasma of sheep after subcutaneous injection of 0.13 mg Se/kg as Se^{75}-sodium selenite. Precipitation was performed with 20 % TCA-solution.

Table 2. Amount of Se^{75} in some organs of sheep after injection of Se^{75}-sodium selenite subcutaneously.
Results expressed in per cent of dose per whole organ

Dose, mg Se/kg	0.002	0.01	0.13					
Days after injection	13	13	2		8	13		17–22
Number of animals (sex)	2(1♀,1♂)	2(2♂)	5(3♀,2♂)		3(2♀,1♂)	5(3♀,2♂)		2(2♀)
	$\bar{x}$	$\bar{x}$	$\bar{x}$	s	$\bar{x}$	$\bar{x}$	s	$\bar{x}$
Skeletal muscle	9.8	12.9	3.7	0.7	3.6	2.6	0.4	2.9
Whole blood	6.2	7.4	5.4	1.7	4.7	3.8	0.2	4.5
Liver	3.8	4.0	12.2	5.3	8.8	6.9	1.7	6.0
Kidneys	4.2	4.5	1.0	0.2	1.0	0.8	0.1	1.2
Lungs	1.6	2.2	1.2	0.4	1.1	0.6	0.1	0.8
Myocardium	0.8	0.9	0.3	0.05	0.2	0.2	0.05	0.3
Spleen	0.4	0.4	0.2	0.05	0.1	0.1	0.05	0.1
Pancreas	0.4	0.4	0.1	0.05	0.15	0.1	0.05	0.1
Total	27.2	32.8	24.1	4.4	19.6	15.1	2.1	15.9

proportion of the two doses per g than of the highest dose, with the exception of the liver (Tables 1 and 2). The values for the liver showed a tendency to rise with increasing doses. The differences were not significant, however.

The Se^{75}-concentration in most tissues decreased only slightly during the period of observation after subcutaneous injection of 0.13 mg of selenium per kg as Se^{75}-sodium selenite (Table 1). The mean value for the liver at 2 and 8 days was higher than at 13 and 17—22 days, but the differences were not significant. Renal cortex, muscle, and lungs showed significantly ($P < 0.01$, < 0.05, and < 0.01, respectively) higher Se^{75}-values at 2—8 days than at 13—22 days. In the rumen wall the Se^{75}-concentration was higher on the second day after the injection than it was later. The eight organs listed in Table 2 also contained a significantly ($P < 0.01$) higher percentage of the Se^{75}-dose at 2 days than at 13 days.

When Se^{75}-sodium selenite was injected intraruminally in a dose of 0.002 mg of selenium per kg, the Se^{75}-content in the tissues was lower than after subcutaneous injection of the same dose (Tables 1—4). The difference was smaller after the highest dose. The liver, lung, and blood, on the other hand, contained significantly ($P < 0.01$, < 0.05, and < 0.01, respectively) greater amounts of Se^{75} after the subcutaneous than after the intraruminal injection of this dose, too. The Se^{75}-contents in the

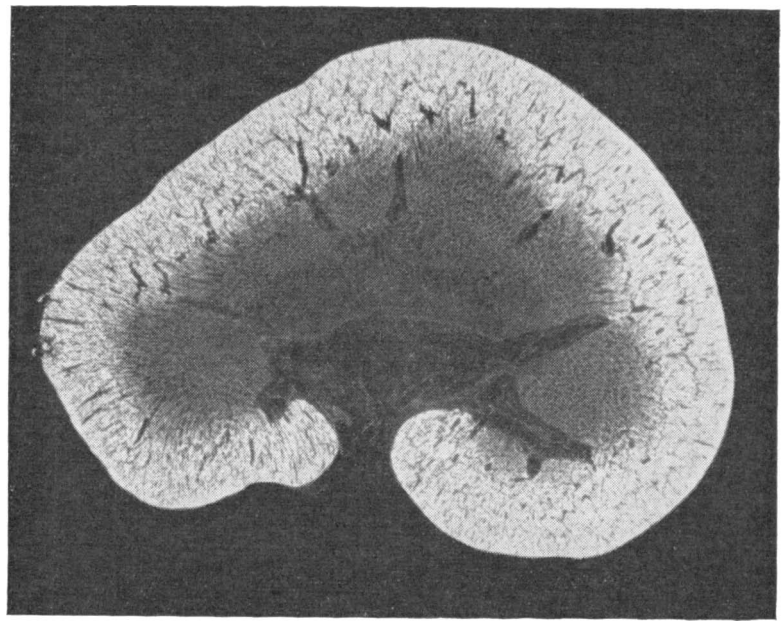


Figure 4. Autoradiogram showing the distribution of Se^{75} in the kidney of a lamb 2 days after subcutaneous injection of Se^{75}-sodium selenite. White areas correspond to high levels of radioactivity. Note high Se^{75}-concentration in the cortex. × 2

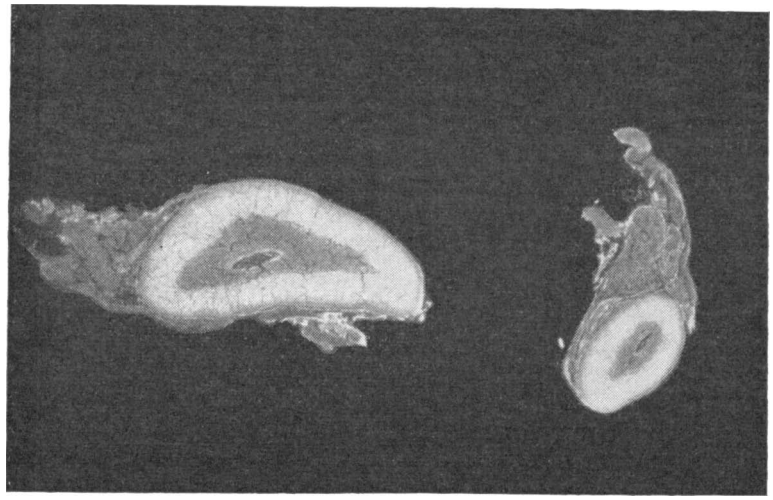


Figure 5. Autoradiogram showing the distribution of Se^{75} in a sagittal and a transverse section through the adrenals of a lamb 1 hour after subcutaneous injection of Se^{75}-sodium selenite. White areas correspond to high levels of radioactivity. Note high Se^{75}-concentration in the cortex. × 2

Table 3. Se^{75}-concentration in tissues of sheep after injection of Se^{75}-sodium selenite intraruminally (i. r.), Se^{75}-selenomethionine subcutaneously (s. c.), and Se^{75}-selenocystine intravenously (i. v.). Results expressed in per cent of dose per gram wet tissue $\times 10^3$

Se^{75}-compound and route of administration	Se^{75}-sodium selenite (i. r.)			Se^{75}-methionine (s. c.)		Se^{75}-cystine (i. v.)
Dose, mg Se/kg	0.002	0.13		0.005		1.4
Days after injection	13	13		2-6	13-24	13-18
Number of animals (sex)	2(1 ♀,1 ♂)	5(4 ♀,1 ♂)		2(2 ♀)	2(1 ♀,1 ♂)	2(1 ♀,1 ♂)
	$\bar{x}$	$\bar{x}$	s	$\bar{x}$	$\bar{x}$	$\bar{x}$
Kidney	26.1	7.2	1.9	32.6	43.1	50.5
„ cortex	—	11.5	3.0	57.0	69.8	76.1
„ medulla	—	2.1	0.6	8.6	8.8	22.1
Liver	3.2	3.1	0.4	9.2	5.4	8.5
Pancreas	4.0	2.3	0.6	27.8	16.5	18.4
Parotis	3.6	1.9	0.6	7.7[1])	6.0[1])	8.3
Abomasum wall	1.2	1.0	0.1	4.8	2.9[1])	4.4
Intestinal wall	2.0	1.2	0.2	8.6[1])	4.8[1])	—
Rumen wall	1.0	0.6	0.2	4.2[1])	2.2[1])	3.0[1])
Rumen content	0.03[1])	0.03	0.03	—	0.01[1])	—
Lung	2.0	1.3	0.2	4.4	4.3	7.6
Myocardium	1.4	1.1	0.4	3.7	3.1	4.1
Skeletal muscle	0.2	0.23	0.06	0.8	1.4	1.1
Spleen	2.4	1.6	0.5	6.2	8.1	9.3
Thymus	2.9[1])	1.2	0.3	5.0	6.2	10.2[1])
Mesenteric lymph node	7.6	1.9	0.4	9.5[1])	8.0[1])	10.8[1])
Lymph node	4.1	1.6	0.4	7.2	6.6	6.3
Adrenal	6.6	2.0	0.5	16.2	16.4	17.6
Thyroid	3.1	1.5	0.4	8.0	10.9	7.1
Skin + wool	1.0	0.6	0.07	2.3[1])	3.2	10.0[1])
Testis	6.6[1])	1.1[1])	—	—	21.8[1])	—
Epididymis	1.2[1])	0.5[1])	—	—	11.1[1])	—
Ovary	3.7[1])	1.3	0.1	6.2[1])	—	5.5[1])
Uterus	2.5[1])	1.0	0.2	5.2[1])	—	3.3[1])
Whole blood[2])	1.3	0.7	0.3	2.8	2.8	3.2
Plasma[2])	1.4	0.8	0.4	3.4	2.0	3.2

[1]) Only one tissue sample examined.
[2]) Expressed per ml.

eight organs listed in Tables 2 and 4 were also significantly ($P < 0.001$) higher after subcutaneous than after intraruminal injection.

The Se^{75}-concentrations in the tissues after injection of Se^{75}-selenomethionine and Se^{75}-selenocystine subcutaneously and in-

Table 4. Amount of Se^{75} in some organs of sheep after injection of Se^{75}-sodium selenite intraruminally (i. r.), Se^{75}-selenomethionine subcutaneously (s. c.), and Se^{75}-selenocystine intravenously (i. v.). Results expressed in per cent of dose per whole organ

Se^{75}-compound and route of administration	Se^{75} sodium selenite (i. r.)			Se^{75}-methionine (s. c.)		Se^{75}-cystine (i. v.)
Dose, mg Se/kg	0.002	0.13		0.005		1.4
Days after injection	13	13		2-6	13-24	13-18
Number of animals (sex)	2(1 ♀,1 ♂)	5(4 ♀,1 ♂)		2(2 ♀)	2(1 ♀,1 ♂)	2(1 ♀,1 ♂)
	$\bar{x}$	$\bar{x}$	s	$\bar{x}$	$\bar{x}$	$\bar{x}$
Skeletal muscle	2.8	2.7	0.7	8.7	15.4	11.4
Whole blood	4.1	2.4	0.8	7.6	8.8	9.0
Liver	1.8	1.7	0.5	4.8	3.7	4.4
Kidneys	1.8	0.7	0.2	2.8	4.7	4.5
Lungs	0.8	0.5	0.2	1.5	1.4	2.9
Myocardium	0.2	0.2	0.1	0.6	0.5	0.7
Spleen	0.2	0.1	0.05	0.3	0.4	0.5
Pancreas	0.2	0.2	0.05	1.5	0.8	1.0
Total	11.9	8.5	1.8	27.8	35.7	34.4

Table 5. Protein-bound Se^{75} in homogenized tissues of sheep 2 and 13 days after subcutaneous injection of 0.13 mg Se/kg as Se^{75}-sodium selenite. Proteins were precipitated with TCA-solution.

Days after injection	2				13			
	Analyzed number		% of Se^{75} in precipitate		Analyzed number		% of Se^{75} in precipitate	
	animals	samples	$\bar{x}$	range	animals	samples	$\bar{x}$	range
Intestinal wall	3	6	76	73—81	3	6	82	75—90
Liver	3	6	73	68—77	3	6	80	76—83
Kidney	3	8	83	77—87	3	8	94	91—97
Pancreas	2	4	77	74—79	3	6	80	68—86
Myocardium	3	6	71	66—73	3	8	83	78—89

travenously, respectively, are shown in Table 3. The accumulation of Se^{75} was high in kidneys, pancreas, and adrenals after injection of the two selenium compounds. Pancreas and liver showed higher Se^{75}-concentrations at 2—6 days than at 13—24 days. In the other organs the time factor had little influence on the tissue concentration of Se^{75} (Tables 3 and 4). The uptake of Se^{75} in pancreas was higher after injection of Se^{75}-selenomethionine and Se^{75}-selenocystine than after injection of Se^{75}-sodium selenite.

Precipitation of the protein in blood plasma showed that 1 hour after the injection an average of 53 per cent of its Se^{75} was precipitated with the protein. This proportion increased to an average of 90 per cent after 7 days (Fig. 3). Most of the radioactivity of the homogenized tissues was also present in the protein fraction (Table 5). This percentage increased only slightly between the 2nd and the 13th day.

DISCUSSION

The results showed that the percentage of the Se^{75}-dose retained per g of tissue was higher when the sheep received small selenium doses as Se^{75}-sodium selenite than when they received large doses. The tissues also showed much higher retention of a subcutaneous than of an intraruminal dose of Se^{75}, when selenite was administered in a tracer dose, whereas a small difference was noted when it was given in a therapeutic dose. There is, thus, much evidence, as has been shown by *Cousins & Cairney* (1961), that in sheep the storage of inorganic selenium is limited. There is also evidence indicating that *Lindberg & Lannek*'s (1965) conclusion as regards the retention of a selenium dose in pigs is relevant for sheep as well, namely that a smaller proportion of the administered selenium dose will be retained if the selenium concentration of the basal diet has already filled the physiological stores of the treated animal. In contrast with that of other tissues, however, the percentage retention of the liver was relatively constant, irrespective of the size of the selenium dose administered by the respective route. This suggests a metabolic difference between the liver and the rest of the body tissues as far as selenium is concerned, as has earlier been demonstrated for rats by *Hopkins et al.* (1966).

The Se^{75}-concentration in most tissues decreased only slightly from the 2nd to the 13th day after a subcutaneous injection of Se^{75}-sodium selenite. *Lindberg & Tanhuanpää* (1965) reported similar observations in pigs after intramuscular injection of sodium selenite. An earlier investigation in sheep (*Jacobsson* 1966) also showed that more than 50 per cent of a corresponding dose was excreted in the first 48 hours, as against only 13 per cent over the next 11 days. From the data in Table 1 the difference between the amounts retained at 2 days and at 13 days can be estimated at 0.42 p.p.m. in liver and 0.006 p.p.m. in skeletal muscle. This suggests that the meat from a sheep slaughtered a

few days after treatment with a single dose of selenite may be used as human food. *Lindberg & Lannek* reported that this also holds true after oral administration of selenite to pigs.

The selenium in various tissues is to a very great extent protein-bound (*Smith et al.* 1938; *McConnell & van Loon* 1955; *Rosenfeld & Eppson* 1964). *Schwarz & Sweeney* (1964) showed that when physiological amounts of selenium were added as Se^{75}-selenite to liver homogenates, 75 per cent was bound non-enzymatically to TCA-precipitable material. They also demonstrated that effective binding appeared in the area of S-S but not in that of the SH form, when reaction of selenite with amino acids was studied. The selenium reaction products showed RF values only faintly different from those for the S-S compounds. The investigation presented here also showed that, on an average, 71—83 per cent of the tissue Se^{75} were protein-bound after no more than 48 hours. This extensive, relatively firm, binding can possibly explain the slight decrease of the Se^{75}-concentration during the observation period. A similar view relating to pigs has been expressed by *Lindberg & Tanhuanpää.*

The high radioactivity in plasma shortly after a subcutaneous injection of the Se^{75}-solutions suggests that these are rapidly absorbed. The cause of the temporary decrease in the Se^{75}-content of the blood plasma which occurs in the first few hours after injection of a low selenium dose may be that the selenium is bound to the cells or enters into them, as maintained by *Nelp & Blumberg* (1965). The subsequent increase of the plasma-Se^{75} content has been explained by a release from the liver of absorbed selenium into the circulating blood in protein-bound form (*Oldendorf & Kitano* 1963; *Nelp & Blumberg*).

The Se^{75}-content in the blood cells is calculated by means of the normal value for the packed cell volume (PCV), the blood volume, and the measured values for the Se^{75}-concentration in blood plasma and whole blood. As the PCV and blood volume in each animal were not known at the time of sampling, the reported values represent only estimations of the Se^{75}-content in the blood cells. The obtained results indicate, however, that the selenium can enter the blood cells and then leave them again during the first 24 or 48 hours after subcutaneous as well as after intraruminal administration of selenium as selenite and selenomethionine. This is in accordance with the observations in sheep reported by *Wright* (1965). He found that the red cells rapidly

accumulated a limited proportion of an intravenous dose of radioactive selenium. In his experiments the uptake of Se^{75} by the intact erythrocytes reached a peak within 3 hours and did not rise above this level during the next 12 hours. According to *Wright,* the selenium was incorporated into the globin of the haemoglobin and remained in the red cells throughout their lifespan. *McConnell & Cooper* (1950) showed that in dogs selenium was also incorporated into haemin. In the present study the Se^{75}-content of the blood cells rose, whereas that of the plasma fell during the observation period. This also suggests that incorporation of selenium takes place.

The results of this investigation show that the kidney and liver had high concentrations of Se^{75}. This verifies earlier observations in sheep (*Cousins & Cairney*; *Kuttler et al.* 1961; *Wright & Bell* 1964). *Rosenfeld* (1964) showed that testicular tissue and adrenals also had relatively high Se^{75}-concentrations after administration of tracer doses of Se^{75}-selenite in rats. The same result was obtained for testicular tissue of the sheep in the present experiments with low doses of selenium both as Se^{75}-sodium selenite and as Se^{75}-selenomethionine. In earlier studies with mice (*Jacobsson & Hansson* 1965; *Hansson & Jacobsson* 1966) as well as in the present investigation it was found that the Se^{75}-activity was concentrated mainly in the cortex of the adrenals. The significance of an accumulation of selenium in this endocrinically important tissue is difficult to interpret. The uptake of Se^{75} in heart muscle was much higher than that in skeletal muscle, especially after injection of Se^{75}-sodium selenite. This accumulation of selenium in the heart has been described in, for instance, sheep, pigs, and mice (*Rosenfeld & Beath* 1945; *Ekman et al.* 1963; *Jacobsson & Hansson*). The toxic damage to the myocardium in selenium poisoning (*Rosenfeld & Beath* 1946; *Glenn et al.* 1964) would probably be explained by the relatively high selenium concentration at this site. *Rosenfeld & Beath,* in toxicity studies, found a correlation between the tissue damage in the organs and the selenium concentration.

In the present study, the Se^{75}-concentration in the blood cells was slightly higher after injection of Se^{75}-selenomethionine than after injection of Se^{75}-sodium selenite. This, as well as the relatively high radioactivity demonstrated in the bone-marrow of mice after injections of Se^{75}-selenomethionine (*Hansson & Jacobsson*), suggests a rapid incorporation of the organic selenium

compounds into the blood cells. The distribution of Se^{75}-selenomethionine and Se^{75}-selenocystine differed from that of Se^{75}-sodium selenite in a tracer dose, notably in that injection of the first-named compounds resulted in a higher Se^{75}-level in the pancreas. A high uptake of S^{35} and Se^{75} in the pancreas has been demonstrated in rats, cats, and mice after injection of S^{35}-methionine, S^{35}-cystine, Se^{75}-selenomethionine, and Se^{75}-selenocystine (*Hansson* 1959; *Hansson & Blau* 1963; *Anghileri & Marqués* 1965; *Hansson & Jacobsson*). That, in other respects, the distribution of selenite, selenomethionine, and selenocystine follows a relatively similar pattern may possibly be attributable to the tendency of selenium to be protein-bound.

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SUMMARY

A total of 34 sheep of both sexes were used in the experiments. Se^{75}-selenomethionine in a tracer dose and Se^{75}-sodium selenite at three dose-levels (from a tracer dose to a therapeutic dose) were injected by the subcutaneous route. Se^{75}-selenocystine in a dose that may be regarded as higher than a therapeutic dose of selenium was given intravenously. Se^{75}-sodium selenite in a tracer dose and a therapeutic dose was injected intraruminally.

High levels of Se^{75} were noted mainly in renal cortex, liver, and adrenal cortex after injection of Se^{75}-sodium selenite, whereas the values for skeletal muscle were low. Injections of Se^{75}-selenomethionine and Se^{75}-selenocystine resulted in higher Se^{75}-levels in pancreas than did tracer doses of Se^{75}-sodium selenite.

The tissues retained a greater percentage of a tracer dose than of a therapeutic dose of Se^{75}-labelled sodium selenite, with the exception of the liver, in which the percentage retention was relatively constant, irrespective of the dose level. The amount of Se^{75} recovered in the tissues was higher after subcutaneous than after intraruminal injection of a small selenium dose. The Se^{75}-levels differed very little or not at all when a therapeutic dose was injected by these two routes. These results suggest that in sheep the storage of inorganic selenium is limited.

In most tissues the difference between the Se^{75}-concentration at 2 days and that at 13 days was small after subcutaneous injection of a therapeutic dose of selenium. The Se^{75}-content in renal cortex, muscle, and lung was higher, however, at 2—8 days than at 13—22 days. It was also higher in the rumen wall at 2 days than later.

When Se^{75}-sodium selenite was injected subcutaneously, most of the radioactivity in the tissues was present in the protein precipitated with trichloracetic acid, the recoveries in the precipitate being 71—83 per cent at 2 days and 78—94 per cent at 13 days. The proportion of plasma-Se^{75} bound with the proteins averaged 53 per cent at 1 hour and 90 per cent at 7 days.

ZUSAMMENFASSUNG

Aufnahme von Se^{75} in Gewebe beim Schaf nach der Zufuhr einer einfachen Dosis von Se^{75}-Natriumselenit, Se^{75}-Selenmethionin oder von Se^{75}-Selencystin.

In diesen Versuchen wurden insgesamt vierunddreissig Schafe beiden Geschlechts angewandt. Subkutan wurde eine Spurdosis von Se^{75}-Selenmethionin und drei verschiedene Dosen (von der Spurdosis bis zur therapeutischen Dosis) von Se^{75}-Natriumselenit injiziert. Intravenös wurde Se^{75}-Selencystin in einer Dosis injiziert, die vermutlich eine therapeutische Selendosis übersteigen dürfte. Intraruminal wurde Se^{75}-Natriumselenit in Spurdosis und in therapeutischer Dosis injiziert.

Eine hohe Se^{75}-Konzentration wurde vor allem in der Nierenrinde, in der Leber und in der Nebennierenrinde nach Injektion von Se^{75}-Natriumselenit erzielt. In der Skelettmuskulatur wurden dagegen niedrige Werte gemessen. Die Injektion von Se^{75}-Selenmethionin und von Se^{75}-Selencystin gab eine höhere Se^{75}-Konzentration im Pankreas als Spurdosen von Se^{75}-Natriumselenit.

Die Gewebe nahmen einen grösseren Teil einer Spurdosis als einer therapeutischen Dosis Se^{75}-gemerkten Natriumselenits auf. Eine Ausnahme bildete die Leber, die einen relativ konstanten Teil der Dosis, abgesehen von deren Grösse, aufnahm. Die Se^{75}-Konzentration war in den Geweben bei subkutaner Injektion höher als bei intraruminaler Injektion einer kleinen Selendosis. Der Unterschied war gering oder ein solcher fehlte, wenn eine therapeutische Dosis verabfolgt wurde. Diese Verhältnisse scheinen auf ein begrenztes Retentionsvermögen anorganischen Selens beim Schaf hinzudeuten.

Der Unterschied zwischen den Se^{75}-Konzentrationen der meisten Gewebe war nach subkutaner Injektion einer therapeutischen Selendosis nach zwei und dreizehn Tagen gering. Die Se^{75}-Konzentration in der Nierenrinde, in der Muskulatur und Lunge war jedoch höher nach 2—8 Tagen als nach 13—22 Tagen. Dieselbe war auch in der Pansenwand nach 2 Tagen höher als später.

Bei subkutaner Injektion von Se^{75}-Natriumselenit fanden sich durchschnittlich nach 2 Tagen 71—83 % und nach 13 Tagen 80—94 % des Se^{75} der Gewebe in dem mit Trichloressigsäure ausgefällten Eiweiss. Vom Se^{75} des Plasmas waren durchschnittlich nach 1 Stunde 53 % und nach 7 Tagen 90 % von den Proteinen gebunden worden.

SAMMANFATTNING

Upptaget av Se^{75} i vävnader hos får efter tillförsel av en enstaka dos av Se^{75}-natriumselenit, Se^{75}-selenmetionin eller Se^{75}-selencystin.

Sammanlagt användes trettiofyra får av båda könen i försöken. Subkutant injicerades en spårdos av Se^{75}-selenmetionin och tre olika doser (från spårdos till terapeutisk dos) av Se^{75}-natriumselenit. Intravenöst injicerades Se^{75}-selencystin i en dos, som får anses ligga över en terapeutisk selendos. Intraruminalt injicerades Se^{75}-natriumselenit i spårdos och terapeutisk dos.

Hög Se^{75}-koncentration erhölls i framför allt njurbark, lever och binjurebark efter injektion av Se^{75}-natriumselenit. I skelettmuskulaturen uppmättes däremot låga värden. Injektion av Se^{75}-selenmetionin och Se^{75}-selencystin gav högre Se^{75}-koncentration i pankreas än spårdoser av Se^{75}-natriumselenit.

Vävnaderna tog upp en större del av en spårdos än av en terapeutisk dos Se^{75}-märkt natriumselenit. Ett undantag var levern, som tog upp en relativt konstant del av dosen oavsett dess storlek. Se^{75}-koncentrationen var högre i vävnaderna vid subkutan än vid intraruminal injektion av en liten selendos. Skillnaden var liten eller ingen om en terapeutisk dos tillfördes. Dessa förhållanden får anses tyda på en begränsad retentionsförmåga av oorganiskt selen hos får.

Skillnaden mellan de flesta vävnadernas Se^{75}-koncentration vid två och tretton dygn var liten efter subkutan injektion av en terapeutisk selendos. Se^{75}-koncentrationen i njurbark, muskulatur och lunga var emellertid högre efter 2—8 dygn än efter 13—22 dygn. Den var även högre i våmväggen efter 2 dygn än senare.

När Se^{75}-natriumselenit injicerades subkutant fanns i medeltal efter 2 dygn 71—83 % och efter 13 dygn 80—94 % av vävnadernas Se^{75} i den med triklorättiksyra utfällda äggvitan. Av plasmans Se^{75} bands i medeltal efter 1 timme 53 % och efter 7 dygn 90 % till proteinerna.

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