AN ABSTRACT OF THE THESIS OF

William Robert Wise Jr. (Name) for the M. S. (Degree) in Animal Science (Major)

presented on August 5, 1967

Title NUTRITIONAL INTERRELATIONSHIP OF COBALT AND SELENIUM IN THE LAMB AND WEANLING RAT

Abstract approved [redacted]
P. H. Weswig

The purpose of this thesis is to determine if dietary and metabolic relationships exist between cobalt and selenium. Two groups of lambs were compared. The dams of one group of lambs received an alfalfa hay adequate in selenium and the dams of the other group received an alfalfa hay deficient in selenium. At six weeks of age the lambs were sacrificed and examined for lesions of white muscle disease. The heart, liver, kidney, and skeletal muscle were analyzed for cobalt and selenium. The lambs in the low selenium group had a much lower concentration of this element in these tissues and organs than lambs fed the normal selenium hay. There was no difference in selenium concentration of tissues and organs between low selenium lambs with lesions of white muscle disease and healthy low selenium lambs. The cobalt concentration of tissues was unaffected by selenium intake, but when symptoms of white muscle disease appeared, the cobalt concentration of the kidney

was significantly lowered.

In a second experiment, groups of weanling rats were fed a basal diet deficient in selenium. One group received a selenium supplement, a second group received a cobalt supplement, a third group received both the selenium and cobalt supplements, and a fourth group served as controls. Rats from these groups were injected with selenium-75 (as selenite) and cobalt-60 (as cobaltous chloride) and the effect of dietary treatments on the uptake of the radiotracers by various organs was determined. Dietary selenium did not affect the uptake of cobalt-60, while dietary cobalt did increase the uptake of selenium-75 in the muscle (significant at the 90% level).

Nutritional Interrelationship of Cobalt and Selenium in Lambs and Weanling Rats

by

William Robert Wise, Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1968

APPROVED:

[redacted]

Professor of Animal Science

[redacted]

Head of Department of Animal Science

[redacted]

Dean of Graduate School

Date thesis is presented August 5, 1960

Typed for William Robert Wise, Jr. by Ruth Baines

ACKNOWLEDGMENTS

The author wishes to thank his major professor, Dr. Paul H. Weswig for his assistance and guidance during this research project. Thanks are also due to Dr. O. H. Muth for supplying the lamb tissues and doing histological studies, to Dr. Phillip Whanger for his helpful suggestions, and to Miss Julie Jirel for her invaluable assistance in the preparation of the manuscript. The assistance of the many other members of the Agricultural Experiment Station who have contributed to this project is appreciated.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Selenium in Nutrition of Sheep	2
Selenium in Nutrition of Rats	5
Relationships Between Selenium and Other Elements	7
Cobalt in Animal Nutrition	9
The Possible Interrelation of Cobalt and Selenium	10
NUTRITIONAL INTERRELATIONSHIP OF SELENIUM AND COBALT IN LAMBS	13
Introduction	13
Experimental	13
Results and Discussion	15
Selenium Concentrations of Tissues	15
Cobalt Concentrations of Tissue	18
Growth of Lambs	19
SGOT Values	19
Summary	20
SELENIUM AND COBALT STUDIES WITH RATS	22
Introduction	22
Experimental	23
Results and Discussion	27
Uptake of Selenium-75 and Cobalt-60 by Tissues and Organs	27
Growth and Feed Consumption	35
Summary	38
DISCUSSION AND CONCLUSION	39
BIBLIOGRAPHY	42
APPENDIX	49

LIST OF FIGURES

Figure No.		Page
1	Average feed consumption per day per rat	36
2	Average postweaning growth of rats	37

LIST OF TABLES

Table No.		Page
1	Average selenium concentrations of lamb tissues	16
2	Ratios of the selenium concentration of tissues or organs to the selenium concentration of the diet	17
3	Average cobalt concentrations of lamb tissue	18
4	Dietary supplements	24
5	Preweaning diet	25
6	Torula depletion diet	26
7	Average corrected total CPM per tissue or organ for each treatment	29
8	Average corrected CPM per gram of tissue for each treatment	30

NUTRITIONAL INTERRELATIONSHIP OF COBALT AND SELENIUM IN LAMBS AND WEANLING RATS

INTRODUCTION

Intensive research into the metabolism and nutrition of selenium was stimulated by the discovery (Schwarz and Foltz, 1957) that selenium protected against dietary liver necrosis in the rat. This was followed by the discovery of preventive effects of selenium against exudative diathesis in the chick (Schwarz et al., 1957 and Patterson, Milstrey and Stokstad, 1957) and against white muscle disease in lambs (Muth et al., 1958 and Proctor, Hogue and Warner, 1958). Now, selenium responsive diseases have been observed (Schwarz, 1961) in mice, rabbits, mink, swine, cattle, horses and humans.

The nutrition and metabolism of selenium cannot be discussed without also discussing other dietary factors which influence selenium. The most notable is the interrelationship that exists between selenium and vitamin E. Sulfur has an antagonistic effect, decreasing the biological availability of selenium (Schubert et al., 1961 and Hogue, 1964). Subtoxic doses of arsenic will protect against toxic doses of selenium (Rosenfeld and Beath, 1964). Selenium has been shown to be a factor that will protect against cadmium damage to the testes (Gunn and Gould, 1966 and Mason and Young, 1966).

Results of several researchers (Andrews, Grant and Stephenson, 1964, Bunyan, Edwin and Green, 1953 and Gardiner, 1966) have suggested a dietary relationship between selenium and cobalt. It is the purpose of this thesis to investigate this possibility.

Selenium in Nutrition of Sheep

A deficiency of selenium in lambs and calves results in white muscle disease (WMD). This economically important myopathy (Muth, 1955) occurs in parts of the United States, Scotland, Sweden and New Zealand. This disease has been known to affect up to 50% of some lamb flocks and has resulted in losses of nearly all calves of some herds.

The external symptoms of WMD (Muth, 1955) are dyspnea and general weakness, which results in impaired voluntary movement. Autopsy reveals lesions of the skeletal and cardiac muscle, which vary from a slightly lightened to a markedly bleached appearance. Under microscopic examination the calcification of the muscle shows up as rows of granules, sometimes filling up the sarcolemma. Cardiac lesions frequently appear as complete loss of sarcoplasm, with only the connective tissue remaining.

Muth et al. (1959) found that WMD in lambs, caused by feeding a basal ration consisting mainly of hay from an area in Oregon

where WMD is a problem, could be prevented by addition of 0.1 ppm selenium (as selenite) to the ration. Weekly injection of 770 IU vitamin E per day to the ewes had little or no effect in preventing WMD in the lambs. In a later experiment, Oldfield, Muth and Schubert (1960) found that injecting the lamb with selenite or feeding a massive dose of vitamin E protected against WMD, but only selenium administration resulted in an improved growth.

Hartley and Grant (1961) have reviewed selenium responsive diseases in New Zealand livestock. WMD, as it occurs in New Zealand, is prevented or cured by selenite, while large doses of tocopherol are only partially protective.

In contrast, Hogue, et al. (1962) found that WMD, caused by feeding a ration of hay and raw cull kidney beans, was markedly reduced by the addition of either 1 ppm selenium as selenite or 100 IU vitamin E per ewe per day to the ration. The addition of both vitamin E and selenium completely prevented WMD.

Hopkins, Pope and Bauman (1964) fed lambs a liquid diet composed mainly of torula yeast, sucrose and stripped lard. This diet was deficient in selenium and vitamin E. The addition of vitamin E to this ration prevented all symptoms of WMD, but these lambs exhibited a poor growth response. Selenite added to the basal ration delayed the onset of the disease, moderated the

severity, and resulted in a better growth response up to nine weeks of age. Serum glutamic oxaloacetic transaminase (SGOT) levels in the selenium supplemented animals were extremely high and several of the animals were lethargic. Selenium plus vitamin E prevented all symptoms of the disease and resulted in an improved growth rate.

The differences in results between these researchers can be explained by differences in the experimental diets used. Muth et al. (1959) used basal diets which were extremely low in selenium but continued an adequate level of vitamin E. The prophylactic doses of vitamin E used were far above normal dietary levels. Diets used by Hogue et al. (1962) were not as low in selenium as Muth's, and the cull kidney beans (Hogue, 1964) have been shown to contain a vitamin E antagonist. This may explain why these experimental animals responded more to vitamin E while Muth's experimental animals gave better responses to selenium. WMD, as it occurs in Oregon, is caused by the extremely low levels of selenium in forages, and WMD on Hogue's kidney bean diet is largely a vitamin E deficiency. Hopkins, Pope and Bauman (1964) used a diet which is deficient in both selenium and vitamin E and showed that lambs needed both selenium and vitamin E to maintain normal health and growth.

Selenium in Nutrition of Rats

In the rat, a simultaneous lack of both selenium and vitamin E (Schwarz, 1965) results in liver necrosis. This disease is experimentally produced by feeding weanling rats on a diet which has torula yeast as its sole protein source. After about twenty-four days on this diet, death occurs. Autopsy reveals gross macroscopic lesions of the liver.

The induction period (Schwarz, 1965) for liver necrosis lasts six to ten days, during which time the vitamin E and selenium stores of the body are being depleted and no symptoms are evident. The latent phase lasts an additional ten to fourteen days. During this time there are no visible lesions, but electron microscopy reveals damage to the endoplasmic reticulum and mitochondria. During the terminal stage, gross macroscopic lesions of the liver develop rapidly and death occurs within a few hours to several days.

While liver slices or homogenates of normal liver (Schwarz, 1962) will maintain respiration in a Warburg respirometer for four to six hours, liver slices or homogenates from necrotic animals will maintain respiration for only about twenty to thirty minutes, and then oxygen consumption declines to 10 to 20% of initial consumption. Dietary vitamin E or vitamin E injected prior to extirpation of the liver will prevent respiratory failure. Vitamin E placed in the

Warburg flask will not prevent respiratory failure, but tocopherol metabolites isolated from urine will prevent respiratory breakdown. Supplementation with selenium compounds, either dietary or injected into the rat, has no effect on liver homogenates, but when liver slices are used, dietary selenium or selenium given by stomach tube several hours before extirpation of the liver is partially effective in prevention of respiratory decline. The liver maintains normal respiration for 30 to 60 minutes and then undergoes a 20% decline. Selenium added to the Warburg flask or injected immediately before extirpation of the liver has no effect on respiratory decline. Schwarz interprets these results as meaning that the physiological roles of selenium and tocopherol differ and that selenium exerts its effect only after a relatively slow biochemical conversion process in the body. The difference between tissue slices and homogenates may indicate that selenium acts at the cell surface.

The involvement of trace elements other than selenium in respiratory decline was demonstrated by prevention of respiratory decline by EDTA (McLean, 1960). Addition of 0.01 to 0.20 μmole Cd^{++} or 0.10 to 0.30 μmole AsO_2^- was shown to induce respiratory failure while Mn^{++} will prevent respiratory failure.

Relationships Between Selenium and Other Elements

Severe outbreaks of WMD following use of sulfur containing fertilizers (Muth et al. 1959) led to the suspicion that sulfur interfered with selenium in animal nutrition. It has been shown (Trelease and Beath, 1949) that sulfur interferes with the uptake of selenium by plants. Muth, Schubert and Oldfield (1961) demonstrated that sulfate, added to a ration, removed the protective level of added selenite.

Sulfate has been shown to reduce the toxic effects of high levels of selenite (Bornhorst and Palmer, 1957, and Halverson, Guss and Olson, 1962). Urinary excretion of doses of ^{75}Se is substantially increased when rats are treated with sulfate and retention of the dose by the tissues is reduced (Ganther and Baumann, 1962b). Bornhorst and Palmer (1957) hypothesize that sulfur and selenium compete for a transport mechanism.

Ganther (1965) explains that while the chemical similarity of sulfur and selenium suggests parallel metabolic pathways, major differences do exist. Sulfur compounds generally follow oxidative pathways, while selenium compounds become reduced. Therefore, sulfur compounds such as methionine are metabolized to sulfate, while selenite is reduced to organo-selenium compounds.

Cadmium chloride increases the retention of selenite in the

body and decreases excretion of selenium in the urine, feces, and expired air (Ganther and Baumann, 1962a).

It has been shown (Mason, 1966) that selenium as selenium dioxide and zinc as zinc acetateare effective against cadmium induced testicular damage in the rat. Present theories suggest that these elements may compete for binding sites on plasma protein, may cause alterations in lipoproteins of cell membranes, or may cause enzymatic disturbances in testes. Gunn and Gould (1966) in reviewing evidence that cadmium, zinc, and selenium are all involved in vascular reactions, hypothesized that cadmium damages a site in the vascular endothelium of the testes, and zinc and selenium are capable of inactivation of cadmium at that site.

Sublethal doses of arsenic compounds (Rosenfeld and Beath, 1964) have been shown to protect against lethal doses of sodium selenate. The deposition of selenium in tissues, when arsenic is included in the diet, is not affected. Arsenite injected prior to the injection of selenite into rats affects the metabolism of selenium by inhibiting the formation of volatile selenium compounds, and by increasing intestinal secretion of this element (Ganther and Baumann, 1962a).

Cobalt in Animal Nutrition

The essentiality of cobalt in animal nutrition (Underwood, 1962) was shown when certain enzootic diseases of sheep and cattle were cured by addition of cobalt to the ration. Symptoms of cobalt deficiency are extreme emaciation and listlessness, giving the appearance of a starved animal. The skin and mucous membranes become pale from a progressively developing anemia. Symptoms of mild deficiency are an unthriftiness in young stock, and diminished lactation in mature animals. Lambs or calves are weak when born and will usually die within a few weeks.

Rumen microorganisms influence the metabolism of cobalt in ruminants (Smith and Loosli, 1957). This was suggested by the fact that horses survived with no ill effects on pastures where cattle and sheep produced cobalt deficiency symptoms, and that efforts to produce cobalt deficiency (Houk, Thomas and Sherman, 1946) in rats, using purified rations, proved unsuccessful. Dietary or oral doses of cobalt (Marston and Lee, 1949) were effective in preventing deficiency symptoms in sheep and cattle, but parenterally administered cobalt was ineffective except in extremely large quantities (Ray et al., 1948; Keener et al. 1950; Keener, Baldwin and Percival, 1951). The effect of the large doses was attributed to some of the dose entering the rumen.

With the isolation of vitamin B_{12} it was shown (Smith, Koch and Turk, 1951) that cobalt deficient sheep and cattle responded to B_{12} supplementation. Injected doses were effective (Kercher and Smith, 1955) in far smaller quantities than oral doses. It was decided that vitamin B_{12} was synthesized by the rumen microorganisms. This is a rather inefficient process (Comar and Bronner, 1962) since much of the cobalt is converted into purine containing analogs of the vitamin which are of no value to the animal.

In the nonruminant animal, cobalt deficiency has never been clearly demonstrated (Underwood, 1962). Rate of gain has been improved by addition of cobalt (Dinusson et al., 1953) to diets of growing and fattening pigs, but rats have been maintained on (Houk, Thomas and Sherman, 1946) diets containing as little as 0.3 μg cobalt per day and rabbits have been grown (Thompson and Ellis, 1947) on diets containing as little as 0.1 μg cobalt per day. A decreased mortality in rats fed a soya flour and corn meal diet with cobalt supplementation has been reported (Cobalt in rats, 1952) but, since vitamin B_{12} gave the same effect, this effect was attributed to conversion of cobalt to vitamin B_{12} by gut organisms.

The Possible Interrelation of Cobalt and Selenium

The possible interrelationship between selenium and cobalt

(Andrews et al., 1964) in sheep nutrition was suspected by New Zealand workers. Unthriftiness, or failure to grow at a normal rate, is a major cause of economic loss in the New Zealand livestock industry and forms of unthriftiness have been shown to respond to selenium dosing and to cobalt dosing. Similarities occur between the selenium responsive and cobalt responsive unthriftiness; both affect particularly the young sheep, both can have high death rate, and the more spectacular response to selenium dosing often occurs where soils are known to be cobalt deficient.

To further study any possible relationship of cobalt to selenium, Andrews et al. (1964) placed lambs for ten months on a pasture that was low in both selenium and cobalt. One group of lambs received a 1.25 mg per week selenium (as selenite) dose, a second group received a 7 mg per week cobalt dose (as cobalt chloride), a third group received both the selenium and cobalt dose, and the fourth group served as controls and were not dosed.

The cobalt dose caused a significant growth response, while the selenium dose caused a much smaller response. Selenium dosing had no effect on vitamin B_{12} concentrations in either the liver or kidney. Cobalt dosing had no effect on selenium concentrations in the liver but cobalt deficient sheep accumulated significantly more selenium in the kidney than those in which cobalt

deficiency was prevented. Andrews explains this as probably being an impairment of renal function due to the cobalt deficiency.

Gardiner (1966) determined that a cobalt deficiency in sheep increases their susceptibility to a selenium toxicity.

Bunyan et al. (1957) showed that cobalt supplementation would partially protect against liver necrosis when rats were fed a basal diet of 30% baker's yeast, sucrose, minerals and vitamins. On the other hand, Schwarz (1959) reported that rats did not respond to cobalt supplementation when they were fed a similar diet with torula yeast as the protein source.

From these experiments, it would appear there might be a metabolic interrelationship between selenium and cobalt. The requirements for cobalt and selenium by various species of animals seem to be different, and these requirements may also depend on type of diet.

NUTRITIONAL INTERRELATIONSHIP OF SELENIUM AND COBALT IN LAMBS

Introduction

Since Andrews, Grant and Stephenson (1964) have determined that cobalt dosing can affect selenium concentrations in the kidney of sheep, the inverse should be determined. Therefore, the objective of this study was to determine if selenium intake can affect cobalt concentrations of various organs.

Experimental

Corriedale ewes were pastured in the spring and summer on a mixture of grass and clover which contained 0.02 ppm selenium. On November 1 they were divided into two groups; one was fed a low selenium alfalfa hay (0.01 ppm Se) and the other was fed a high selenium alfalfa hay (0.40 ppm Se). The high selenium hay contained 0.50 ppm cobalt while the low selenium hay contained 0.41 ppm cobalt. These are normal values for cobalt content of hay (Underwood, 1962). Lambs from these ewes were born from February 10 to March 24.

At six weeks of age the lambs from each group were weighed and a blood samples were taken. The animals were then autopsied. The skeletal and cardiac muscles were examined for visible lesions

of WMD and sections of these muscles were saved for histological studies.

Serum glutamic oxaloacetic transaminase (SGOT) values were determined on the blood (Sigma Chemical Company, 1961), since elevated levels of this enzyme are used as clinical indication of WMD. (Blincoe and Dye, 1958, Kuttler and Marble, 1958, and Swingle, Young and Dang, 1959).

At the time of autopsy, the kidneys, liver and sections of the muscle (semitendinosis) and heart were saved and later freeze dried. Tissues from eight lambs from the high selenium group and twelve lambs from the low selenium group, half of which had lesions of WMD, were selected and analyzed for both selenium and cobalt. Both of these elements concentrate in the liver and kidney while the heart and skeletal muscle reflect a deficiency of selenium.

The selenium analysis (Allaway and Cary, 1964) involves oxidation of the dried sample in a Schoniger flask and coprecipitation of the selenium with arsenic, followed by complexing the selenium with 1, 3-diaminonapthalene and determination of the flourescence of the complex. The cobalt analysis (AOAC, 1960) involves dry ashing of the sample, followed by extraction of the cobalt with dithiazone. The dithiazone is digested, the cobalt is complexed with nitroso-R-salt, and the optical density of the complex is

determined. The cobalt analysis was done in duplicate because of the low levels of cobalt present and the variability of the analytical results.

Analyses for each element and each tissue were grouped according to histopathological criteria, high selenium lambs (normal), low selenium lambs (normal) and low selenium lambs (WMD). Appropriate statistical analysis was applied to the data and repeat analyses were performed on the samples if possible and if thought necessary.

Results and Discussion

Selenium Concentrations of Tissues

Certain of the values for the selenium analysis did not seem to agree with the mean, and insufficient tissue was available to repeat the analysis. These were tested by the R test for rejection of extreme values and the following values were discarded; lamb No. 791, heart and liver and lamb No. 747, liver, and muscle (see appendix, Tables 2 and 3).

Lambs from the low selenium group (see Table 1 and appendix Tables 2, 3, and 4) have significantly lower concentration of selenium in the heart, liver, kidneys and skeletal muscle than the lambs from the high selenium group. These values are in

good agreement with those of Allaway et al.(1966). Within the low selenium group, there are no significant differences between the selenium concentrations of tissues of the normal lambs and lambs with WMD. The apparent higher value for the muscle in normal low selenium lambs (0.04 ppm Se) as compared to the lambs with WMD (0.11 ppm Se) is due mainly to two high values in the normal group.

Table 1. Average selenium concentrations of lamb tissues

Tissue	low selenium lambs		high selenium lambs
	with WMD	normal	normal
heart, ppm dry basis	.06 ± .10	.04 ± .03	1.18 ± .38
kidney, ppm dry basis	.57 ± .23	.64 ± .18	3.29 ± 1.08
liver, ppm dry basis	.05 ± .02	.05 ± .03	3.60 ± 1.06
muscle, ppm dry basis	.11 ± .12	.04 ± .03	.84 ± .09

It is curious that although the tissues of normal low selenium lambs contain enough selenium for proper functioning, the tissues of high selenium lambs store so much more selenium. The selenium content of the tissues of the high selenium lambs is not particularly high. Allaway et al. (1966) reported 14.7 ppm selenium in the liver and 7.7 ppm selenium in the kidney in normal lambs. The question then arises as to why so much selenium is taken up when so little is needed.

Low selenium content of tissues is indicative of WMD but is not a positive diagnosis. Selenium concentration of liver is more sensitive to differences in selenium intake than selenium concentration of the kidney (Andrews et al., 1964). Andrews et al. (1964) state that 0.12 ppm selenium in the liver indicates a moderately selenium responsive sheep. In this experiment there are healthy lambs containing the same concentration of selenium in their tissues as lambs with WMD.

Table 2. Ratios of the selenium concentration of tissues or organs to the selenium concentration of the diet

	low selenium lambs		high selenium lambs
	with WMD	normal	
heart	4	6	3
kidney	64	57	8
liver	5	5	9
muscle	4	11	2

Table 2 demonstrates that while the kidney, heart, and skeletal muscle take up selenium with increasing efficiency while the selenium concentration of the feed is decreased, the efficiency with which the liver takes up selenium is actually decreased. This is probably due to the fact that the liver functions in the detoxification of excess selenium (Ganther, 1966).

Cobalt Concentrations of Tissue

Table 3. Average cobalt concentrations of lamb tissue

Tissue	low selenium lambs		high selenium lambs
	with WMD	normal	normal
heart, ppm dry basis	.05 ± .02	.04 ± .03	.03 ± .03
kidney, ppm dry basis	.12 ± .04	.27 ± .11	.26 ± .08
liver, ppm dry basis	.29 ± .08	.26 ± .08	.27 ± .09
muscle, ppm dry basis	.03 ± .01	.06 ± .06	.04 ± .03

An 0.04 to 0.06 ppm liver cobalt level indicates a cobalt deficiency, while 0.08 to 0.12 ppm or more indicates a satisfactory cobalt status (McNaught, 1948). The kidney and heart normally contain 0.25 ppm and 0.06 ppm cobalt respectively and in a deficiency contain 0.05 and 0.01 ppm cobalt respectively (Underwood, 1962). By these criteria, the tissues of the animals in this experiment (Table 3 and appendix Tables 2, 3, and 4) must be considered normal in respect to cobalt status.

There was no difference in cobalt concentration in the tissues analyzed between lambs in the high selenium group and the normal lambs in the low selenium group. However, within the group of lambs on the low selenium ration, the lambs affected by WMD accumulated only 0.12 ppm cobalt in kidney as compared with 0.27 ppm cobalt in kidneys of lambs without WMD. This more than twofold difference was significant at the 95% level ($t = 2.4$).

This reduction in kidney cobalt concentration could not be used as a diagnosis for WMD since the kidney cobalt concentration remains in the normal range.

Andrews et al. (1964) explains the greater accumulation of selenium in cobalt deficiency to be most likely due to an impairment of renal function caused by the deficiency. In the case of this experiment, the decreased cobalt in a lamb with WMD may also be due to impaired renal function of a different nature.

Growth of Lambs

The mean weight for the normal low selenium lambs (34.6 lb) was the same as for the high selenium lambs (33.5 lb) while the low selenium lambs with WMD weighed slightly, but not significantly, less (28.5 lb). Selenium deficiency does result in a failure to grow at normal rate (Hartley and Grant, 1961). By comparison of these results with those of Brody (1945), who raised Suffolk sheep to a weight of 20 lb in six weeks, these growth rates are excellent.

SGOT Values

The SGOT values for the high selenium lambs at six weeks of age averaged 79 with a range from 45 to 103. The normal low selenium lambs had slightly higher SGOT values with an average

of 126 with a range of 70 to 200 and the lambs with WMD had markedly elevated SGOT values which averaged 2575 and ranged from 380 to 10, 201.

The normal SGOT range in sheep is under 200, and a value above this is an indication of WMD (Blincoe and Dye, 1958). Diagnosis of WMD by elevated SGOT values in this experiment agreed exactly with histological determination of the lesions. Glutamic oxaloacetic transaminase is plentiful in skeletal muscle, liver and myocardium. Destruction of the cells of these tissues releases the enzyme into the blood (Mason and Wroblewski, 1957).

Summary

A low level of selenium in the tissue does not necessarily mean that an animal will have an elevated SGOT. For the lambs in the low selenium group, the lambs with WMD, all of which had elevated SGOT, contain the same amount of selenium in the tissue as normal low selenium lambs, all of which had SGOT values in the normal range.

The results from this experiment indicate that lambs fed low selenium alfalfa hay have a much lower concentration of this element in their kidneys, hearts, liver, and skeletal muscle than lambs fed high selenium hay. There was no difference in selenium

concentration in the tissues and organs of lambs showing WMD as compared with the normal low selenium lambs. The cobalt concentration of these tissues does not seem affected by the selenium intake, but when symptoms of WMD are observed, the cobalt concentration of the kidney is significantly lowered. It would seem that a metabolic interrelationship between cobalt and selenium deficiency as characterized by WMD does exist in lambs.

SELENIUM AND COBALT STUDIES WITH RATS

Introduction

Since the results of the experiment previously discussed in this thesis and those of Andrews et al. (1964) and Gardiner (1966) suggest an interrelationship between selenium and cobalt in the kidney, it was decided to determine the effect of dietary cobalt intake on the uptake of selenium-75 and the effect of dietary selenium intake on the uptake of cobalt-60 in various tissues of the rat.

Subcutaneously injected selenium-75 (as selenate) (McConnell, 1941) concentrates mostly in the liver, and to a lesser extent in the total muscle, blood, lung, spleen and heart. This injected dose initially is present in the blood in the plasma, but the plasma selenium declines rapidly as the selenium incorporated in the red blood cells increases. Mice excrete 83% of a sublethal dose of selenium-75 (as selenite) in 48 hours mostly in the urine and to a lesser extent in the feces and expired air (Heinrich and Kelsey, 1955). Chicks fed a selenium deficient ration retain considerably more of a dose of selenium-75 (as selenious acid) than chicks fed a ration with 1 ppm selenium (Jensen, Walter, and Dunlap, 1963).

An oral dose of cobalt-60 in the rat will accumulate mostly in the liver, and to a lesser extent in the kidney and spleen (Cuthbertson, Free, and Thornton, 1950). Orally administered doses

of radioactive cobalt chloride are poorly absorbed by the tissues in comparison with parenteral doses (Comar and Davis, 1947). Injected cobalt 60 (as cobalt chloride) is mainly excreted in the urine, with a small amount appearing in the feces (Ulrich and Copp, 1951), while an orally administered dose is excreted mainly in the feces, with a small amount appearing in the urine (Comar, Davis, and Taylor, 1946). Excretion increases as the size of the dose increases (Comar, Davis and Taylor, 1946) but previous dietary intake of cobalt has no effect.

Experimental

A 2 x 2 factorial experiment was designed to determine the effect of dietary cobalt on selenium uptake, the effect of dietary selenium on cobalt uptake, and if any interaction occurred between selenium and cobalt.

Two nursing rats were placed on a low vitamin E diet (Table 5) at six days postpartum. At 21 days of age the young were weaned and separated into four groups, with two females and two males in each group. The groups were placed on a basal ration (Table 6) deficient in selenium and vitamin E, with the dietary supplements shown in Table 4. Weights of the rats and feed consumed were recorded at two day intervals.

Table 4. Dietary supplements

Group	No. of rats	Dietary supplement
1	2 males, 2 females	None
2	2 males, 2 females	1.0 ppm Co as $CoSO_4$
3	2 males, 2 females	0.1 ppm Se as Na_2SeO_3
4	2 males, 2 females	1.0 ppm Co as $CoSO_4$ and 0.1 ppm Se as Na_2SeO_3

At 38 days of age, two rats from group one and two rats from group two (Table 4), a male and a female from each group, were injected with 1 μc selenium-75 as sodium selenite and placed in metabolism cages. The remaining two rats in group one and the remaining two rats in group two (Table 4) were injected with 5 μc cobalt-60 as cobaltous chloride and placed in metabolism cages. Feces and urine were collected and expired air was passed through a trapping solution (6% $Hg(NO_3)_2$ saturated with $HgCl_2$) in order to absorb volatile selenium excretion products (Schultz and Lewis, 1940).

Forty-eight hours after injection the animals were removed from the metabolism cages, anesthetized, and blood was taken by open heart puncture. The following tissues were collected and placed in tared counting tubes: heart, liver, spleen, pancreas, bone (femur) muscle (quadriceps femoris) and eye.

The following day this procedure was repeated using the rats

Table 5. Preweaning diet[1]

Constituent	Weight (grams)
rice flour	2900
torula yeast	1870
skim milk	613
cellulose	323
salt mix	161
gelatin	129
liver residue (Nutritional Biochemicals Corp.)	32
stripped lard (Distillation Products Industries)	400
cod liver oil	13
choline	6

[1] The composition of this diet was obtained from Dr. Klaus Schwarz, Section on Experimental Liver Disease, National Institutes of Health, Bethesda, Md.

[2] Salt mixture H. M. W., purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. This salt mixture contains the following percentages of salts: $CaCO_3$ 54.3; $Mg\ CO_3$, 2.5; $MgSO_4$, 1.6; NaCl, 6.9; KCl, 11.2; KH_2PO_4, 21.2; $FePO_4 \cdot 4\ H_2O$, 2.05; KI, 0.008; $MgSO_4$, 0.035; NaF 0.1; $Al_2(SO_4)_3K_2SO_4$, 0.017; $CuSO_4$, 0.09. (Hubbell, Mendel, and Wakeman, 1937).

Table 6. Torula Depletion Diet (Schwarz and Foltz, 1958)

Constituent	Weight (grams)
torula yeast	2400
salt mix (Table 5, reference 1)	400
sucrose	4120
lard	400
vitamin mix[1]	80
vitamin A	80 mg
vitamin D	800 μg

[1] The vitamin mix is composed of the following: lactose, 88.68 gm, thiamine HCl, 40 mg, riboflavin, 25 mg, pyridoxine HCl, 20 mg, d-calcium pantothenate, 200 mg, choline chloride, 10 gm, niacin, 1 gm, menadione, 10 mg, folic acid, 20 mg, biotin 10 mg, vitamin B_{12}, 1 mg.

from groups three and four.

The samples were weighed and counted in a Packard Model 500D autogamma counter. Data was calculated as cpm per entire tissue and as cpm per gram of tissue. Statistical tests were run to determine the effect of dietary cobalt on the uptake of cobalt-60, the effect of dietary selenium on the uptake of selenium-75 and any interaction between cobalt and selenium.

Results and Discussion

Uptake of Selenium-75 and Cobalt-60 by Tissues and Organs

The total counts per minute for each tissue are given in appendix Table 5 and summarized in Table 7. The total counts per minute per gram of tissue are given in appendix Table 6 and summarized in Table 8. Results of cpm per gram are quite variable in some of the smaller tissues, apparently due to the drying of the tissues. The data are discussed in order of decreasing uptake of the radioactive element by the tissue. Weights of tissues are given in appendix Table 7.

The livers from animals receiving only the cobalt supplements had greater uptake of selenium-75 than livers from animals of the other groups (Tables 7 and 8). This suggested an interaction but any interaction did not approach significance.

The uptake of selenium-75 (all as selenite) was less in the kidney in the groups of rats receiving selenium dietary supplements. This decrease was significant at the 80% level ($t = 1.73$) when tested on a basis of cpm per tissue (Table 7). The group of rats receiving only the cobalt dietary supplements had a greater uptake of selenium-75 than the other groups, which suggested an interaction. However, this interaction did not approach significance.

Uptake of selenium-75 by the blood was unaffected by either dietary cobalt or dietary selenium. One value was lost when a male rat on the diet supplemented with both selenium and cobalt had a spasm and died as the needle of the syringe entered the heart. An injected dose of 75-selenite (as selenite) is initially present in the blood mainly in the plasma (McConnell, 1941) but radioactivity in the plasma declines rapidly as the radioactivity in the red blood cells increases. After two to six hours, most of the injected dose in the blood is in the red blood cell fraction.

Hearts of those animals on selenium supplemented diets took up more selenium-75 than those animals on the selenium deficient diet. This difference was significant at the 90% level ($t = 2.32$) when calculated on a basis of cpm per tissue (Table 7). When calculated on a basis of cpm per gram of tissue, this difference disappeared (Table 8). Examination of the weights of the hearts showed

Table 7. Average corrected total CPM per tissue or organ for each treatment

Tissue	CPM ^{75}Se			CPM ^{60}Co		
Blood (per ml)	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	5420	5400	-Co	816	887
	+Co	5120	6800	+Co	574	517
Heart	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	1420	1800	-Co	943	1480
	+CO	1190	1990	+Co	755	1140
Liver	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	53400	49800	-Co	79500	74800
	+Co	77000	56300	+Co	47000	57600
Spleen	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	796	587	-Co	685	535
	+Co	1000	454	+Co	484	507
Pancreas	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	694	544	-C	4590	3450
	+Co	737	736	+Co	2020	2730
Kidney	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	18500	16800	-Co	14500	14600
	+Co	32500	15800	+Co	8440	9890
Muscle	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	5080	3890	-Co	294	348
	+Co	6310	7560	+Co	233	272
Bone	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	707	693	-Co	595	1190
	+Co	616	832	+Co	724	941
Eye	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	85	97	-Co	59	90
	+Co	113	83	+Co	45	60

Table 8. Average corrected CPM per gram of tissue for each treatment

Tissue	CPM ^{75}Se			CPM ^{60}Co		
Blood	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	5450	5270	-Co	994	1010
	+Co	5470	5310	+Co	581	775
Heart	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	3310	3380	-Co	3180	3650
	+Co	4200	5010	+Co	2360	2810
Liver	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	12500	12900	-Co	20700	31100
	+Co	20600	12100	+Co	13800	15400
Spleen	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	7510	14980	-Co	4970	4820
	+Co	11135	14200	+Co	4780	6070
Pancreas	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	2280	3250	-Co	23200	16500
	+Co	2900	3940	+Co	9010	13800
Kidney	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	14800	18000	-Co	17700	17400
	+Co	28600	14500	+Co	8700	10700
Muscle	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	884	706	-Co	718	611
	+Co	1350	1760	+Co	427	575
Bone	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	1830	1970	-Co	407	227
	+Co	1570	2070	+Co	167	188
Eye	Treatment	-Se	+Se	Treatment	-Se	+Se
	-Co	896	821	-Co	1840	1157
	+Co	1370	1424	+Co	803	834

that those rats with selenium supplemented diets had slightly larger hearts, averaging .469 g as compared with .404 g for the low selenium groups. This difference can probably be attributed to three day age difference at time of injection.

Spleens of rats that had not received dietary selenium supplementation had a greater uptake of selenium-75 than animals that had received dietary selenium supplementation. This difference was significant at the 90% level (t = 2.24) when calculated on a basis of cpm per tissue (Table 7). Calculated as cpm per gram, results became quite variable (Table 8). This is probably due to some drying of the tissue which would affect the small mass of the spleen considerably. Results on the pancreas are also quite variable. This organ is quite diffuse in the rat and it is difficult to distinguish it from the adipose tissue. These factors make it difficult to quantitatively remove the organ.

The quadriceps femoris was selected for this study since this muscle could be rather easily quantitatively removed. Rats receiving a dietary cobalt supplement had a greater uptake of selenium-75 in this muscle. This was significant at the 90% level (t = 2.23) when calculated on a basis of cpm per tissue (Table 7) and at an 80% level (t = 1.92) when calculated on a basis of cpm per gram of tissue (Table 8). McConnell and Roth (1961) have shown that ^{75}Se-selenite is incorporated into muscle myosin and aldolase

in rabbits.

The femur was chosen for study not only because it is a large bone, but also because of ease of removal of the entire bone. No differences in the uptake of selenium-75 were noted between any of the dietary treatments. Selenite is taken up by the bone only in the marrow (Jacobsson and Hansson, 1965).

The uptake of selenium-75 by the eye was very low and did not show any differences due to dietary treatments.

The eye was included in this study because selenium has been shown to concentrate in the retina of the eye in several species (Siren, 1964). It is theorized that selenium functions in vision, but the nature of any possible function remains unknown.

The liver took up more cobalt-60 (all as cobaltous chloride) than any other organ. This uptake was reduced by dietary cobalt supplementation. This reduction was significant at the 80% level ($t = 1.72$) when calculated on a basis of cpm per tissue (Table 7).

Dietary supplementation with cobalt decreased the retention of the dose of cobalt-60 in the kidney. This decrease was significant at the 90% level (Tables 7 and 8) both when calculated on a basis of cpm per tissue ($t = 2.02$) and on a basis of cpm per gram of tissue ($t = 2.20$).

There was less uptake of cobalt-60 by pancreases from

animals fed the cobalt supplemented diet than from those receiving no dietary cobalt supplement. This decrease was significant at the 80% level ($t = 1.82$) when calculated on a basis of cpm per tissue (Table 7), but the results when calculated on a basis of cpm per gram of tissue were highly variable (Table 8). This is probably due to some drying of the tissue before it was weighed, even though care was taken to prevent evaporation.

In the heart there was a greater uptake of cobalt-60 in those groups of rats receiving selenium supplementation when calculated on a basis (Table 7) of cpm per tissue ($t = 1.89$, 80% significant). Examination of the weights of the hearts showed those animals receiving dietary selenium supplementation to have larger hearts (Appendix Table 7), averaging .403 g as compared with .315 g for the rats on the low selenium diets. Again, this difference is probably due to the three day difference in age between the low and high selenium groups at time of dissection. When calculated on a basis of cpm per gram (Table 8) of tissue there was a decrease in the uptake of the cobalt-60 in those rats receiving dietary cobalt (Table 8). This was 80% significant ($t = 1.90$).

Dietary cobalt had no effect on uptake of cobalt-60 by either the bone or the spleen.

The blood from animals fed cobalt supplemented diets took

up less cobalt-60 than blood from animals receiving no dietary cobalt supplement (Tables 7 and 8). This difference was significant at the 90% level both when calculated as cpm per ml ($t = 2.38$) and when calculated as cpm per gram ($t = 2.48$).

The dietary treatments had no effect on the uptake of cobalt-60 by either the skeletal muscle or the eye. The number of counts taken up by the eye was very small.

The fact that dietary supplementation of an element can decrease retention of that element suggests either of two possibilities or their combination. First, the element is adsorbed on binding sites on protein. The element supplied by the diet simply occupies many of these sites so that there are less binding sites available on which the radioisotope can be adsorbed.

Secondly, if the element is incorporated into tissue or used in metabolic functioning, the metabolic pathways using the element will be largely filled by previous dietary supplementation of the element. This would decrease retention of the radioactive isotope.

The lowered uptake of cobalt-60 by several organs due to dietary cobalt supplementation seems in conflict with the result of Comar (1941) that rats on a diet with a normal amount of cobalt excrete the same amount of a dose of radioactive cobalt as rats on a low cobalt ration. These differing results may be due to one of

two reasons. First, the difference in cobalt concentration between diets was not as great in Comar's (1946) experiment (0. 29 ppm) as in this experiment (1. 0 ppm). Secondly, Comar (1946) was comparing results with a stock laboratory ration with results on a special low mineral ration which was thought to have been lacking in other factors.

In no case did the interaction between cobalt and selenium approach significance. This test for interaction was made more difficult by the missing values due to the two experimental animals that died before the completion of the experiment. Had these values been present, tests for interaction would have been simpler and perhaps more significant.

Growth and Feed Consumption

Growth and feed consumption in all four groups of rats was the same except a reduction in weight and food consumed was noticed in the group with the unsupplemented diet (see Figures 1 and 2). Several of the rats of this group were entering the terminal stage of liver necrosis at this time, as shown by the death of two rats shortly after the last weighing. The onset of this stage is quite sudden and death occurs within 48 hours (Schwarz, 1962).

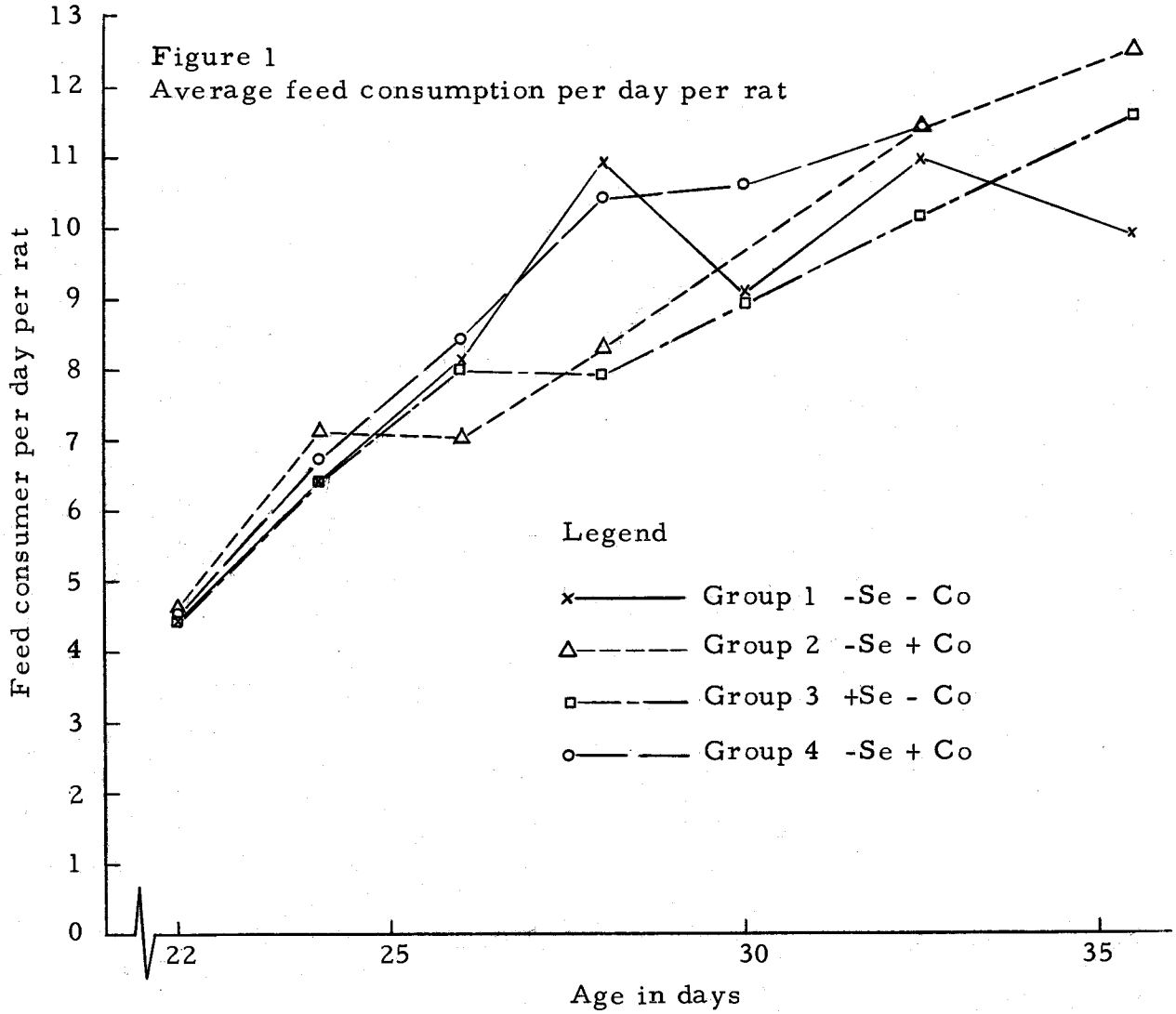
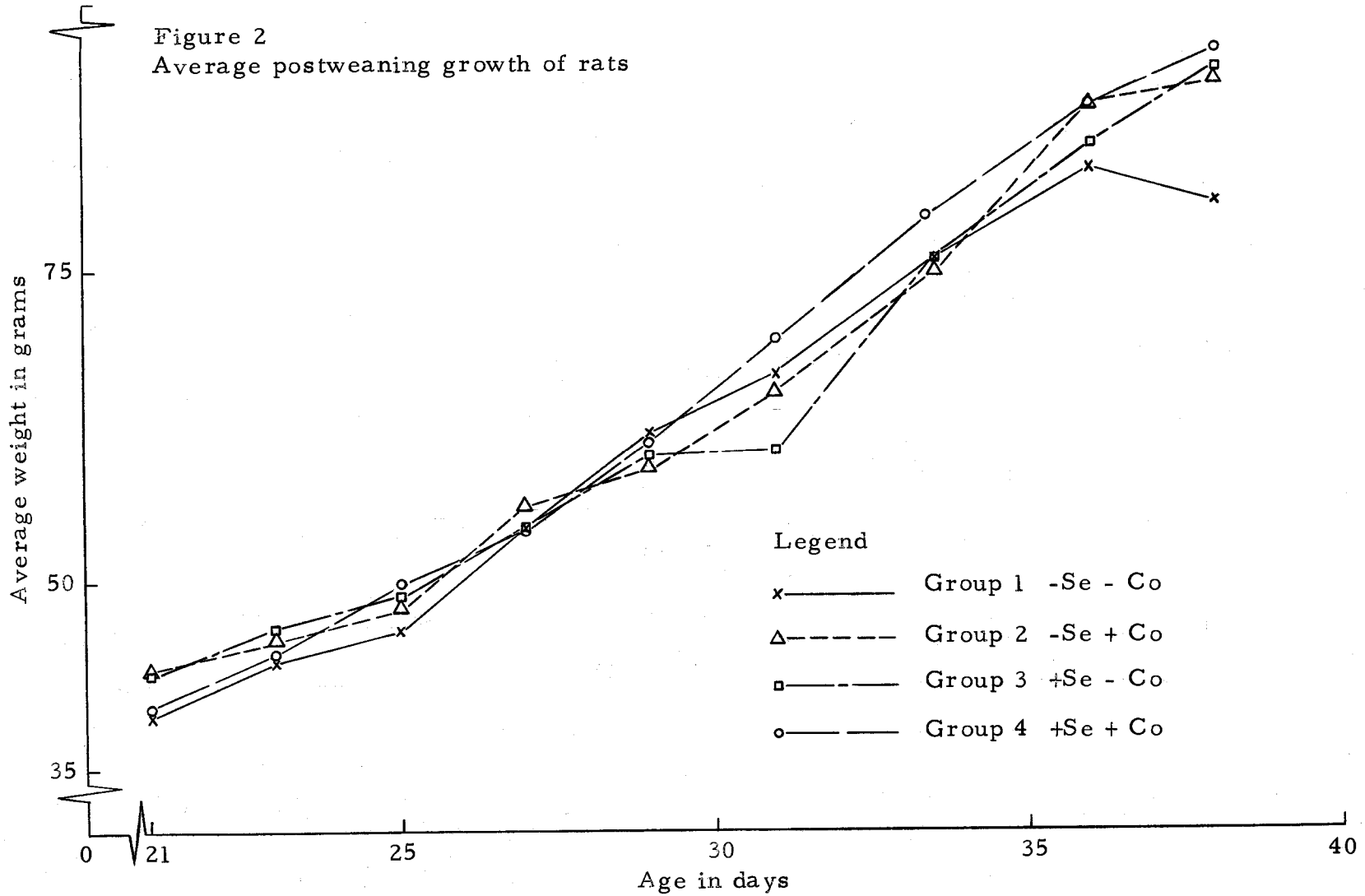


Figure 1
Average feed consumption per day per rat
Feed consumer per day per rat
13
12
11
10
9
8
7
6
5
4
3
2
1
0
22
25
30
35
Age in days
Legend
Group 1 -Se - Co
Group 2 -Se + Co
Group 3 +Se - Co
Group 4 -Se + Co

Figure 2
Average postweaning growth of rats



Summary

The only result of this experiment that suggests a relationship between selenium and cobalt is the increased uptake of ^{75}Se-selenite by the muscle in rats which had been fed diets supplemented with cobalt. This suggests that cobalt stimulates a metabolic pathway which involves selenium. Some possible metabolic effects will be discussed in the following section.

DISCUSSION AND CONCLUSION

Evidence has been presented in the previous sections that there may be a metabolic interrelation between cobalt and selenium in some species. The question now arises of what is the nature of any possible interrelation.

The possibility of cobalt and selenium competing in the same metabolic pathways seems rather unlikely due to their chemical dissimilarity. The partially filled 3d and filled 4s outer subshells gives cobalt typical metallic characteristics, while selenium has filled 4s and 4p outer subshells and nonmetallic characteristics (Kleinberg, Augersinger and Griswold, 1960). Cobalt has oxidation states of +2 and +3; the most stable being the +2. Selenium has oxidation states of +6, +4, +2 and -2; the most common being the +4 (Rosenfeld and Beath, 1964).

It is possible that cobalt is needed for the activation of an enzyme which affects the metabolism of selenium since cobalt is known to function in the activation of several enzymes. Yeast aldolase requires either Co^{++}, Fe^{++} or Zn^{++} for activation and glycylglycine dipeptidase requires either Co^{++} or Mn^{++} for activation (White, Handler, and Smith, 1964).

Inorganic selenium compounds have been shown to be incorporated into selenium analogs of sulfur containing compounds.

McConnell and Wabnitz (1957) found that after administration of $^{75}Se\ Cl_4$ to a dog, that selenium-75 had been incorporated into selenocystine and selenomethionine. Selenium-75 as selenious acid is incorporated also into cytochrome c (McConnell and Dallam, 1962). Therefore, whenever a sulfur compound occurs in metabolism it is likely that a selenium analog may also exist. Perhaps selenium is required as a nutrient because one of the selenium analogs will perform a needed function that the sulfur compound cannot perform.

Vitamin B_{12} has been shown to function in the metabolism of certain sulfur compounds. Vitamin B_{12} is known to function in the methylation of homocysteine to form methionine (White, Handler and Smith, 1964). Therefore it is possible that vitamin B_{12} also functions in transmethylation to form selenomethionine.

There is a definite requirement for vitamin B_{12} in reaction of methylmalonyl CoA to succinyl CoA. This suggests that B_{12} may function in a similar reaction with the selenium analog of coenzyme A.

One of the major pathways for excretion of subacute doses of selenite is the synthesis of dimethylselenide (Schultz and Lewis, 1940). Since vitamin B_{12} functions in methyl group metabolism it is possible that vitamin B_{12} is needed in the formation of dimethylselenide. The results of Gardiner (1966), indicating that a cobalt

deficiency increased the susceptibility of sheep to selenium toxicity, might be explained on the basis there was less detoxication of selenium by methylation under cobalt deficiency. This may explain why there is an increased level of selenium in the kidneys of cobalt deficient sheep due to selenium being in a nonexcretable form or in an inefficiently excreted form. In conclusion, therefore, the hypothesis that vitamin B_{12} functions in the formation of methylated selenium compounds appears to be the most logical explanation for a metabolic interrelationship between cobalt and selenium.

These two studies emphasize the importance of studying metabolic interrelationships on the whole organism and also various species, as there may be different metabolic requirements which vary with species, age, sex, heredity, as well as environmental influences.

BIBLIOGRAPHY

Allaway, W. H. and E. E. Cary. 1964. Determination of submicrogram amounts of selenium in biological material. Analytical Chemistry 26:1359-1362.

Allaway, W. H. et al. 1966. Movement of physiological levels of selenium from soils through plants to animals. Journal of Nutrition 88:411-418.

Andrews, E. D., A. B. Grant and B. J. Stephenson. 1964. Weight responses of sheep to cobalt and selenium in relation to vitamin B_{12} and selenium concentrations in the liver and kidney. New Zealand Journal of Agricultural Research 7:17-27.

Association of Official Agricultural Chemists. 1960. Official methods of analysis of the Association of Official Agricultural Chemists. 9th ed. Washington, D. C. 832p.

Blincoe, Clifton and W. B. Dye. 1958. Serum transaminase in white muscle disease. Journal of Animal Science 17:224-226.

Bonhorst, C. W. and I. S. Palmer. 1957. Metabolic interactions of selenate, sulfate and phosphate. Journal of Agricultural and Food Chemistry 5:931-933.

Brody, Samuel. Bioenergetics and growth. 1945. New York, Reinhold, 1023p.

Bunyan, J., E. E. Edwin and J. Green. 1958. Protective effect of trace elements other than selenium against dietary necrotic liver degeneration. Nature 181:1801.

Cobalt in rats. 1952. Nutrition Reviews 10:238-239.

Comar, C. L., G. K. Davis and R. F. Taylor. 1946. Cobalt metabolism studies: Radioactive cobalt procedures with rats and cattle. Archives of Biochemistry 9:149-158.

Comar, C. L. and G. K. Davis. 1947. Tissue distribution of radioactive cobalt administered to rabbits, swine and young calves. Journal of Biological Chemistry 170:379-389.

Comar, C. L. and F. Bronner. 1962. Mineral metabolism. New York, Academic, 623p.

Cuthbertson, W. F. J., A. F. Free and D. M. Thornton. 1950. Distribution of radioactive cobalt in the rat. British Journal of Nutrition 4:42-48.

Dinusson, W. E., et al. 1953. Cobalt, alfalfa and meat scraps in drylot rations for growing fattening pigs. Journal of Animal Science 12:623-627.

Ganther, H. E. and C. A. Baumann. 1962a. Selenium metabolism. I. Effects of diet, arsenic, and cadmium. Journal of Nutrition 77:210-216.

Ganther, H. E. and C. A. Baumann. 1962b. Selenium metabolism. II. Modifying effects of sulfate. Journal of Nutrition 77:408-414.

Ganther, H. E. 1965. The fate of selenium in animals. World Review of Nutrition and Dietetics 5:338-366.

Ganther, H. E. 1966. Enzymatic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts. Biochemistry 5: 1089-1098.

Gardiner, M. R. 1966. Chronic selenium toxicity studies in sheep. Australian Veterinary Journal 42:442-448.

Gunn, S. A. and T. C. Gould. 1966. Specificity of response in relation to cadmium, zinc, and selenium. Paper presented at the First International Symposium on Selenium in Biomedicine, Oregon State University, Corvallis, Oregon, (in press).

Halverson, A. W., P. L. Guss and O. E. Olson. 1962. Effect of sodium salts on selenium poisoning in the rat. Journal of Nutrition 77:459-464.

Harley, W. J. and A. B. Grant. 1961. A review of selenium responsive diseases of New Zealand livestock. Federation Proceedings 20:679-688.

Heinrich, Max and F. E. Kelsey. 1955. Studies on selenium metabolism: The distribution of selenium in the tissues of the mouse. Journal of Pharmacology and Experimental Therapeutics 114:28-32.

Hogue, D. E., et al. 1962. Relation of selenium, vitamin E, and an unidentified factor to muscular dystrophy in the lamb. Journal of Animal Science 21:25-29.

Hogue, D. E. and H. F. Hinz. 1964a. Anti-vitamin E activity of kidney beans in nutritional muscular dystrophy in the chick. Federation Proceedings 23:395.

Hogue, D. E. 1964b. Factors contributing to muscular dystrophy in the lamb. Paper presented at a Research Conference on Selenium-sulfur Interrelationships, Cornell University, Ithaca, N. Y., 1964. (Preprint)

Hopkins, L. L., A. L. Pope and C. A. Baumann. 1964. Contrasting nutritional responses to vitamin E and selenium in lambs. Journal of Animal Science 23:674-681.

Houk, A. E. H., A. W. Thomas and H. C. Sherman. 1946. Some interrelationships of dietary iron, copper and cobalt in metabolism. Journal of Nutrition 31:609-620.

Hubbell, R. B., L. B. Mendel and A. J. Wakeman. 1937. A new salt mixture for use in experimental diets. Journal of Nutrition 14:273-285.

Jacobsson, S. O. and E. Hansson. 1965. Distribution of selenium in rats studied by whole body radiography after injection of ^{75}-Se selenite. Acta Veterinaria Scandinavica 6:287-298.

Jensen, L. S., E. D. Walter and J. S. Dunlap. 1963. Influence of dietary vitamin E and selenium on the distribution of ^{75}Se in the chick. Proceedings of the Society for Experimental Biology and Medicine 112:899-901.

Keener, H. A., et al. 1950. A study of the function of cobalt in the nutrition of sheep. Journal of Animal Science 9:404-413.

Keener, H. A., R. R. Baldwin and G. P. Percival. 1951. Cobalt metabolism studies with sheep. Journal of Animal Science 10: 4238-433.

Kercher, C. J. and S. E. Smith. 1955. The response of cobalt deficient lambs to orally administered vitamin B_{12}. Journal of Animal Science 14: 458-464.

Kleinberg, Jacob, W. J. Argersinger, E. Griswold. 1950. Inorganic chemistry. Boston, Heath, 680p.

Kuttler, K. L. and D. W. Marble. Relationship of serum oxaloacetic transaminase to naturally occurring and artificially induced white muscle disease. American Journal of Veterinary Research 19: 632-636. 1958.

McConnell, K. P. 1941. Distribution and excretion studies in the rat after a single subtoxic subcutaneous injection of sodium selenate containing radioselenium. Journal of Biological Chemistry 141: 427-437.

McConnell, K. P. and R. D. Dallan. 1962. Time distribution examination of the in vivo incorporation of selenium into cytochrome c of the rat and its turnover. Nature 193:746-748.

McConnell, K. P. and D. M. Roth. 1961. Selenium in rabbit skeletal muscle myosin. Federation Proceedings 20:299.

McConnell, K. P. and C. H. Wabnitz. 1957. Studies on the fixation of radioselenium in proteins. Journal of Biological Chemistry 226: 765-776. 1957.

McLean, A. E. M. 1960. Phenergan and versene in dietary liver necrosis. Nature 185:191-192.

McNaught, K. J. 1948. Spectrophotometric determination of cobalt in pastures and animal tissues. New Zealand Journal of Science and Technology, sec. A, 30:109-115.

Marston, H. R. and H. J. Lee. 1949. Primary site of action of cobalt in the ruminant. Nature 164:529-530.

Mason, K. E. and J. O. Young. 1966. Effectiveness of selenium and zinc in protecting against cadmium induced injury of the rat testis: Paper presented at the First International Symposium on Selenium in Biomedicine, Oregon State University, Corvallis, Oregon, 1966. (In Press)

Mason, J. H. and F. Wroblewski. 1957. Serum glutamic oxaloacetic transaminase activity. Archives of Internal Medicine 99: 245-252.

Muth, O. H. 1955. White muscle disease (myopathy) in lambs and calves. I. Occurrence and nature of the disease under Oregon conditions. Journal of the American Veterinary Medical Association 126:355-360.

Muth, O. H., et al. 1958. Effects of selenium and vitamin E on white muscle disease. Science 128:1090.

Muth, O. H., et al. 1959. White muscle disease in lambs and calves. VI. Effects of selenium and vitamin E on lambs. American Journal of Veterinary Research 20:231-234.

Muth, O. H., J. R. Schubert and J. E. Oldfield. 1961. White muscle disease in lambs and calves. VII. Etiology and prophylaxis. American Journal of Veterinary Research 22:466-469.

Oldfield, J. E., O. H. Muth and J. R. Schubert. 1960. Selenium and vitamin E as related to white muscle disease in lambs. Proceedings of the Society for Experimental Biology and Medicine 103:799-800.

Patterson, E. L., R. Milstrey and E. L. R. Stokstad. 1957. Effect of selenium in preventing exudative diathesis in chicks. Proceedings of the Society for Experimental Biology and Medicine 95:617-620.

Proctor, J. F., D. E. Hogue and R. G. Warner. 1958. Selenium, vitamin E and linseed oil meal as preventatives of muscular dystrophy in lambs. Journal of Animal Science 17:1183-1184.

Ray, S. N., et al. 1948. Studies on the role of cobalt in sheep nutrition. Journal of Animal Science 7:3-25.

Rosenfeld, Irene and O. A. Beath. 1964. Selenium-geobotany, biochemistry, toxicity and nutrition. New York, Academic. 411p.

Schubert, J. R., et al. 1961. Experimental results with selenium in white muscle disease of lambs and calves. Federation Proceedings 20:689-694.

Schultz, Julius and H. B. Lewis. 1940. The excretion of volatile selenium compounds after the administration of sodium selenite to rats. Journal of Biological Chemistry 133:199-207.

Schwarz, Klaus, et al. 1957a. Prevention of exudative diathesis in chicks by factor 3 and selenium. Proceedings of the Society for Experimental Biology and Medicine 95:621-625.

Schwarz, Klaus and C. M. Foltz. 1957b. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. Journal of the American Chemical Society 79:3292-3293.

Schwarz, Klaus and C. M. Foltz. 1958. Factor 3 activity of selenium compounds. Journal of Biological Chemistry 233:245-251.

Schwarz, Klaus, W. Mertz and E. J. Simon. 1959a. In vitro effect of tocopherol metabolites on respiratory decline in dietary necrotic liver degeneration. Biochimica et Biophysica Acta. 32:484-491.

Schwarz, Klaus, E. E. Roginski and C. M. Foltz. 1959b. Ineffectiveness of molybdenum, osmium, and cobalt on dietary liver degeneration. Nature 183:472-473.

Schwarz, Klaus. 1961. Development and status of experimental work on factor 3- selenium. Federation Proceedings 20:666-673.

Schwarz, Klaus. 1962. Vitamin E, trace elements, and sulfhydryl groups in respiratory decline. Vitamins and Hormones 20:463-484.

Schwarz, Klaus. 1965. Role of vitamin E selenium and related factors in experimental nutritional liver disease. Federation Proceedings 24:58-67.

Sigma Chemical Company. 1965. The colorometric determination of glutamic-oxaloacetic and glutamic pyruvic transaminases. 3rd ed. St. Louis. 12p. (Sigma Technical Bulletin No. 505)

Siren, M. J. 1964. Is selenium involved in the excitation mechanism of photoreceptors? Science Tools 11:37-43.

Smith, S. E., B. A. Koch and K. L. Turk. 1951. The response of cobalt deficient lambs to liver extract. Journal of Nutrition 44:455-464.

Smith, S. E. and J. K. Loosli. 1957. Cobalt and vitamin B_{12} in ruminant nutrition: a review. Journal of Dairy Science 40:1215-1227.

Swingle, K. F., S. Young and H. C. Dang. 1959. The relationship of serum glutamic oxaloacetic transaminase to nutritional muscular dystrophy in lambs. American Journal of Veterinary Research 20: 75-77.

Thompson, J. F. and G. H. Ellis. 1947. Is cobalt a dietary essential for the rabbit? Journal of Nutrition 34:121-127.

Trelease, S. F. and O. A. Beath. 1949. Selenium, its geological occurrence and its biological effects in relation to botany, chemistry, agriculture, nutrition and medicine. New York, published by authors, 292p.

Ulrich, Frank and D. H. Copp. 1951. Metabolism of radioactive cobalt in normal and alloxan diabetic rats. Archives of Biochemistry and Biophysics 31:148-153.

Underwood, E. J. 1940. The significance of trace elements in nutrition. Nutrition Abstracts and Reviews 9:515-534.

Underwood, E. J. 1962. Trace elements in human and animal nutrition. 2d ed. New York, Academic. 429p.

White, Abraham, P. Handler and E. L. Smith. 1964. Principles of biochemistry. 3d ed. New York, McGraw Hill. 1106p.

APPENDIX

Table 1. Weights and SGOT values of lambs at six weeks of age

High Selenium Lambs

Number	Weight at 6 weeks (pounds)	SGOT at 6 weeks (sigma frankel units)
745	39	83
746	33	45
747	48	103
748	28	64
764	36	97
769	$26\frac{1}{2}$	79
770	30	99
772	28	60
Average	34	79

Low Selenium Lambs

without white muscle disease

Number	Weight at 6 weeks (pounds)	SGOT at 6 weeks (sigma frankel units)
781	-	112
785	40	86
789	$36\frac{1}{2}$	147
791	39	70
801	24	143
810	34	200
Average	35	126

with white muscle disease

Number	Weight at 6 weeks (pounds)	SGOT at 6 weeks (sigma frankel units)
784	26	380
786	$23\frac{1}{2}$	973
787	$32\frac{1}{2}$	2744
803	30	10201
805	33	435
808	26	715
Average	29	2575

Table 2. Selenium and cobalt concentration of tissues from high selenium lambs

Lamb	Selenium (ppm dry weight)				Cobalt (ppm dry weight)			
	heart	kidney	liver	muscle	heart	kidney	liver	muscle
745	.68	2.36	2.50	.90	.01	.28	.32	.05
746	1.18	3.08	3.02	.92	.01	.35	.38	.02
747	.58	4.60	.96	.06	.00	.36	.40	.08
748	1.15	2.95	4.37	.76	.03	.28	.15	.01
764	1.35	4.05	3.12	.86	.08	.31	.19	-
769	1.56	1.28	3.60	.78	.05	.21	.21	-
770	1.35	3.99	5.70	.96	.02	.12	.25	.02
772	1.61	4.02	3.50	.71	.00	.18	.23	.05
Average	1.18	3.29	3.60	.84	.03	.26	.27	.04

Table 3. Selenium and cobalt concentration of tissues from normal low selenium lambs

Lamb	Selenium (ppm dry weight)				Cobalt (ppm dry weight)			
	heart	kidney	liver	muscle	heart	kidney	liver	muscle
781	.00	.42	.06	.04	.01	.23	-	.06
785	.03	.73	.03	.04	.05	.40	.37	.12
789	.24	.42	.06	.26	1.06	.35	.30	.07
791		.96		.36	.01	.29	.23	.05
801	.00	.39	.04	.00	.07	.26	.22	-
810	.04	.52	.06	.04	.06	.11	.18	.01
Average	.06	.57	.05	.11	.04	.27	.25	.06

Table 4. Selenium and cobalt content of tissues from low selenium lambs with white muscle disease

Lamb	Selenium (ppm dry weight)				Cobalt (ppm dry weight)			
	heart	kidney	liver	muscle	heart	kidney	liver	muscle
784	.04	.63	.07	.03	-	.14	.39	.05
786	.06	.79	.09	.06	.03	.18	.30	.02
787	.06	.87	.07	.05	.04	.14	.34	.03
803	.01	.38	.02	.01	.07	.12	.29	-
805	.08	.46	.01	.08	.06	.07	.23	.03
808	.00	.72	.04	.00	.04	.07	.21	.03
Average	.04	.64	.05	.04	.05	.12	.29	.03

Table 5. Total counts per minute per tissue

Tissue	CPM ^{75}Se			CPM ^{60}Co		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Blood per ml	-Co	5417	5399	-Co	816	865
		-	5483		-	909
	+Co	5422	-	+Co	557	559
		4819	6803		590	574
Heart	-Co	1420	1841	-Co	944	1440
		-	1757		-	1529
	+Co	1427	1969	+Co	795	1389
		947	2012		716	881
Liver	-Co	53386	43023	-Co	79474	93025
		-	56578		-	56545
	+Co	91835	42741	-Co	47938	70907
		62179	69908		46122	44378
Spleen	-Co	796	401	-Co	685	677
		-	773		-	394
	+Co	1019	468	+Co	379	563
		990	439		589	451
Pancreas	-Co	694	595	-Co	4587	3733
		-	493		-	3176
	+Co	968	595	+Co	2055	3571
		505	876		1989	1885
Kidney	-Co	18450	15920	-Co	14472	16306
		-	17667		-	12800
	+Co	39411	13809	+Co	7836	12557
		25547	17880		9046	7232
Muscle	-Co	508	430	-Co	294	341
		-	348		-	356
	+Co	612	602	+Co	281	343
		649	910		185	202

Table 5. Continued

Tissue	CPM ^{75}Se			CPM ^{60}Co		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Bone	-Co	708	608	-Co	595	1164
			778		-	1211
	+Co	699	938	+Co	765	1157
		533	727		684	726
Eye	-Co	85	95	-Co	59	109
			99			71
	+Co	149	69	+Co	73	68
		78	98		18	52

Table 6. Counts per minute per gram of tissue

Tissue	CPM ^{75}Se			CPM ^{60}Co		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Blood	-Co	5448	5206	-Co	994	1130
			5344			888
	+Co	6166		+Co	555	548
		4768	7023		607	574
Heart	-Co	3310	3365	-Co	3178	3591
			3405			3710
	+Co	3630	3593	+Co	2641	3179
		2420	6429		2069	2435
Liver	-Co	12479	10813	-Co	20729	41437
			14988			20911
	+Co	24148	9008	+Co	13576	18332
		16970	15211		13997	12519
Spleen	-Co	7508	3715	-Co	4966	6151
			6443			3489
	+Co	7899	7547	+Co	4159	4855
		11135	20924		5400	7277
Pancreas	-Co	2283	2127	-Co	23169	19962
			4365			13081
	+Co	3951	2805	+Co	8422	11519
		1857	5066		9606	16113
Kidney	-Co	14796	15308	-Co	17670	19717
			20741			15041
	+Co	35157	12844	+Co	9273	12645
		22042	16143		8121	8846
Muscle	-Co	884	725	-Co	718	639
			687			583
	+Co	1212	1192	+Co	449	683
		1486	2328		405	467

Table 6 - continued

Tissue	CPM ^{75}Se			CPM ^{60}Co		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Bone	-Co	1828	2840	-Co	4075	2477
			1096			2070
	+Co	1625	1568	+Co	1809	2183
		1521	2576		1533	1577
Eye	-Co	896	853	-Co	1840	1448
			790			867
	+Co	1842	1006	+Co	1174	1013
		899	1842		432	656

Table 7. Tissue Weights

Tissue	Weights of tissue from ^{75}Se injected rats (grams)			Weights of tissue from ^{60}Co injected rats (grams)		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Blood	-Co	1.001	2.071	-Co	.821	.583
			2.049			2.049
	+Co	1.756		+Co	1.546	2.042
		.502	1.257		1.012	1.899
Heart	-Co	.429	.547	-Co	.297	.401
			.516			.412
	+Co	.393	.548	+Co	.301	.437
		.391	.313		.346	.362
Liver	-Co	4.278	3.979	-Co	3.834	2.245
			3.775			2.704
	+Co	3.803	4.745	+Co	3.531	3.868
		3.664	4.596		3.295	3.545
Spleen	-Co	.106	.108	-Co	.138	.110
			.120			.113
	+Co	.029	.062	+Co	.091	.116
		.171	.021		.109	.062
Pancreas	-Co	.304	.280	-Co	.198	.187
			.113			.243
	+Co	.245	.212		.244	.310
		.272	.173		.207	.170
Kidney	-Co	1.247	1.040	-Co	.819	.827
			.853			.851
	+Co	1.121	1.075	+Co	.845	.993
		1.159	1.103		1.114	.818
Muscle	-Co	.575	.593	-Co	.409	.533
			.507			.611
	+Co	.505	.505	+Co	.625	.502
		.437	.391		.457	.432

Table 7 - continued

Tissue	Weights of tissue from ^{75}Se injected rats (grams)			Weights of tissue from ^{60}Co injected rats (grams)		
	Treatment	-Se	+Se	Treatment	-Se	+Se
Bone	-Co	.387	.214	-Co	.146	.470
			.486			.583
	+Co	.430	.598	+Co	.425	.530
		.350	.282		.446	.460
Eye	-Co	.095	.116	-Co	.032	.075
			.099			.082
	+Co	.081	.052	+Co	.062	.067
		.087	.053		.041	.079