Retention of Selenium by Growing Lambs

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The retention of selenium by lambs deficient in vitamin E and selenium or deficient in selenium was estimated using labeled selenate. No differences were observed in the rate of excretion of selenium by the lambs, although there was a tendency for lambs receiving vitamin E to excrete more selenium during the first few days after administration. The liver of lambs given radioactive selenate retained more selenium than any other tissue studied. Selenium-75 in the tissues decreased with time, although red blood cells and wool increased during the first 4 to 8 weeks after ingestion of selenate and then decreased. Leg muscle retained only small amounts of selenium, and after 30 weeks less than 2% of the total selenium originated from the therapeutic dose. When lambs were fed increasing levels of selenium and/or vitamin E, dietary selenium significantly increased the total selenium in muscle and liver. Vitamin E significantly decreased the selenium content of the lamb muscle when 1.0 p.p.m. of selenium was present in the diet.

Selenium is effective in prevention and treatment of nutritional muscular dystrophy (NMD) in lambs in many areas where the selenium content of the forage is low (Hartley, 1961; Hopkins *et al.*, 1964; Muth *et al.*, 1958). However, like some other nutrients, selenium is toxic if ingested in excessive amounts (Rosenfeld and Beath, 1964). In view of the toxic properties of selenium, its use as a therapeutic agent must be limited until information is available on its distribution and retention. In addition to the therapeutic use of selenium, it is effective in preventing NMD when small amounts are added to the diet of sheep (Muth *et al.*, 1958). The effect of such additions on the level of selenium in the tissues is also of interest.

The present report deals with four experiments designed to provide data pertinent to these questions. In experiment I, labeled selenate was administered orally to lambs, the excreta were collected, and the rate of excretion and the distribution of the labeled dose were determined. Other animals were slaughtered in experiment II after ingestion of labeled selenate, and the amount of radioactivity was measured in a number of tissues. In experiments III and IV, graded levels of selenium were fed to lambs in the presence and absence of vitamin E, and the amount of total selenium in the tissue was determined.

METHODS

Experiment I. Twelve lambs were weaned from their dams at 2 days of age and distributed at random to one of three groups. Two lambs from each group were placed in square (72 cm.) elevated, galvanized pens which allowed collection of urine and feces. All animals were fed an artificial milk diet which was low in vitamin E and selenium and was composed primarily of Torula yeast, stripped lard, and glucose (Table I). The artificial milk, which contained 20% solids, was prepared by adding the fat-soluble vitamins to the dry diet (1500 I.U. of vitamin A and 150 I.U. of vitamin D per 100 grams of dry diet), and homogenizing in a Waring Blendor with warm distilled water. The diet was fed from bottles four to five times daily. Dry diet in the form of meal or pellets (Table I) was provided continuously after the first 5 days.

In this study, four lambs were each given one capsule of sodium selenate labeled with selenium-75 3 weeks after the start of the experiment and supplemented with 100 mg. of vitamin E per week; four other lambs were each given a capsule of labeled selenate at 3 weeks; and two lambs were allowed to develop clinical signs of NMD before administration of one capsule per lamb of the selenium-75–labeled selenate.

After the capsules were given, an aliquot of the daily urinary excretion was taken, and the radioactivity was determined by measurement in an Armac whole body counter. Feces were collected and stored under refrigeration until the radioactivity was determined. The total selenium content of the urine and feces from the administered dose was calculated.

Table I. Composition of Diets

Component	Expt. I		Expts. II and III	
	Milk	Pellets	Milk	Pellets
Torula yeast[a]	60.0	50.0	60.0	40.0
Glucose monohydrate	21.4	21.2	10.7	35.7
Sulka floc[b]	...	15.0	...	15.0
Stripped lard[c]	15.0	10.0	25.0	5.0
Mineral premix[d]	3.5	3.5	...	...
Mineral premix B	...	...	3.5	3.5
Vitamin premix A[e]	0.2	0.2	...	...
Vitamin premix B	...	...	0.2	0.2
Choline chloride	0.1	0.1	0.1	0.1
dl-Methionine	...	...	0.5	0.5

[a] State Lakes Yeast Corp., Rhinelander, Wis. [b] Brown Co., Berlin, N. H. [c] Distillation Products Industries, Rochester, N. Y. [d] Mineral premix A and B in per cent, respectively: $CaCO_3$, 62.5, 71.0; NaCl, 28.0, 28.5; $FeSO_4 \cdot 7H_2O$, 2.7, 0.0; KIO_3, 0.15, 0.05; $MnSO_4 \cdot H_2O$, 0.08, 0.0; $CoCl_2 \cdot 6H_2O$, 0.02, 0.12; $CuSO_4$, 0.15, 0.0; $NaMoO_4 \cdot 2H_2O$, 0.0, 0.36. [e] Vitamin premix A and B in per cent, respectively: inositol, 10.0, 10.0; calcium pantothenate, 2.0, 0.6; menadione, 0.40, 0.40; riboflavin, 0.3, 0.0; pyridoxine, 0.25, 0.0; folic acid, 0.02, 0.0; biotin, 0.01, 0.0; B_{12} (0.1% trituration), 1.00, 1.0; sucrose, 86.0, 88.0.

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Sodium selenate was prepared from ^{75}Se selenite by the method of Gilbertson and King (1950). Aliquots containing 3.35 mg. of selenium as selenate (86 μc.) were absorbed on aluminum oxide in gelatin capsules and administered at the appropriate time.

Experiment II. Twenty-six cross-bred lambs between 17 and 30 days of age were given a single oral capsule containing 3.35 mg. of selenium as sodium selenate and 86 μc. of selenium-75. After administration of the capsules, the lambs were confined in dry lot with their dams. The lambs were creep-fed a mixture of 5 parts of ground corn, 3 parts of ground oats, 1 part of linseed meal, and 1 part of wheat bran and were weaned when they were 10 weeks old. After weaning, the lambs were fed a ration of mixed hay (0.14 p.p.m. of selenium) and grain (0.25 p.p.m. of selenium).

The lambs were sacrificed by exsanguination at 1, 2, 4, 8, 12, 21, and 30 weeks after the administration of the radioactive selenate, and 26 tissues were freed of extraneous material, rinsed in water, and weighed. Blood was collected with sodium citrate as an anticoagulant and separated into plasma and red blood cells. Trichloroacetic acid (TCA) was added to the plasma, and the radioactivity of the precipitate and the supernate was measured.

The radioactivity of all samples was determined in an Armac whole body liquid scintillation counter, and was expressed as micrograms of selenium per 100 grams of tissue.

Experiments III and IV. In experiment III, 48 two-day-old lambs were fed the basal diet shown in Table I supplemented with 0.0, 0.1, and 1.0 p.p.m. of selenium as selenite and/or 0.0, 2.2, 5.5, and 11.0 mg. of *dl*-α-tocopherol per kg. of body weight per week in a factorial design. In experiment IV, 16 lambs were fed the basal diet supplemented with 0.0 or 1.0 p.p.m. of selenium as selenite and 0.0 or 22.0 mg. of *dl*-α-tocopherol per kg. of body weight in a factorial design. The lambs were fed the diet as an artificial milk containing 20% solids as in experiment I. Tocopherol was administered orally as a drench in olive oil.

Samples of muscle and liver were obtained from the lambs that did not survive the 8-week experimental period as soon as possible after death. The surviving lambs were sacrificed by exsanguination at the conclusion of the experimental period, and samples of muscle and liver were obtained. The tissue samples were analyzed for selenium by the method of Ewan *et al.* (1968b). Performance data for these experiments are to be published (Ewan *et al.*, 1968a).

RESULTS AND DISCUSSION

Table II presents the excretion of selenium in the urine and feces of lambs given selenate orally. Most of the administered selenium was excreted in the urine, and was eliminated during the first week after administration. Sizable amounts of selenium appeared in the feces during the early intervals, and small amounts persisted throughout the experimental period. The supplementation of the deficient diet with α-tocopherol appeared to increase the excretion of selenium during the first few days. However, by the second week, similar amounts of selenium had been excreted by all groups.

The data presented are in contrast to results obtained by others (Cousins and Cairney, 1961; Ehlig *et al.*, 1967; Paulson *et al.*, 1966; Peterson and Spedding, 1963; Wright and Bell, 1964) with older, ruminating sheep. With these older sheep, the major portion of orally administered selenium-75 was excreted in the feces and only small

Table II. Distribution of Selenium-75 in the Urine and Feces of Lambs Given Selenate[a] Orally

Interval, Days	Basal			Basal + Vitamin E			Basal (Dystrophic)		
	Urine	Feces	Total excreted	Urine	Feces	Total excreted	Urine	Feces	Total excreted
0–1	243.3[b]	7.7	251.0	808.8	127.2	936.0	425.0	28.2	453.2
1–2	290.3	66.1	607.4	294.0	115.1	1345.1	409.0	59.9	922.1
2–3	285.0	118.9	1011.3	176.8	82.2	1604.1	332.0	85.0	1339.1
3–4	129.0	95.6	1235.9	127.3	43.6	1775.0	252.5	89.4	1681.0
4–5	172.5	66.6	1475.0	48.2	18.5	1841.7	84.1	57.7	1822.8
5–6	101.1	75.1	1651.2	22.0	21.5	1885.2	42.2	57.4	1922.4
6–7	95.8	88.8	1835.8	19.8	34.0	1939.0	38.3	48.4	2009.1
7–14	132.4	193.9	2162.1	95.6	100.8	2135.4	93.3	86.4	2188.8
14–21	38.3	53.4	2253.8	41.8	43.1	2220.3	30.9[c]	44.5	2264.2
21–28	30.7[c]	24.2	2308.7	32.7	31.9	2284.9	...	...	...
28–35	42.6[c]	23.8	2375.1	30.7	30.0	2345.6	...	...	...
35–42	61.0	21.0	2457.1	25.4	23.8	2394.8	...	...	...
42–49	57.4[c]	21.0	2535.5	29.2[c]	20.2	2444.2	...	...	...
49–62	...	...	...	54.3	49.4	2547.9	...	...	...
62–69	...	...	...	21.2	17.8	2586.9	...	...	...
Total	1679.4	856.1		1827.8	759.1		1707.3	556.9	

[a] Each lamb received one capsule containing 3.35 mg. of selenium as selenate containing 86 μc. of ^{75}Se. Capsules administered at 23 days for group fed basal (4 lambs) or basal + vitamin E (4 lambs). Selenate administered to dystrophic group (2 lambs) after clinical symptoms of NMD appeared.
[b] All values in μg. of selenium from original dose per lamb per interval.
[c] Death loss.

amounts appeared in the urine. This may suggest that selenium fed to ruminants is converted by the rumen microorganisms to a form of selenium that is not readily utilized by the animal. Peterson and Spedding (1963) and Ehlig *et al.* (1967) have indicated that elemental selenium may be in this unavailable form. Wright (1964) found that intravenous injection of selenite into wethers resulted in greater excretion in the urine (12% of the dose) than in the feces (10% of the dose) during a 28-day collection period. With intravenous injection, the results are in better agreement with the data obtained in the present study when young lambs were given selenate orally.

The amount of selenium from the orally administered selenate which was retained in various lamb tissues is shown in Table III (experiment II). The retained selenium decreased continuously throughout the experimental period in most of the tissues (plasma TCA precipitate, plasma TCA supernate, skin, brain, liver, lungs, kidney, spleen, pancreas, stomach, small and large intestine and their contents, muscle heart, diaphragm, fat, bile, and bone). This was not true in the blood, where the red blood cells increased to maximum values at 4 and 8 weeks, or wool, which was highest at 4 weeks. Similar changes have been demonstrated in blood (McConnell *et al.*, 1960; Wright, 1964) and wool (Rosenfield and Beath, 1964; Wright, 1964). In contrast to work with more mature sheep (Kuttler *et al.*, 1961; Paulson *et al.*, 1966; Wright, 1964; Wright and Bell, 1964), the liver was found to retain the greatest quantity of the oral selenate initially. This observation is in agreement with distribution studies after selenium injection into rats (Hopkins *et al.*, 1966), and may further support the idea that since the young lamb does not have a functional rumen, selenium follows metabolic pathways that are similar to the monogastric animals. Andrews *et al.* (1964) and Allaway *et al.* (1966) have reported greater increases in the concentration of selenium in the liver than the kidney when the dietary selenium intake was increased. The kidney retained the greatest amount of selenium when compared to the other tissues, except liver.

The leg muscle retained very small amounts of selenium throughout the experiment. At 30 weeks, the muscle samples were analyzed by the method of Kelleher and Johnson (1961), and found to contain 0.065 μg. of total selenium per gram of fresh tissue. Thus, approximately 2% of the total selenium in the muscle originated from the labeled dose of selenate.

The total selenium content of liver and muscle tissue of lambs fed increasing levels of selenium as selenite and *dl*-

Table III. Selenium Retention in Tissues of Lambs Following Oral Administration of Selenate[a]

Tissue	Weeks after Administration						
	1	2	4	8	12	21	30
Blood, whole	6.86[1,2,b]	8.64[1]	8.08[1,2]	6.42[2,3]	4.87[3]	2.07[4]	0.88[4]
Red blood cells	3.41[3,4]	5.76[1,2]	6.57[1]	6.07[1,2]	4.38[2,3]	1.95[4,5]	0.65[5]
Plasma, TCA ppt.	3.01[1]	2.39[1]	1.22[2]	0.52[2,3]	0.44[2,3]	0.22[2,3]	0.11[3]
Plasma, TCA sup.	0.26[1]	0.10[2]	0.01[2]	0.09[2]	0.01[2]	0.01[2]	0.03[2]
Skin	6.67[1]	4.99[1]	2.27[2]	0.74[2,3]	0.66[2,3]	0.26[3]	0.16[3]
Wool	0.93[3]	1.94[3]	8.06[1]	5.62[1,2]	5.72[1,2]	2.09[2,3]	1.43[3]
Brain	4.15[1]	3.76[1]	3.18[1,2]	2.31[2]	2.23[2]	0.72[3]	0.31[3]
Adrenal	5.24[1,2]	9.52[1]	6.15[1,2]	3.15[2]	6.16[1,2]	1.05[2]	0.80[2]
Liver	178.15[1]	186.33[1]	93.60[1,2]	18.58[3]	13.22[3]	2.07[2,3]	0.55[3]
Lungs	11.12[1]	8.81[1]	4.18[2]	2.36[2,3]	2.10[3]	0.95[3]	0.39[3]
Kidney	39.85[1]	34.38[1]	19.13[2]	8.88[3]	7.79[3]	2.65[4]	1.17[4]
Spleen	13.00[1]	10.16[2]	5.98[3]	3.20[4]	2.26[4,5]	2.41[4,5]	0.78[5]
Pancreas	10.17[1]	6.15[2]	4.05[3]	1.33[4]	1.38[4]	1.01[4]	0.21[4]
Stomach	4.67[1]	4.86[1]	1.77[2]	0.74[2]	0.67[2]	0.28[3]	0.11[3]
Small intestine	10.47[1]	6.82[2]	2.82[3,4]	3.94[3]	1.34[3,4]	0.56[5]	0.16[5]
Small intestine contents	8.70[1]	3.78[2]	1.57[2,3]	0.31[2,3]	0.35[2,3]	0.12[3]	0.05[3]
Large intestine	6.39[1]	4.11[2]	1.82[3]	0.90[3,4]	0.99[3,4]	0.36[4]	0.11[4]
Large intestine contents	15.57[1]	1.60[2]	0.89[2]	0.17[2]	0.18[2]	0.08[2]	0.13[2]
Muscle, leg	1.84[1]	1.75[1]	1.81[1]	0.77[2]	0.56[2]	0.31[2]	0.13[2]
Heart	6.37[1]	5.10[2]	3.87[2]	2.31[3]	1.76[3,4]	0.81[4,5]	0.29[5]
Diaphragm	3.66[1]	3.08[1]	2.13[1]	0.88[2]	0.70[2]	0.33[2]	0.16[2]
Fat, kidney	5.22[1]	3.69[1]	1.07[2]	0.34[2]	0.28[2]	0.09[2]	0.04[2]
Bile	7.80[1,2]	13.22[1]	10.57[1,2]	0.68[2]	2.25[2]	0.08[3]	0.18[3]
Bone	6.86[1]	4.86[2]	2.54[3]	0.89[4]	0.86[4]	0.30[4]	0.09[4]

[a] Each lamb received 3.35 mg. of selenium as selenate containing 86 μc. of ^{75}Se orally.
[b] Values expressed as μg. of selenium from administered dose per 100 grams of tissue or 100 ml. of fluid and were averages of two lambs at first week and four lambs at all other periods. Any two means with same numerical superscript are not significantly different ($P < 0.05$) by Duncan's multiple range test.

α-tocopherol are presented in Table IV (experiments III and IV). Increasing the level of selenium in the Torula yeast diet significantly ($P < 0.005$) increased the amount of selenium present in the liver and muscle. Increasing the level of vitamin E in the diet significantly decreased ($P < 0.05$) the total selenium present in the muscle when the basal diet or the basal diet supplemented with 1.0 p.p.m. of selenium was fed. Vitamin E had no significant effect on the level of selenium in the liver.

Since the muscle and liver samples were obtained either at the time of death of the lambs or at the conclusion of the 56-day experimental period, a correlation was calculated between the survival of the lambs fed the basal diet supplemented with only vitamin E and the selenium content of their muscle. The correlation was highly significant ($r = 0.62$, $P < 0.01$ for 15 d.f.), and when expressed as a linear regression, there was a loss of 0.0037 p.p.m. per day and an estimated initial level of selenium in the muscle of 0.25 p.p.m. Samples of muscle from 2-day-old lambs with similar genetic and nutritional background were found to contain 0.32 p.p.m. of selenium. Thus, the decrease in selenium content of muscles of lambs fed the basal diet supplemented with tocopherol can be explained by the length of time the lambs were fed the diet. Among the groups of lambs fed the basal diet supplemented with 1.0 p.p.m. of selenium, a similar decrease was observed as the level of tocopherol in the diet increased. Since the lambs in this group survived the 56-day experimental period, there is no apparent explanation for this change.

While the level of selenium in the diet appeared to be the major factor affecting the level of selenium in the liver, a decrease in the levels was observed with an increase in survival time of lambs supplemented with vitamin E alone and the selenium content of the liver ($r = 0.758$, $P < 0.01$ for 15 d.f.), and when expressed as a linear regression, the rate of depletion was 0.0095 p.p.m. per day and the estimated initial value was 0.72 p.p.m. The livers of lambs sacrificed at 2 days from ewes consuming a diet containing 0.14 p.p.m. of selenium were found to contain 1.08 p.p.m.

Allaway *et al.* (1966) have suggested from their data and data of Cousins and Cairney (1961) that a critical concentration of selenium in liver for development of nutritional muscular dystrophy is 0.2 p.p.m. (dry basis). Among the groups of lambs not given selenium supplementation, those receiving low levels of vitamin E died of NMD before the liver levels of selenium were depleted to this critical concentration. With the highest level of vitamin E supplementation in experiments III and IV, liver selenium values were lower than the critical level suggested by Allaway *et al.* (1966). These results would suggest that the selenium level in the tissues is not the only factor involved but that the levels of both tocopherol and selenium determine the appearance of NMD.

Table IV. Effects of *dl*-α-Tocopherol and Selenium on the Selenium Content of Liver and Muscle

Level of Se, P.P.M.	Level of Vit. E, Mg./Kg./ Wk.	P.P.M. Selenium (Dry Basis) Muscle[a,b]	Liver[a]	Av. Survival,[c] Days
		EXPERIMENT III		
0.0	0.0	0.196 ± 0.061[d]	0.455 ± 0.114	26.8(0)
0.0	2.2	0.140 ± 0.048	0.283 ± 0.070	40.5(2)
0.0	5.5	0.114 ± 0.041	0.306 ± 0.128	34.3(1)
0.0	11.0	0.024 ± 0.019	0.173 ± 0.040	46.0(3)
0.1	0.0	0.154 ± 0.048	0.712 ± 0.146	39.3(2)
0.1	2.2	0.159 ± 0.050	0.653 ± 0.076	45.0(2)
0.1	5.5	0.156 ± 0.032	0.633 ± 0.028	56.0(4)
0.1	11.0	0.159 ± 0.015	0.673 ± 0.108	56.0(4)
1.0	0.0	0.457 ± 0.039	3.57 ± 0.70	49.0(3)
1.0	2.2	0.318 ± 0.027	3.38 ± 0.84	56.0(4)
1.0	5.5	0.282 ± 0.031	3.00 ± 0.70	50.0(3)
1.0	11.0	0.292 ± 0.063	3.07 ± 0.60	56.0(4)
		EXPERIMENT IV		
0.0	0.0	0.284 ± 0.022	0.418 ± 0.073	38.3(0)
0.0	22.0	0.064 ± 0.015	0.159 ± 0.041	56.0(4)
1.5	0.0	0.550 ± 0.125	3.91 ± 0.42	39.0(2)
1.5	22.0	0.373 ± 0.018	3.59 ± 0.67	56.0(4)

[a] Significant effect of selenium ($P < 0.005$).
[b] Significant effect of vitamin E ($P < 0.05$).
[c] Average number of days lambs survived; number of lambs that completed 56-day trial in parenthesis.
[d] Average of four lambs plus or minus standard error.

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