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To the Graduate Council:

I am submitting herewith a thesis written by Robert G. Buescher entitled "The Effect of Excessive Dietary Calcium on the Absorption and Utilization of Selenium-75, Molybdenum-99, Copper-64 and Iron-59 in Swine." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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THE EFFECT OF EXCESSIVE DIETARY CALCIUM UPON THE ABSORPTION AND UTILIZATION OF SELENIUM-75, MOLYBDENUM-99, COPPER-64 AND IRON-59 IN SWINE

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Robert G. Buescher

August 1961

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CHAPTER I

INTRODUCTION

The known essential mineral elements for swine are: calcium, phosphorus, copper, iron, sodium, chlorine, magnesium, manganese, cobalt, iodine, zinc, potassium and sulfur. Richert and Westerfield (1953) and De Renzo et al. (1953) were first to discover a role of molybdenum in animal nutrition and first to associate molybdenum with the enzyme, xanthine oxidase. More recently, Eggert et al. (1957), observed a partial role of selenium in connection with alleviating vitamin E deficiency in swine. Even though a deficiency of these two elements does not normally occur, the possibility arises as ingredients of swine rations are constantly being varied in new formulations.

Copper and iron are two essential elements with which workers have compiled volumes of data and yet they are still found to be deficient in many areas in which swine are produced. Iron has been known to be a constituent of hemoglobin for years. Likewise, copper was found to be essential in the process of hemoglobin formation. With the development of continuously feeding swine on concrete floors and the rapid growth rate of baby pigs, iron deficiencies have produced numerous cases of anemia and general unthriftiness. Copper may also be involved in these deficiencies.

Quantitatively, calcium is the major mineral constituent of the body. Studies of poor bone growth date back to the mid-nineteenth century. Voluminous data have been compiled since this era. Optimum

growth levels and proper balancing with phosphorus have been reported periodically. Calcium deficiencies, excesses and unbalanced calcium-phosphorus ratios are not uncommon. Various studies have been conducted on the interrelationships of calcium and other elements. These studies mainly have arisen due to nutritional difficulty, as in the case of calcium and zinc. The outbreak of parakeratosis brought about many studies of calcium and zinc, (Tucker and Salmon, 1955; Conrad and Beeson, 1957; Luecke et al., 1957; Lloyd and Bell, 1958; Berry et al., 1960; and many others). This relationship, however, was mentioned to possibly exist by Kletzien (1940).

Recently, increasing the calcium level in the diet of growing rats has been shown to be a decontaminant in that it decreases the retention of strontium in the body (Wasserman et al., 1957; Mraz and Cragle, 1960). Working with human patients, Spencer et al., (1956), Spencer et al. (1957), Spencer et al. (1958) and Samachson et al. (1958), have confirmed that high urinary calcium excretion levels result in higher radiostrontium excretions. This is a forward drive in the protection of humans and animals in this "Atomic Age", however, further elucidation of excessive calcium effects on other elements is necessary.

Calcium is a relatively low-cost item in practical swine rations. Because of this and the decontamination aspect of the fission product strontium-90, this study was undertaken. To date, little is known of the effects of excessive calcium upon the other mineral elements of swine rations.

The objectives of this study were:

1. To determine the absorption and utilization of Selenium-75, Molybdenum-99, Copper-64 and Iron-59 when feeding excessive calcium to growing swine.

2. To further determine the distribution of the radioactive elements in the tissues of these animals.

3. To determine growth rate, feed consumption and feed efficiency when feeding excessive levels of calcium.

These isotopes were selected for this study because of their properties, which made it possible to employ the "multiple dosing technique". Se-75 and Mo-99 were used together because both are gamma emitters and Se-75 has a half-life of 120 days whereas Mo-99 has a half-life of 2.8 days. Likewise copper and iron were used simultaneously since Fe-59 has a half-life of 45.1 days and Cu-64 has a half-life of 12.8 hours and both are gamma emitters. Each of the samples could be counted twice and the short half-life isotope corrected for decay and determined by difference.

CHAPTER II

LITERATURE REVIEW

Selenium

The role of selenium. Selenium toxic properties have been studied to a great extent. An essential function for selenium in plants, animals or microorganisms has not been clearly shown. Selenium is known to be readily absorbed from seleniferous diets and to accumulate in the body tissues and fluids. In a study by Levine (1925), selenium was shown to have direct effect on certain unicellular organisms and enzyme systems. Also selenium was reported to inhibit alcoholic fermentation in yeast by Potter and Elvehjem (1936). Klug et al. (1950) demonstrated that the liver succinic dehydrogenase levels of rats fed seleniferous diets are reduced below normal. Wright (1940) found that an inhibiting effect of selenium has been observed on urease but not on cholinesterase, catalase or liver arginase.

Schwarz and Foltz (1957) reported the presence of organically bound selenium in active preparation of "factor 3". Inorganic selenium was found to protect rats completely against the development of necrotic liver degeneration. Protection was obtained on levels as low as 4.0 micrograms of selenium per 100 grams of diet. The quantity of selenium given one rat per day was only a few tenths of one microgram. From these results, the investigators stated that selenium is an essential trace element and that from other observations it was stated that selenium may function in oxidation-reduction reactions. Scott et al. (1957)

demonstrated that selenium in amounts of 10 to 100 micrograms per 100 grams of diet prevented mortality and exudative diathesis and promoted growth of chicks fed a vitamin E deficient torula yeast diet. Likewise, Stokstad et al. (1957) confirmed the above scientists' work by stating that selenium as selenite at the level of 0.3 p.p.m. gave complete protection against exudative diathesis in chicks fed a torula yeast diet.

A partial role of vitamin E and selenium in the pig was found by Eggert et al. (1958). These workers, using trios of littermate pigs, fed a basal ration low in vitamin E, basal plus 40 p.p.m. vitamin E, and basal plus 1.0 p.p.m. selenium as sodium selenite. Four of the six pigs on the basal ration died in 53 days and upon autopsy showed a marked necrosis of the liver and two of the four showed a yellow discoloration of body fat. None of the pigs receiving selenium or vitamin E died. There were no differences in weight gains up to the time of death. All pigs were sacrificed at 57 to 60 days with no evidence of liver necrosis or discoloration of body fat. Likewise, Proctor et al. (1958), corroborated by Oldfield et al. (1960) and Welch et al. (1960), found selenium to be a factor in prevention of nutritional muscular dystrophy in lambs which can also be prevented and cured by administering vitamin E. McLean et al. (1959) injected 1.0 milligram selenium as sodium selenate subcutaneously every 7 to 10 days for 105 days in lambs and found a significant growth response. In contrast, Maplesden and Loosli (1960) reported that selenium would not prevent nutritional muscular dystrophy in dairy calves, but they were able to prevent it with vitamin E.

Selenium and reproduction. Rat fertility can be influenced by selenium (selenite). Rosenfeld and Beath (1954) supplied 7.5 p.p.m. selenium in the drinking water of rats and found that selenite prevented reproduction in the females but had no effect on fertility of the males. At levels of 2.5 p.p.m. the selenite only reduced the number of young reared by the second generation of mothers.

The effect of selenium on swine reproduction was studied by Wahlstrom and Olson (1959) who discovered that 10 p.p.m. of selenium (selenite) lowered the conception rate and increased the number of services required per conception. Sows fed the selenium ration farrowed a higher percentage of dead pigs and their pigs were smaller and weaker at birth. Fewer of these pigs were weaned and the weaning weights were reduced.

Absorption and excretion of selenium. On a selenium-free ration, no selenium can be detected in the tissues or organs of animals. In selenium poisoning, the concentrations present in the tissues depends upon the amounts and forms administered and the length of time of administration. Little is known of the factors influencing selenium absorption in animals and apparently nothing is known of the main site or sites of absorption of this element within the gastro-intestinal tract.

Studies have indicated that selenium is rapidly absorbed from naturally seleniferous diets and from soluble salts of selenium in purified diets. Anderson and Moxon (1941) observed no differences in retention of naturally occurring selenium from seleniferous wheat and

of sodium selenite administered at the same level. Smith et al. (1938), using higher levels of dietary selenium, obtained greater retention from naturally occurring organic selenium than from sodium selenite.

The breath, urine and feces are channels of excretion for selenium. Only with very high levels are appreciable amounts of selenium exhaled. In a study by McConnell (1942), rats were found to have exhaled 3-10 per cent of a single subtoxic dose of radioactive selenium as sodium selenate within 24 hours after injection. At ordinary intakes, the urine is reported to be the primary pathway of selenium excretion. In more recent studies, McConnell and Martin (1953), found in two experiments that rats excreted 62 and 44 per cent of the total selenium during the first four hours. These investigators reported that the destruction and elimination of selenium-bound hemoglobin may take place in part of the liver, and the degradation products appear in the bile, where a fraction of the selenium is combined with the bile pigments. Moxon et al. (1940) found that the administration of bromo-benzene increased the excretion of selenium from the animal body.

Selenium toxicity. Selenium toxicity dates back over 100 years. It is a naturally occurring disease of livestock known as "alkali disease" and "blind staggers". It is most prevalent in the semi-arid areas of the Great Plains of North America. Intensive studies, therefore, have been undertaken by a multitude of workers. A more detail review is presented by Trelease and Beath (1949), Underwood (1956) and Shrift (1957).

Sulfate has consistently been found to counteract selenate

toxicity in microorganisms and higher plants. High protein levels are known to decrease the toxicity of seleniferous diets in rats, but the reports on results obtained with methionine are conflicting. In a paper by Klug et al. (1952), methionine failed to protect rats on seleniferous wheat. However, methionine, as well as choline and betaine, which also are methyl donors, offered partial protection to rats against selenite toxicity in work by McConnell (1952). Arsenic, at a level of 5 p.p.m. in the drinking water of rats, completely prevented all symptoms of selenium toxicity as demonstrated by Moxon and Rhian (1943). In their research, they found that at a concentration of 5 p.p.m., fluorine, molybdenum, chromium, vanadium, cadmium, zinc, cobalt, nickel and uranium increased mortality, whereas, lead had no effect and tungsten decreased mortality and prevented, to some extent, the typical liver lesions.

In more recent swine work by Wahlstrom et al. (1955 and 1956) it was discovered that excellent protection against up to 10 p.p.m. of selenite selenium, was given by 0.02 per cent arsanilic acid or 0.005 per cent 3-nitro, 4-hydroxyphenylarsonic acid.

Molybdenum

The role of molybdenum. Molybdenum, because of its toxic properties has been investigated by many scientists. A disorder known as "teart" or "molybdenosis" has been known for more than one hundred years and associated with certain pasture areas in England and United States. The molybdenum toxicity has been shown to be alleviated by

administering copper and inorganic sulfur.

Molybdenum has been shown to be required by all nitrogen-fixing organisms in legumes and higher plants. Arnon and Stout (1939) investigated molybdenum as an essential element for higher plants. Using purified nutrient solutions, these workers discovered that tomatoes required 0.01 p.p.m. molybdenum to suffice their needs, however, they would tolerate up to 10.0 p.p.m.

The role of molybdenum in animal nutrition is not clearly understood. The first indication of importance in animal nutrition arose in the present decade. Richert and Westerfield (1953) and De Renzo et al. (1953) isolated and identified the xanthine oxidase factor to contain molybdenum. These studies were conducted with rats, whose requirement was not determined, but 0.02 micrograms of molybdenum per gram of diet were sufficient to produce a "saturation level" of xanthine oxidase.

Mo-99 was found in the xanthine oxidase fraction of milk from dairy cows by Totter et al. (1953), when they administered Mo-99. A study of the effect of molybdenum upon growth, hemoglobin, reproduction and lactation of rats was conducted by Jeter and Davis (1954). These workers observed no effect on hemoglobin with any of the copper levels (5 and 20 p.p.m.) or molybdenum levels (less than 1.0, 20, 80 and 140 p.p.m.) used. With 80 p.p.m. molybdenum and 20 p.p.m. copper, growth was not significantly retarded, but when the copper level was reduced to 5 p.p.m., the growth of the male rats was retarded at all levels of molybdenum. These workers also noted that 75 per cent of the male rats

on diets of 80 and 140 p.p.m. molybdenum were sterile. Limited studies also indicated degeneration of the testis.

Shirley et al. (1954) demonstrated the placental transfer of Mo-99 and Ca-45 in swine. In this study Mo-99 was readily absorbed in sows and widely distributed in their tissues. Indications of a placental barrier were found upon sampling various tissues of the fetuses where little or no Mo-99 was found. The Ca-45 was readily transferred to the developing fetus.

Experimenting with molybdenum as a dietary essential for lambs, Ellis et al. (1958) added molybdenum and copper to rumen microorganisms from alfalfa-fed lambs. They discovered a prominent increased (in vitro) cellulose degradation to the same degree as when alfalfa ash was added. These workers also state that in feeding lambs a ration with added molybdenum, they made faster gains than did the controls. The addition of molybdenum increased the digestibility of cellulose but did not alter the digestibility of other proximate ration constituents. Their research demonstrated a nutritional role for molybdenum in growing lambs for optimum cellulose digestion by rumen flora. Reid et al. (1956) using purified diets on chicks and poults indicated that addition of molybdenum resulted in a growth of 14-19 per cent over the unsupplemented controls. A requirement was not determined but was reported to possibly exist.

A stimulation of growth and an increase in xanthine dehydrogenase activity of liver and intestinal tissues of chicks were observed by Kurnick et al. (1957). In these studies, molybdenum was added as

sodium molybdate to purified and practical diets of chicks. These researchers also stated that availability studies with rats, chicks and turkeys indicated that the availability of molybdenum was influenced not only by the genus of the animal but also by the direction and magnitude of the genus difference.

In a study using 0.51 to 0.80 p.p.m. molybdenum in a casein-cerelose diet, Leach and Norris (1957) reported no effect on growth of chicks and that tungsten reduced growth and molybdenum content of the livers. Also in this study, molybdenum failed to promote growth in hatchery chicks fed a soybean meal cerelose diet. Teekell and Watts (1959) found a significant growth response of chicks on a practical type ration with supplemental molybdenum levels of 1, 5, and 10 p.p.m. whose dams had received supplemental tungsten. They suggested a synergistic effect between molybdenum and tungsten for growing chicks.

Calcium and molybdenum. The metabolism of phosphorus-32 and molybdenum-99 in rats receiving high calcium diets was demonstrated by Arrington and Davis (1955). The levels of calcium were not given; however, it was stated that the control diet contained a calcium to phosphorus ratio of 1.3 to 1.0. The high calcium diet was prepared by adding calcium carbonate to the basal ration so that the calcium phosphorus ratio was 3.0 to 1.0. The phosphorus level, likewise, was not given but it was reported that the levels were adequate. The metabolism of Mo-99 was not altered by the presence of excess calcium. More than 50 per cent of the Mo-99 oral dose was excreted in the urine and approximately 8 per cent in the feces within 24 hours after administering

the dose. It appeared that the urinary excretion of Mo-99 by the rats receiving excess calcium in their diets was less than the control. This difference was not statistically significant. The amount of Mo-99 in the tissues of the rat were similar on both diets. The kidney and blood contained the most Mo-99 followed by the bones, liver and muscle. There was considerable variations in the quantity of Mo-99 in the excreta and tissues between individuals of both groups. It was concluded that increased calcium intake had no effect on absorption, retention or excretion of Mo-99. In this experiment, these workers also state that there was a decreased urinary excretion and an increase in tissue deposition of P-32. A significant retention of P-32 was demonstrated when the rats were fed the high calcium diet.

In the case of plants, Marston (1952), stated that alkaline conditions favor liberation of molybdenum from unavailable "fixed" state in which it occurs within certain soils and so greatly increases the uptake of this element by plants grown in them. Absorption of molybdenum by plants from culture solutions is increased by the addition of phosphate and is decreased by sulfate addition.

Molybdenum toxicity. Molybdenum toxicity, as previously stated, has been investigated by many scientists. The fact that copper alleviates molybdenum toxicity as well as interacting with inorganic sulfur in the same function is discussed with the review of copper.

Neilands et al. (1948) studied the toxic effect of molybdenum in a purified diet fed to rats. In this study, copper additions to the diet largely overcame the molybdenum toxicity. Iron, zinc, and cobalt

appeared without effect on molybdenum toxicity. The fate of molybdenum was determined by use of radioactive molybdenum. Analysis at 2.5 hours, 1 day and two days revealed most of the element to be in the stomach, intestine, feces and urine. At the end of two days, the kidneys and bone contained slightly higher levels per gram than did other tissues examined.

A study was made on the effect of high molybdenum (0.2 per cent) intake on the distribution and excretion of Ca-45 and P-32 in the rabbit by Feaster and Davis (1959). These investigators found that neither soft tissue levels nor urinary or fecal excretion of total or radioactive calcium or phosphorus indicated any impairment in the absorption of these ions from the intestine. Some bone disorders were observed in the rabbits on high molybdenum intake but were declared not to be due to failure in deposition of calcium and phosphorus but a result from a failure in the organic phase of bone metabolism.

Cattle are the least tolerant to molybdenum toxicity, followed by sheep, rats, rabbits, horses and pigs, respectively (Underwood, 1956). Levels of molybdenum as high as 1000 p.p.m. were fed to pigs for 90 days by Davis (1950) with no apparent ill effects. This tolerance may be due to a lower absorption of this element.

Copper

The role of copper. The role of copper in hemoglobin production has been known over 30 years. This was first demonstrated by Hart et al. (1928), working with rats, and has been confirmed many

times by various workers. Elvehjem and Hart (1932), developed anemia in pigs on a whole milk diet and reported that when adding pure iron to the diet only a small temporary improvement in hemoglobin content was observed, but by supplying copper and iron to the diet, the recovery was rapid and complete.

In a review by Elvehjem (1935), it was stated that copper is present in all living matter. It was also pointed out that copper is necessary as a supplement to iron for hemoglobin production and that copper is involved in the transformation of ingested iron into hemoglobin but is not concerned with the assimilation of iron.

Protein compounds containing copper from plant and animals have been shown to be enzymes with an oxidase function, namely tyrosinase, laccase and ascorbic acid oxidase. Also butyryl CoA dehydrogenase has been found to contain copper.

Copper may have a role in thyroxine formation since copper and thyroxine readily complex, (Gemmill, 1960). Copper binding is discussed and reviewed in detail in a book edited by Seven and Johnson, (1960).

Working with swine, Teague and Carpenter (1951) studied copper deficiency and found that in conjunction with a dietary anemia, an unusual leg condition was encountered as shown by a lack of rigidity in the leg joints. They demonstrated copper to be therapeutic and in some cases brought about complete reversal of symptoms.

Somewhat conflicting is the work of Wintrobe et al. (1953) who produced a copper deficiency in swine fed ample iron. They found that

copper therapy without the simultaneous administration of iron was ineffective and also that a deficiency of copper lowers iron absorption.

The low level of copper and iron in mammalian milk reveals the importance of additional sources of these two elements during the suckling period. Lorenzen and Smith (1947) found that in rabbits and guinea pigs, both total amounts and liver copper concentration decreased during suckling, while with rats an increase in total copper in the livers was observed from birth even though the concentration decreased during sucklings.

Bass et al. (1956) obtained the highest average daily gain with growing-fattening swine, using 100 p.p.m. copper while the better feed efficiency resulted when 150 p.p.m. copper was fed. Using 125 p.p.m. copper, Hoefer et al. (1958) demonstrated a high average daily gain of 1.49 pounds by pigs which was equalled only by addition of 50 p.p.m. zinc to the control diet. The calcium content of the ration was 0.5 per cent and additions to the basal included: zinc, iron, zinc and iron and copper. They did not note any apparent difference in hemoglobin levels in this experiment. Hawbaker et al. (1959) found that feeding copper sulfate to swine at 0.1 per cent level resulted in the highest and most efficient gains.

Calcium and copper. Studies on effect of dietary calcium upon copper metabolism are limited. Using mice as the experimental animals, Tompsett (1940), indicated the absorption of copper from the alimentary tract was influenced by the gastric acidity and the calcium content of the diet. Dick (1954), stated that large amounts of calcium carbonate

and ferrous sulfide reduced the assimilation of copper in sheep.

In recent work with dairy calves, Hardison et al. (1958) observed that feeding low levels of 6.8 milligram per day of copper in the presence of calcium, manganese, phosphorus, cobalt, zinc and iron, significantly decreased the concentration of liver copper. These workers also stated that additional work with rats indicates the depression of liver copper may have been due to the presence of added calcium and/or phosphorus. However, the level of calcium and other elements were not given.

Using a semi-purified type ration, Carter et al. (1959) observed bowing of legs and weak pasterns in pigs fed 14 weeks on normal calcium with no added copper. They also reported a decrease in liver cytochrome C oxidase activity in pigs fed rations without added copper. With the same experiment, Miller et al. (1959) detected no effect of added dietary calcium upon the blood, muscle or rib copper concentration. The addition of calcium (0.65 per cent) to a basal ration containing 0.6 per cent calcium, 3 p.p.m. copper and 0.4 per cent phosphorus resulted in an increase in liver copper.

Other copper relationships. Copper has been shown to be either antagonistic or synergistic to elements other than calcium. Marston (1952) has reviewed the roles of copper, cobalt and molybdenum in the nutrition of animals and plants.

Dick (1953 and 1954) has demonstrated the interrelationships of copper, molybdenum and inorganic sulfur in sheep. This worker found that for a given intake of molybdenum, the limitation of copper storage

was proportional to the sulfate content of the diet. It was also stated that with a constant intake the amount of rise in blood level of copper is dependent upon the molybdenum intake.

Molybdenum toxicity can largely be overcome by feeding additional copper in the diet as confirmed by Neilands et al. (1948), Dick (1953 and 1954), Gray and Daniel (1954).

The interrelationships of copper, molybdenum and phosphorus in rats were investigated by Comar et al. (1949) using radioactive isotopes. They demonstrated the dependence of low level molybdenum toxicity upon the dietary copper intake. These workers suggest the toxic action of molybdenum may be due to the following mechanisms: (a) interference, due to a lowered level copper in enzyme systems necessary for skeletal metabolism, (b) inhibition of these enzyme systems by molybdenum, and (c) competition between molybdenum and phosphorus for bone deposition.

Kulwich et al. (1953) studied the interrelationships of copper, molybdenum and zinc in rats and swine. These investigators reported that excessive molybdenum substantially increased tissue copper concentrations and radiocopper accumulations. They also observed that zinc appeared not to be involved in the copper-molybdenum balance.

Testosterone propionate or estradiol benzoate administered to healthy males and post-menopausal women increased the copper content of their serum as reported by Johnson et al. (1959). Cox and Hale (1960) found beef tallow or testosterone having no effect while stilbestrol increased the content of copper in the serum of swine. How

conflicting or minor this relationship may seem, the possibility of increasing the copper or even iron content of mammalian milk or their young's blood level by hormonal treatment may be beneficial.

Additional copper relationships and actions are reviewed and discussed in more detail in Copper Metabolism (1950) and by Underwood in Trace Elements (1956).

Iron

The role of iron. The iron content of the body is only about 0.004 per cent of the body, yet the element plays a central role in life processes. Hemoglobin is known to contain about half the total body iron and insufficient amounts along with copper cause the condition known as anemia. Anemia is more prevalent in baby pigs than any other class of farm livestock. This disease was established to be an iron deficiency by Hart et al. (1928). These workers also discovered that feeding the sow considerable amounts of iron did not increase the content of the sow's milk, nor did it increase the total iron content of the pig's body or the amount of iron stored in its liver. However, in recent studies by Rydberg et al. (1959), the injection of 10 milliliters of iron solution to sows two weeks prior to farrowing, produced pigs which maintained a higher hemoglobin level during the critical post-partum period than did pigs from untreated sows or sows treated four weeks prior to farrowing.

Calcium and iron. Results dealing with the effect of calcium upon the iron utilization are somewhat controversial. In some of the

earlier iron work, Orten et al. (1936) suggested a tendency of increased iron utilization by the addition of calcium to the diet of rats.

Day and Stein (1938) demonstrated that calcium per se had no unique function in hematopoiesis when experimenting with rats fed a purified diet. These workers stated that a relative or absolute excess of phosphorus causes mild anemia and polycythemia and that vitamin D facilitated hematopoiesis probably through its effect upon calcium and phosphorus metabolism.

However, Anderson et al. (1940), using calcium-phosphorus ratios from 0.45 to 7.65, calcium levels from 0.29 per cent to 3.07 per cent of diet and phosphorus levels from 0.648 to 0.402 per cent of diet in rats, concluded that calcium carbonate retarded iron utilization and monobasic sodium phosphate had the opposite effect. That this was utilization and not merely hemoglobin formation was demonstrated by the fact that both hemoglobin regeneration and iron storage in liver increased as the calcium-phosphorus ratio decreased. These workers also noted the pH of the intestinal tract (colon) of animals fed a ration with a calcium-phosphorus ratio of 0.45 was distinctly lower (5.2 to 6.1) than those of animals fed a ration with a calcium-phosphorus ratio of 7.65, (6.55 to 7.18).

Kletzien (1940) fed rats a milk diet which produced a severe anemia. This worker investigated the effect of variation in the supply of calcium on the amount of iron in various organs and the entire body. The addition of 1.0 and 3.0 per cent of calcium carbonate to a dry calcium-deficient diet resulted in lower tissue iron values. The same results were obtained with calcium in the forms of lactate equivalent

to 2.5 per cent and chloride and tri-phosphate equivalent to 3.0 per cent of calcium carbonate when fed. However, when calcium sulfate was used, this was not the case.

Corroborating the latter studies, Freeman and Ivy (1942) found that high calcium carbonate and aluminum hydroxide definitely reduced iron retention by the anemic rat ingesting 0.25 milligram of iron daily.

In rat studies, Gubler (1956) stated that calcium in moderate amounts aided absorption by combining with iron phosphates, but excess amounts in the diet inhibited iron absorption. High levels of bone meal in rat diets were investigated by Chapman and Campbell (1957a), who found that varying levels of iron (2.16 to 2.98 mg./100 gm. diet) in the presence of excessive amounts of bone meal had no effect on food consumption, but retard hemoglobin regeneration. Then at higher levels of iron, (3.52 to 14. 82 mg./100 gm. diet), bone meal did not affect hemoglobin levels, nor did it affect the overall liver iron content. The heart weights decreased as the level of iron increased but the presence of bone meal had no effect on heart weights. The same investigators (1957b) discovered that six common calcium salts interfere with the utilization of iron.

Miller et al. (1959) showed excess calcium (1.25 per cent) did not decrease the liver iron content but the kidney iron was decreased in pigs. In a review, Davis (1959) stated that when high calcium diets are fed, it is possible to meet the animals' need for iron by increasing the dietary intake.

Other iron relationships. Experimenting with copper and iron,

Cunningham (1931) discovered copper feeding had no effect on storage of iron in the fetal rat or in the hen's egg. Likewise, iron feeding has no effect on storage or excretion of copper by the rat. In a review by Matrone (1960), the role of copper and iron and their interactions were discussed as to the nutrition and metabolism of animals.

Hegsted et al. (1949) demonstrated the addition of phosphate salts to the diet of rats revealed that the amount of iron deposited in the liver was inversely related to the phosphorus content of the diet. It was concluded that the absolute amount of iron and/or phosphorus in the diet as well as the iron-phosphorus ratio influences the amount of iron absorbed. Hartman et al. (1955) reported evidence that high levels of manganese reduced iron absorption in rats. Cotzias (1960) has reviewed the effects of excess calcium, iron and other elements upon manganese metabolism.

Calcium and Other Elements

The interrelationship of calcium, phosphorus and vitamin D had probably been the three most studied factors in nutrition research. However, the imbalance of these three factors still presents part of the major problems of livestock feeding. A ration containing excess calcium will not provide efficient assimilation of phosphorus even though sufficient amounts of phosphorus are present in the ration. This same situation is true with excess phosphorus and normal calcium. The ratio of calcium to phosphorus is less essential when adequate amounts of vitamin D are present.

The calcium-zinc relationship has also been studied in various

agricultural colleges due to the outbreak of parakeratosis. Swine rations containing 1.0 to 2.0 per cent calcium, with no zinc added, can cause severe outbreaks of parakeratosis. Forbes (1960) reviewed and discussed the nutritional interactions of zinc and calcium.

Research with rats indicates that high levels of calcium speeds the appearance of magnesium deficiency symptoms, increases mortality and lowers the growth rate if the level of magnesium is low. A recent review was given by O'Dell (1960) on the relationships of calcium and magnesium.

Calcium-strontium relationships have been of much interest because of radioactive "fallout". Wasserman et al. (1957) reported that as the dietary calcium level of growing rats increased, the retention of Ca-45 and Sr-85 decreased. Feeding high levels of calcium to mature rats, Palmer et al. (1958) observed no proportional decrease in the retention of Sr-90. Wasserman (1960) then confirmed that calcium alone will not reduce strontium retention in mature rats. This worker found that adding calcium and phosphorus to the diet increased the excretion of strontium.

Fluorine retention was reviewed and in some cases a high level of calcium seemed to interfere with the absorption of fluorine (Nutr. Revs., 1955). This is of interest in lieu of fluorine toxicity.

Trace elements are of great importance in today's nutritional applications. Much more important, however, are the amounts which are required under variable conditions of other nutrients in the livestock feeds.

In summary, the elements, selenium, molybdenum, copper and iron in regard to excess calcium are separately considered.

1. In this review, no reports on the effect of excessive calcium on selenium were found. The role of selenium in animal nutrition, the absorption and excretion of selenium and selenium toxicity were reviewed.

2. Limited work on the effect of excessive calcium on molybdenum has been reported. In one paper the excess calcium had no effect on the absorption of molybdenum.

3. Excessive calcium is thought to decrease copper absorption under certain conditions.

4. Excessive calcium has been reported to increase, decrease and have no effect on iron absorption. However, in most cases it was shown that the excess calcium decreased the absorption of iron and was thought to do so by decreasing the gastric acidity of the intestine.

CHAPTER III

EXPERIMENTAL PROCEDURE

Experimental Animals and Treatments

Forty eight crossbred barrows, averaging 47 pounds initially, were selected for this study. The barrows were allotted randomly to three treatments varying only in calcium content, (0.6, 1.2 and 2.4 per cent). The pigs were maintained on rations with optimum levels of ingredients (Table I) with the calcium level varied by substituting calcium carbonate (USP) for corn and adding sufficient soybean meal to replenish the protein of the substituted corn.

This study consisted of two experiments of two trials each. Pigs in experiment I were fed the experimental rations for a period of two weeks prior to placing in metabolism stalls for isotope administration. Pigs in experiment II were fed identical rations as in experiment I but were fed these rations for four weeks prior to isotope administration. Trial I was conducted in the fall of 1959 while trial II, a repeat of trial I, was conducted in the winter of 1960. Criteria for the growth phase were feed consumption, weight gains and feed efficiency. The animals were in individual pens during the growth phase in preparation and adjustment for the metabolism phase. At the termination of the growth phases, the animals were selected by weight and placed in individual metabolism stalls for a four day adjustment and preliminary period and a five day metabolism study. Modifications of Hansard's et al. (1951) detailed description of methods of handling

TABLE I

COMPOSITION OF RATIONS

Ingredients	Amount (lb.)		
	Ration I	Ration II	Ration III
Ground yellow corn	1427.4	1390.0	1314.0
Alfalfa meal (17 per cent dehy)	48.8	48.8	48.8
Soybean meal (44 per cent)	430.0	435.6	449.2
Corn oil	40.0	40.0	40.0
Dicalcium phosphate	20.0	20.0	20.0
Salt	10.0	10.0	10.0
Trace minerals*	1.0	1.0	1.0
Vitamin premix**	10.0	10.0	10.0
Calcium carbonate	12.8	44.6	107.0
TOTAL	2000.0	2000.0	2000.0

*Trace mineral mix: Mn, 12.5 per cent ($MnSO_4$); Fe, 8.0 per cent $FeSO_4$); Zn, 5.0 per cent ($ZnSO_4$); Co, 1.25 per cent ($CoSO_4$); Cu, 0.75 per cent ($CuSO_4$); I, 0.40 per cent (KI).

**Vitamin premix, each 10 pounds supplies: riboflavin, 2 gm; niacin, 30 gm; choline chloride, 50 gm; vitamin B_{12}, 10 mg; calcium pantothenate, 10 gm; vitamin A, 1 million USP; vitamin D_3, 0.5 million USP; aureomycin, 20 gm; DL-methionine, 227 gm; butylated hydroxy toluene, 120 gm.

and administration of the isotopes were used in this study. The metabolism stalls were described by Mayo (1961). Pigs in experiment I were orally dosed by stomach tube with Se-75 and Mo-99. Pigs in experiment II were orally dosed by stomach tube with Cu-64 and Fe-59. The amount of radioisotopes and the experimental design are shown in Table II. The Fe-59 and Se-75 were in the chloride form. The Cu-64 was prepared as the nitrate and the Mo-99 as ammonium molybdate. All the radioisotopes were purchased from the Oak Ridge National Laboratory Isotope Sales.

Sampling of Feed, Blood, Excreta and Tissues

Representative samples of the rations were taken at the time of each weekly feed mixing. The samples were placed in pint jars and stored until they were analyzed.

For the duration of the metabolism studies, total excretion of feces and urine were collected and measured separately. The samples were placed in pint jars and sub-sampled immediately or refrigerated until they were processed.

Blood samples were taken by jugular puncture with a 2 inch 18 gauge hypodermic needle and a 30 cubic centimeter syringe. Heparin was added to the syringes and bleeding tubes prior to use to prevent coagulation of the blood. Approximately 15 milliliters of blood were taken at each sampling period. Blood, urine and fecal samples were taken at 2, 4, 8, 16, 24, 36, 48, 72, 96, and 120 hours post-dosing.

At the time of sacrificing, 120 hours post-dosing, representative samples of 17 tissues were collected and placed in pint jars and

TABLE II

EXPERIMENTAL DESIGN

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Experiment I			
No. pigs dosed	6	6	6
Millicuries Mo-99	1	1	1
Microcuries Se-75	250	250	250
Experiment II			
No. pigs dosed	6	6	6
Millicuries Cu-64	2	2	2
Microcuries Fe-59	40	40	40

refrigerated until processed for radiocounting. Of the tissues sampled, total liver, lung, heart, kidney and spleen were obtained. The remaining tissues, bile, small intestine wall and contents, large intestine wall and contents, muscle, rib, tooth, vertebrae, femur, hair, and skin were partially sampled.

Chemical and Radiochemical Analysis

The feed samples were dry ashed in a muffle furnace at 500° centigrade for 24 hours and dissolved in 6 N hydrochloric acid. Calcium was determined with a Beckman flame spectrophotometer according to the methods described by Baker and Johnson (1954). The colorimetric method of Fiske and Subbarow (1925) was employed in determination of phosphorus. Stable copper, iron and molybdenum were determined by a commercial laboratory. Stable selenium was determined by activation analysis at the Oak Ridge National Laboratory.

The radioisotopes were determined in urine, feces, blood, plasma and tissues. Due to the short half-life of Cu-64, no tissue data were obtained. The four isotopes selected for this study, Se-75, Mo-99, Cu-64 and Fe-59 are all gamma emitters. The radioactivity was counted in a well-type scintillation counter and expressed as per cent of total oral dose. All samples were counted fresh by placing 3 milliliters or 3 grams into a scintillation tube (16 X 150 mm.) and placed in the counter for two minutes each. All samples, except plasma, were determined in duplicate. After the blood samples were pipetted into the counting tubes, the remaining blood was centrifuged for 20 minutes at a constant speed. The plasma was then pipetted into a counting tube.

Using the multiple dosing technique with a relatively long and a short half-life isotope in each experiment, the samples were counted and refrigerated until the short half-life isotope decayed over a period of ten half-lives and then recounted. In experiment I, Mo-99 and Se-75 were used. Mo-99 has a half-life of 2.8 days compared with Se-75 which has a half-life of 120 days. Likewise in experiment II, Cu-64 and Fe-59 were selected. The half-life of Cu-64 is 12.8 hours while Fe-59 is 45.1 days. At the time of dosing, one dose of each isotope was placed in a volumetric flask for a standard comparison solution. The sample activity was compared with the standard activity and the per cent of dose was then calculated.

Samples of the small intestine wall and contents were washed and homogenized in a Waring blender and pH of each was determined with a Beckman pH meter.

Analysis of Data

All data were subjected to statistical analysis according to procedures described by Snedecor (1956). Differences less than $P>0.05$ were considered statistically insignificant.

CHAPTER IV

RESULTS AND DISCUSSION

Excessive Calcium Effect on Growth of Pigs

The growth data are summarized and presented in Table III. Due to high variation between trials, the data are shown separately. During trial I conducted in the fall of 1959, the pigs had lower average daily gains ($P < 0.01$) and consumed less feed ($P < 0.01$) per day than did those in trial II which was conducted in the winter of 1960. These differences were attributed to temperature, breeding and source of pigs. The feed per pound of gain was not different between the two trials. Averages of the two trials will be used for discussion as both trials showed a similar trend for the criteria studied. Excessive calcium fed pigs in Lot III (2.4 per cent Ca) depressed ($P < 0.01$) average daily gains, lowered feed consumption and increased the amount of feed required per pound of gain. The differences between these data from pigs in Lot I (0.6 per cent Ca) and Lot II (1.2 per cent Ca) were not significant. At the end of the first week, pigs in both Lots II and III had poor gains, which increased during the second week and plateaued to gains similar to the total average. This difference may have been due to an acidity adjustment in the intestinal tract in the pigs on the high calcium rations. At the termination of the collection period, pH of the intestinal tract was determined and showed no differences due to excessive calcium (Table IV). Reports in the literature are inconsistent as to the effect of excessive calcium on growth at the 1.2 per cent

TABLE III

EFFECT OF EXCESSIVE CALCIUM ON GROWTH OF PIGS

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Trial I			
Number of pigs	8	8	8
Av. init. wt. (lb.)	44.25	44.44	44.50
Av. daily gain (lb.)	1.27	1.15	0.70*
Feed per lb. gain	2.57	2.63	3.83*
Daily feed cons. (lb.)	3.19	3.03	2.68
Trial II			
Number of pigs	8	8	8
Av. init. wt. (lb.)	48.90	48.70	48.50
Av. daily gain (lb.)	2.03	1.72	0.96*
Feed per lb. gain	2.86	2.95	4.28*
Daily feed cons. (lb.)	5.81	5.07	4.12
Combined trials			
Number of pigs	16	16	16
Av. init. wt. (lb.)	46.58	46.57	46.50
Av. daily gain (lb.)	1.65	1.44	0.83*
Feed per lb. gain	2.73	2.81	4.14*
Daily feed cons. (lb.)	4.50	4.05	3.40

*$P < 0.01$

TABLE IV

SMALL INTESTINAL WALL AND CONTENTS pH VALUES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Small intestine wall			
Trial I	5.52	5.63	5.57
Trial II	5.70	5.57	5.58
Average	5.61	5.60	5.58
Small intestine contents			
Trial I	5.50	6.40	5.70

range. Berry et al. (1960) in a similar experiment found no differences in growth of pigs fed rations containing 0.59 and 1.19 per cent calcium. It is generally thought that 2.4 per cent calcium will reduce gains, feed consumption and feed efficiency, however, limited work has been conducted on this.

The dietary results of the collection period are shown in Table V. Gains were minimal due to the restraint of the animal, however, feed consumption continued at a slightly lower rate than in individual pens. Fecal and urine samples were possible at most of the frequent collection periods.

Se-75 Results and Discussion

Se-75 distribution in blood and plasma from an oral dose are shown in Figure 1. Se-75 was rapidly absorbed into the blood stream and reached a peak at 8 hours after dosing in both blood and plasma. Se-75 content of the blood and plasma then declined and at 36-48 hours began to plateau to a level which remained throughout the 120 hour collection period. Se-75 in the blood and plasma was increased ($P<0.01$) by increasing the level of dietary calcium. However, when the total blood volume of the pigs was calculated, there was no difference in the total amount of Se-75 contained in the blood. The average total amount of Se-75 contained in the blood at sacrifice time was 11.7, 13.3 and 11.8 per cent for pigs in Lots I, II and III, respectively. The differences in the blood and plasma on a per milliliter basis were due to the depression of body weight by the excessive calcium in the ration.

The rapid absorption of Se-75 as shown by the peak in blood levels

TABLE V

COLLECTION PERIOD GROWTH DATA

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Experiment I, Trial I			
Number of pigs	3	3	3
Av. wt. (lb.)	58.20	62.20	51.00
Av. daily gain (lb.)	0.72	0.67	0.37
Daily feed cons. (lb.)	2.20	2.45	1.87
Feed per lb. gain	3.06	3.66	5.05
Trial II			
Number of pigs	3	3	3
Av. wt. (lb.)	89.92	86.33	69.75
Av. daily gain (lb.)	0.34	0.46	0.02
Daily feed cons. (lb.)	4.42	4.34	3.15
Feed per lb. gain	13.00	9.43	157.50
Experiment II, Trial I			
Number of pigs	3	3	3
Av. wt. (lb.)	74.50	68.50	57.30
Av. daily gain (lb.)	0.16	0.00	0.08
Daily feed cons. (lb.)	1.91	1.96	1.60
Feed per lb. gain	11.94	___	20.00
Trial II			
Number of pigs	3	3	3
Av. wt. (lb.)	96.33	93.00	76.00
Av. daily gain (lb.)	0.40	0.40	0.13
Daily feed cons. (lb.)	4.33	4.32	2.96
Feed per lb. gain	10.82	10.80	22.77

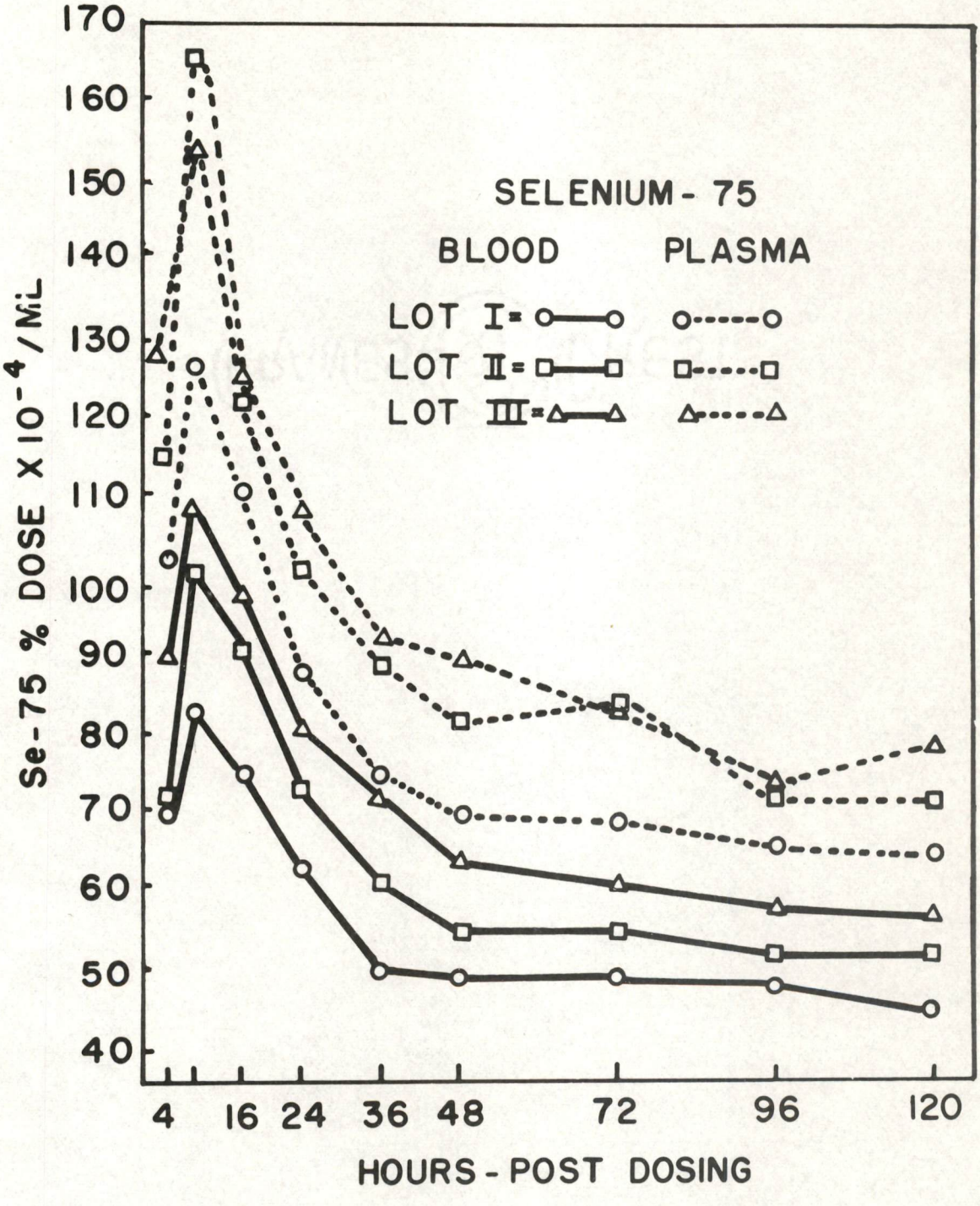


Figure 1. Selenium-75 Content in Blood and Plasma on a Per Milliliter Basis.

at 8 hours after dosing, agrees with rat work conducted by Smith et al. (1938), Anderson and Moxon (1941), McConnell (1942), McConnell and Martin (1953) and others. The latter experiments however, were conducted primarily to study selenium toxicity.

Plasma Se-75 of the pigs amounted to an average of 73 per cent of the total blood Se-75 with a range of 68 to 80 per cent. These values were consistent from 2 hours after dosing to time of sacrifice. There was no difference due to dietary calcium on the percentage of Se-75 contained in the plasma. McConnell and Van Loon (1955) found upon fractionation of dog blood containing Se-75 that between 77 and 89 per cent of the total Se-75 was protein bound.

Total urine output was increased ($P < 0.01$) by the excessive calcium in the ration (Table VI). The distribution of Se-75 in urine was quite variable with no differences due to the excessive calcium in the ration. Within 24 hours after dosing, 44, 40 and 48 per cent of the total urinary Se-75 was excreted by pigs on rations I, II and III, respectively. The accumulative total urinary Se-75 excretions are shown in Figure 2.

The total quantity of feces was decreased ($P < 0.01$) by the high calcium levels (Table VI). Pigs in Lot III excreted 35.4 per cent less feces than did pigs in Lot I. The peak excretion of Se-75 in the feces was 24-36 hours after dosing. By 48 hours after dosing, 60, 57 and 57 per cent (Lots I, II and III, respectively) of the fecal Se-75 had been excreted. Excretion of Se-75 by pigs in Lot II was consistently lower than both Lots I and III. Figure 2 gives the cumulative

TABLE VI

EXCRETION OF SE-75 AND TOTAL VOLUME
AND WEIGHT OF URINE AND FECES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Number of pigs	6	6	6
Feces, kg.	4.08	3.93	2.61*
Urine, l.	9.80*	12.24	13.88
Per cent of Se-75 dose			
Urine	6.40	7.90	7.60
Feces	18.20	16.30	19.00
Total	24.60	24.20	26.60

*$P < 0.01$

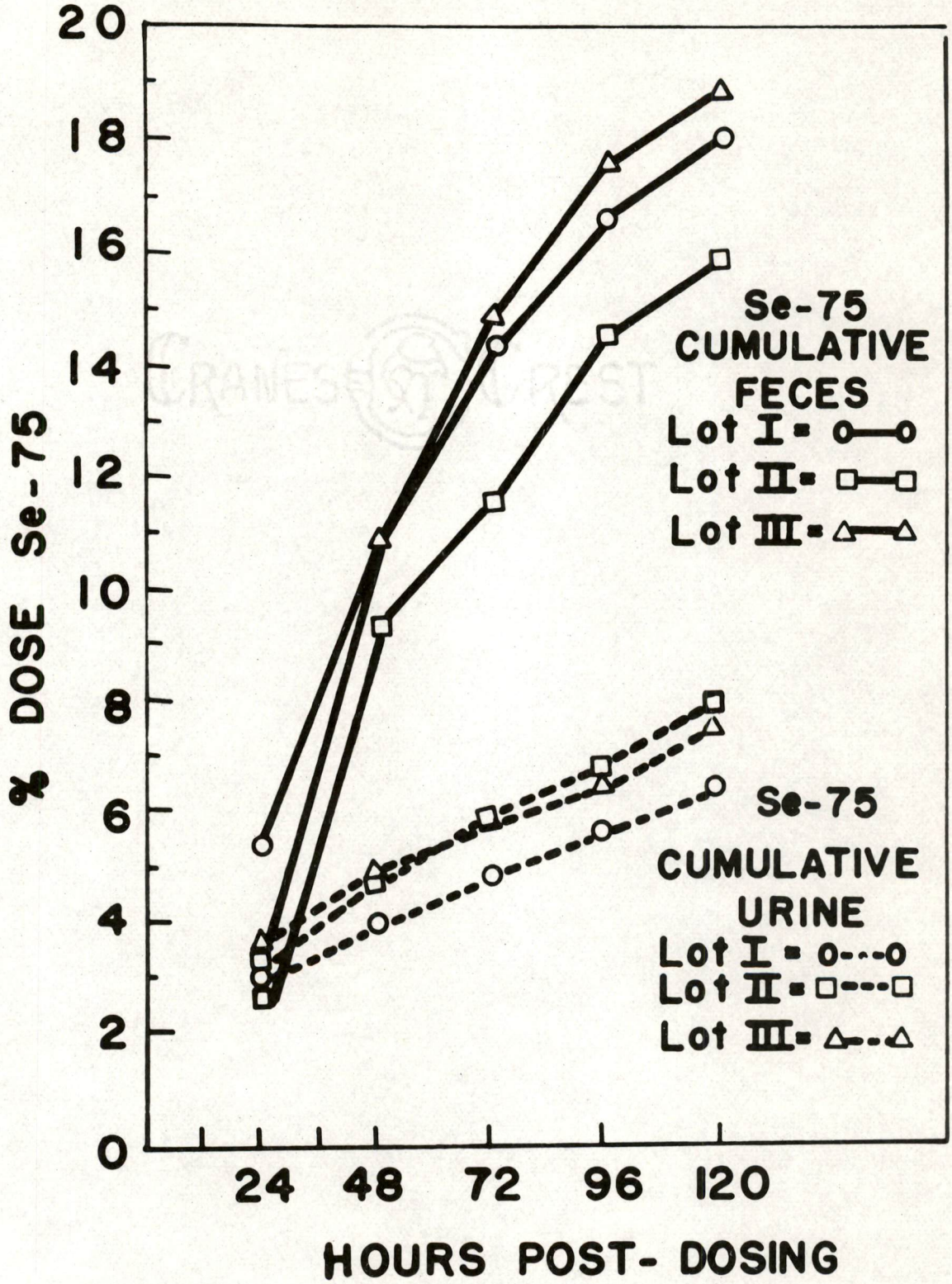


Figure 2. Cumulative Selenium-75 in Feces and Urine.

excretion of Se-75 in the feces. The total fecal output of Se-75 was not affected by ration calcium levels. When the urinary and fecal Se-75 excretion are combined, the total excretion showed no differences. Urine and feces combined, resulted in 24.6, 24.2 and 26.2 per cent of the Se-75 excreted by pigs in Lots I, II and III, respectively. Average of the three lots showed that at 120 hours post-dosing, 71.5 per cent of the total excretion was via the feces. This is in contrast to reports by McConnell (1942) working with rats, in that Se-75 was reported to be eliminated primarily by way of urine at ordinary selenium intakes. Later reports by McConnell and Martin (1953) indicated, in two experiments with dogs, excretion of 62 and 44 per cent of the total selenium in the first four hours after administration. Selenium may be a "high threshold substance", in the form of seleno-cystine or seleno-methionine, due to its high concentration in the kidney, low concentration in the urine and relatively constant concentration in the plasma. The level of dietary selenium and the species difference may have resulted in optimum or sub-optimum plasma levels, therefore obtaining a low amount of renal excretion. No selenium was added to these rations which analyzed 0.6 p.p.m. selenium.

At the termination of the 120 hour collection period the pigs were sacrificed for sampling of various tissues. The tissue results are summarized in Table VII. Excessive calcium in the ration tended to increase the amount of Se-75 in the various tissues, however, due to the large variations, only the femur, large intestine contents ($P < 0.01$), large intestine wall, liver, skin, spleen and rib ($P < 0.05$)

TABLE VII

SUMMARY OF SE-75 TISSUE DATA

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
	Per cent dose/gm X 10^{-4} (6 pigs/lot)		
Heart	31.5 ± 3.2[a]	38.1 ± 5.2	38.1 ± 4.5
Liver	116.0 ± 19.8	137.0 ± 27.3	164.0 ± 33.3*
Bile	7.5 ± 1.2	6.7 ± 0.4	9.3 ± 0.9
Lung	51.8 ± 6.3	64.3 ± 7.6	66.2 ± 7.6
Spleen	67.2 ± 9.8	80.2 ± 9.6	91.2 ± 14.5*
Kidney	401.7 ± 125.7	392.1 ± 136.3	408.1 ± 115.1
Sm. intestine wall	72.2 ± 9.3	70.2 ± 2.4	89.3 ± 9.2
Sm. intestine contents	5.7 ± 1.0	6.5 ± 1.7	12.0 ± 3.7
Large int. wall	43.0 ± 4.7	53.0 ± 5.3	58.5 ± 8.9*
Large int. contents	15.7 ± 3.3	15.2 ± 1.6	26.5 ± 3.2**
Muscle	7.2 ± 1.1	9.0 ± 1.6	9.2 ± 1.5
Skin	10.5 ± 1.9	12.7 ± 2.2	15.7 ± 3.2*
Hair	2.0 ± 0.4	2.0 ± 0.5	5.3 ± 2.3
Rib	24.3 ± 3.7	27.3 ± 3.5	36.5 ± 4.0*
Femur	19.0 ± 2.4	21.0 ± 1.7	29.0 ± 2.4**
Vertebrae	34.2 ± 5.1	39.8 ± 4.3	44.5 ± 4.3
Tooth	20.8 ± 4.3	24.5 ± 2.9	25.2 ± 5.7

[a]Standard error of mean.

*$P < 0.05$ (0.6 vs. 2.4 per cent Ca).

**$P < 0.01$ (0.6 vs. 2.4 per cent Ca).

were different. When the Se-75 was calculated for the total tissue (Table VIII), there were similar amounts of Se-75 in the respective tissues. Differences on a per gram basis were due in part to the depressing effect of calcium on the growth of the pigs. The total liver contained more Se-75 than did any of the other tissues taken. On a per gram basis the kidney had approximately 3 times the concentration of Se-75 than that of the liver. The kidney and liver combined accounted for an average of 8 per cent of the oral dose of Se-75.

Tissue uptake of Se-75 was relatively high for such a minor element of the body. Excessive calcium increased the level of Se-75 per gram of fresh tissue, but basing the data on total tissue weight or body weight of the animal, the levels were similar. The coefficient of variation of the tissues, reveals the kidney variation is 2-3 times higher than the other tissues. It is postulated that selenium contained in the kidney was filtered through the glomeruli from the plasma with a high amount reabsorbed through the tubules. If unequal portions of cortex and medulla of the kidney are obtained in sampling, the variation would be increased. The coefficients of variations of Se-75 for small intestine contents and hair showed a linear increase. The high coefficients of variation for pigs in Lot III was due to one pig which had much greater Se-75 concentration in these tissues than the other pigs. Se-75 in the total liver accounted for 6.8 per cent of the dose while this organ was only 2.1 per cent of body weight. This concurs with work by McConnell and Martin (1953). They also reported that the destruction and elimination of selenium-bound hemoglobin may partly

TABLE VIII

PERCENTAGE SE-75 DOSE IN TOTAL TISSUE

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
	Per cent dose (Trial II only)		
Heart	0.56 ± 0.03*	0.51 ± 0.03*	0.44 ± 0.06*
Kidney	1.46 ± 0.06	1.05 ± 0.02	1.39 ± 0.06
Liver	7.63 ± 0.26	6.87 ± 0.50	5.73 ± 0.31
Lung	1.53 ± 0.14	1.48 ± 0.18	1.16 ± 0.04
Spleen	0.42 ± 0.03	0.43 ± 0.02	0.37 ± 0.05

*Standard error of mean.

take place in the liver and the degradation products appear in the bile, where a fraction of the selenium is combined with the bile pigments. However, upon sampling the bile, the content of Se-75 was very low in comparison with the liver and blood concentration. Total Se-75 retained in the body 120 hours after dosing amounted to about 75 per cent of the administered dose. This would indicate either a need for selenium by the pig or selenium was replacing sulfur in the form of seleno-amino acids or other selenium compounds.

The order of concentration of Se-75 in the tissues was as follows: kidney > liver > spleen > small intestine wall > lung > large intestine wall > vertebrae > rib > heart > femur > teeth > large intestine contents > skin > small intestine contents > muscle > bile > hair. The quantity of Se-75 in the total tissues measured were as follows: liver > lung > kidney > heart > spleen.

Mo-99 Results and Discussion

No differences due to excessive calcium were found in absorption or excretion of Mo-99. Absorption and excretion of Mo-99 was rapid. The first blood sample obtained 2 hours after oral dosing contained the highest concentration of Mo-99 observed for blood and plasma as shown in Figure 3. By 4 hours after dosing, the blood level of Mo-99 had declined by one-half of the 2 hour level and at 8 hours had decreased to a minimal measurable level. The average Mo-99 concentration in the blood was found to be 65 per cent in the plasma at 2 hours after dosing. This level decreased to 42 per cent at 4 hours, 14 per cent at 8 hours and 7 per cent at 16 hours post-dosing, demonstrating rapid movement

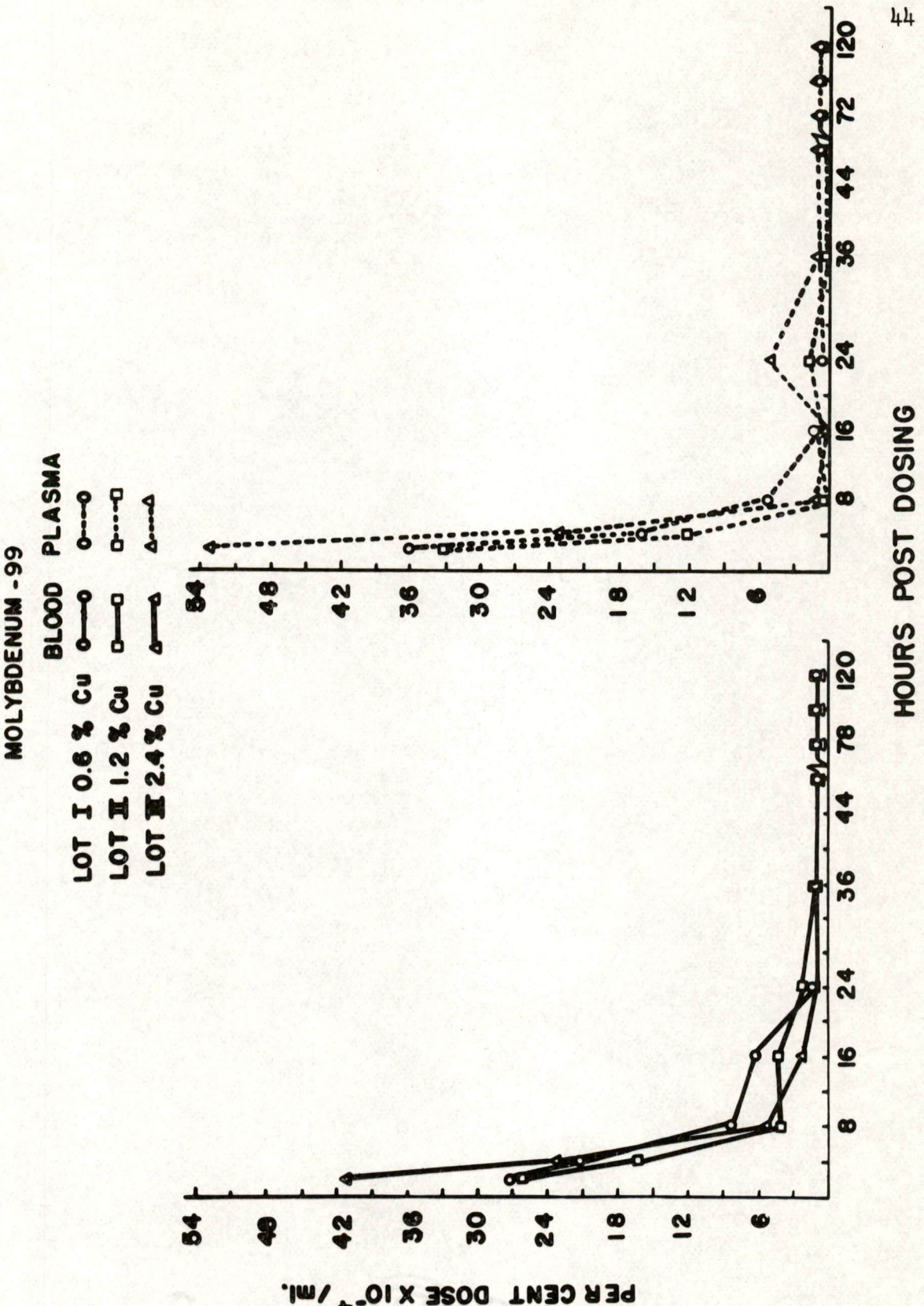


Figure 3. Molybdenum-99 Content in Blood and Plasma on a Per Milliliter Basis.

into the cells. Teekell (1960) observed a similar trend in chicks in the uptake of Mo-99 in the blood; however the plasma portion contained a constant proportion of Mo-99.

Calculating the total blood according to body weight of the pigs revealed that approximately 8 per cent of the total dose was contained in the blood at 2 hours after dosing. At the same time approximately 4 per cent had been excreted in the urine. By 8 hours the blood levels had declined to 1.4 per cent and the urinary output was 27.7 per cent with about 1.0 per cent of the original dose eliminated via the feces. The rapid absorption and urinary excretion indicates the body requirement did not affect the absorption of this element.

The major pathway of excretion of Mo-99 was via the urine which amounted to 51 per cent of the oral dose (Table IX). The average cumulative excretion for 2, 4, 8, 16 and 24 hour post-dosing periods were 4.1, 13.5, 27.7, 38.2 and 43.0 per cent, respectively.

After 24 hours, approximately 9 per cent of the total Mo-99 had been eliminated in the feces. The total fecal elimination of Mo-99 amounted to 27.4 per cent of the total dose for the 120 hour metabolism study. The peak fecal elimination period of Mo-99 was between 24 and 48 hours (Figure 4).

The data from the two trials were variable. In the first trial conducted in the fall of 1959, the pigs excreted Mo-99 primarily in the urine. The second trial conducted in the winter of 1960, showed that about equal quantities were excreted in urine and feces. After the experiments were terminated and analyzed, it was found that the

TABLE IX

EXCRETION OF MO-99 AND TOTAL VOLUME AND WEIGHT OF URINE AND FECES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Number of pigs	6	6	6
Feces, kg.	4.08	3.93	2.61*
Urine, l.	9.80*	12.24	13.88
Per cent of Mo-99 dose			
		Trial I[a]	
Urine	72.13	58.20	63.31
Feces	13.63	15.30	9.48
Total	85.76	73.50	72.79
		Trial II[b]	
Urine	31.79	34.21	45.94
Feces	42.09	57.19	26.88
Total	73.88	91.40	72.82
		Combined Trials	
Av. urine	51.96	46.20	54.63
Av. feces	27.86	36.25	18.18
Av. retained	20.18	17.65	17.19

*$P < 0.01$

[a]Mo-99 specific activity for Trial I at dosing time was 450 mc. per gram of molybdenum.

[b]Mo-99 specific activity for Trial II at dosing time was 61.5 mc. per gram of molybdenum.

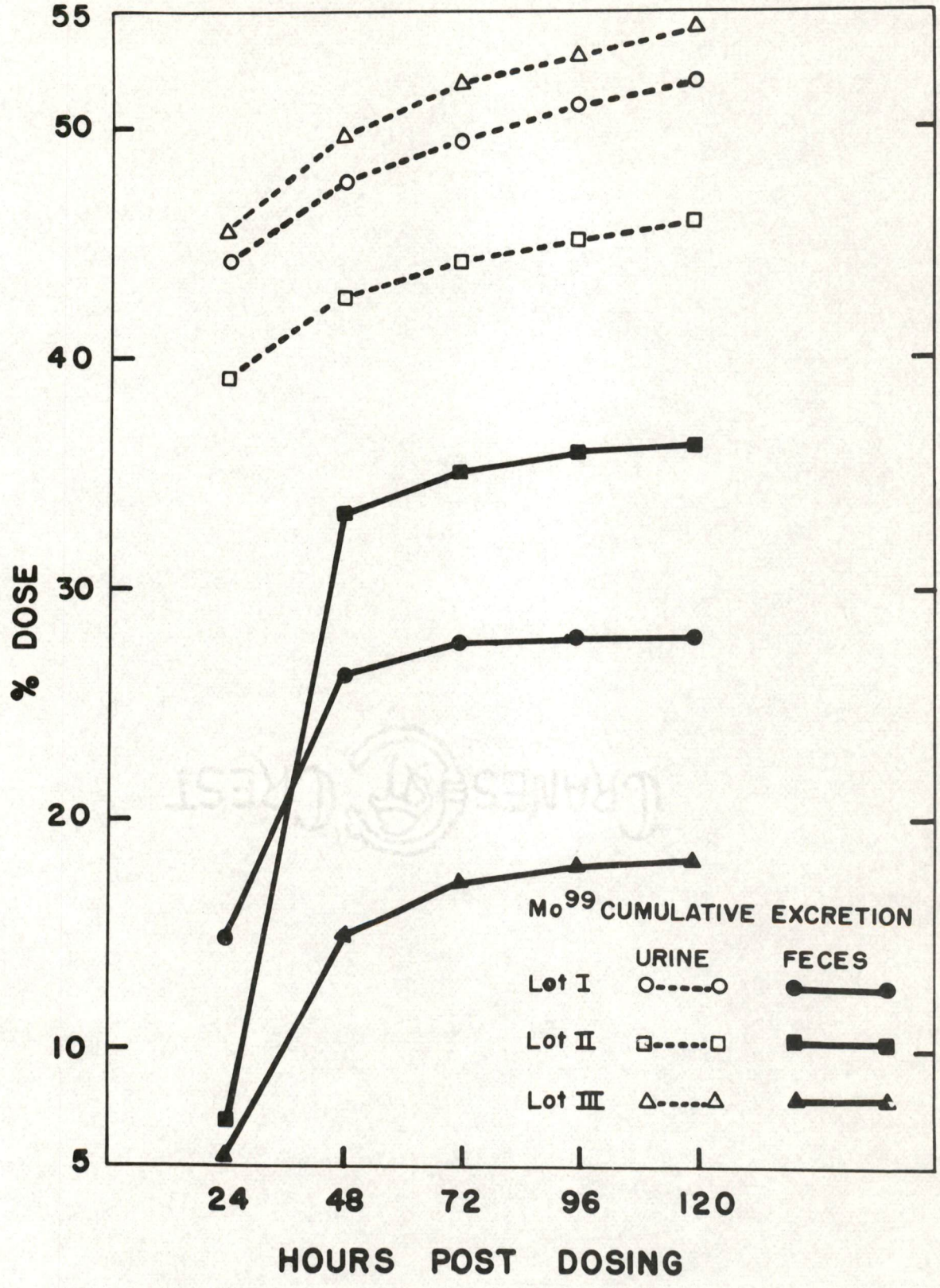


Figure 4. Cumulative Molybdenum-99 in Feces and Urine.

specific activity of the dosing solutions for the two trials varied considerably (Table IX). The inconsistent results from the two trials is thought to be due to the differences in specific activity of the dosing solutions and possibly from temperature differences. In trial I, the specific activity was 450 millicuries per gram whereas in trial II it was 61.5 millicuries per gram. Pigs in both trials received similar amounts of Mo-99 as indicated by comparing counts of standards. From the total dose, the pigs in trial I received 2.2 milligrams of stable molybdenum while the pigs in trial II received 16.5 milligrams of stable molybdenum. Since molybdenum is readily absorbed, the amount of absorption in all probability, is dependent on the amount administered. Therefore in trial II the pigs absorbed less activity but equal amounts of stable molybdenum and consequently more Mo-99 was unabsorbed and excreted in the feces. However, the blood and plasma values should indicate less absorption if specific activity is the explanation for the variation in route of excretion. The blood and plasma Mo-99 levels do not concur with the excretion patterns (Figure 5). Since the blood and plasma do not agree with excretion data, the specific activity may have affected biliary excretion of Mo-99. As the stable molybdenum increased per dose, more Mo-99 was excreted by the bile into the gut and eliminated in the feces.

Shirley et al. (1954) found approximately 55 per cent in urine and 8 per cent in feces of pregnant sows 30 hours after oral dosing with Mo-99. These data are also in accordance with rat data reported by Arrington and Davis (1955), who observed no calcium effect upon

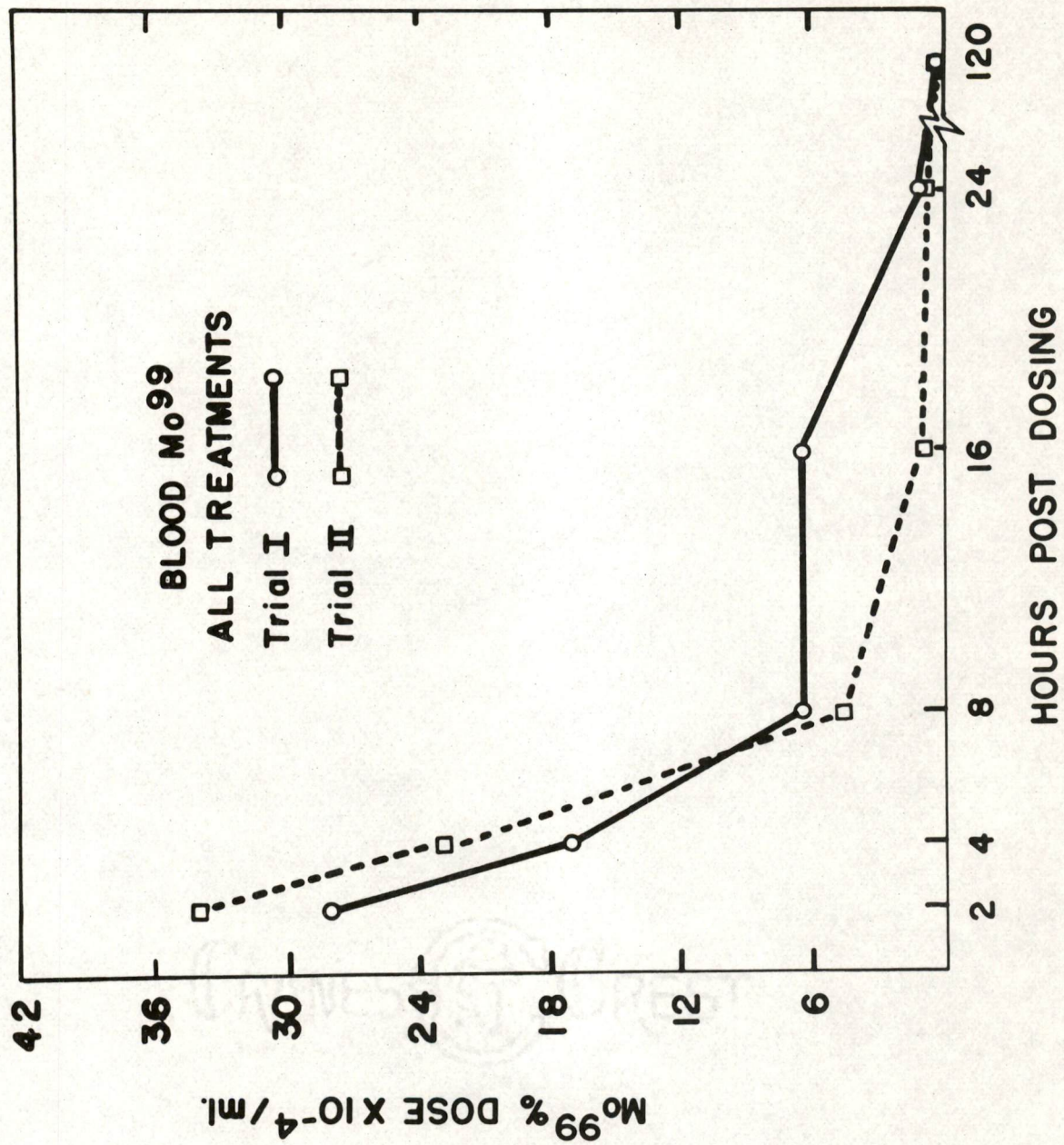


Figure 5. Concentration of Molybdenum-99 in Blood for Trial I and Trial II.

Mo-99 excretion. By 24 hours after oral dosing with Mo-99, an average of 57 per cent was excreted via the urine of rats. While fecal Mo-99 data were limited and variable, they reported about 8 per cent being excreted in the feces in 24 hours. The rapid rate of excretion via the urine has also been corroborated by Fairhall et al. (1945), Neilands et al. (1948) and Comar et al. (1949) in rats.

A summary of tissue concentration of Mo-99 at 120 hours post-dosing is presented in Table X. Concentration in the tissues was relatively low and distributed throughout the body. The kidney contained the highest concentration per gram of fresh tissue which would be expected due to the high urinary excretion. The next highest concentration was found in the liver in which excessive calcium increased the amount of Mo-99 per gram ($P < 0.05$). Mo-99 activity of the remaining tissues was low, showing no effect due to calcium, yet Mo-99 was detected in all 17 tissues sampled. In comparison, Shirley et al. (1954) reported lower concentrations of Mo-99 in pregnant sows after 30 hours than are shown in this study after 120 hours. The Mo-99 concentration of kidney was 50 times and liver 30 times higher under these experimental conditions which indicate a difference in growing and mature swine.

Total Mo-99 retained in the tissues and fluids amounted to approximately 22 per cent of the dose. The total quantity of Mo-99 in the tissues is shown in Table XI. Liver contained the highest quantity of Mo-99 and excessive calcium increased the quantity retained ($P < 0.05$) in this organ. The high concentration and total quantity

TABLE X

SUMMARY OF MO-99 TISSUE DATA

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
	Per cent dose X 10^{-4} / gm. (6 pigs/Lot)		
Heart	1.58 ± 0.26[a]	1.48 ± 0.33	2.63 ± 0.57
Liver	17.17 ± 4.78	23.33 ± 4.41	34.83 ± 1.51**
Bile	1.20 ± 0.41	1.55 ± 0.63	1.55 ± 0.42
Lung	2.18 ± 0.59	1.25 ± 0.21	1.43 ± 0.51
Spleen	1.93 ± 0.29	2.60 ± 0.31	3.32 ± 0.86*
Kidney	31.00 ± 15.8	16.00 ± 7.63	34.00 ± 16.4
Sm. intest. wall	2.97 ± 1.00	2.33 ± 0.52	3.55 ± 0.32
Sm. intest. contents	0.45 ± 0.12	1.15 ± 0.54	0.97 ± 0.35
Large intest. wall	2.00 ± 0.30	2.10 ± 0.25	2.95 ± 1.10
Large intest. contents	0.65 ± 0.15	0.83 ± 0.32	1.20 ± 0.29
Muscle	0.60 ± 0.06	0.67 ± 0.16	0.82 ± 0.04
Skin	0.59 ± 0.06	0.50 ± 0.11	1.10 ± 0.10
Hair	2.57 ± 1.03	1.47 ± 0.73	23.60 ± 17.6
Rib	2.85 ± 0.36	2.43 ± 0.30	3.23 ± 0.37
Femur	2.32 ± 0.25	2.22 ± 0.18	1.95 ± 0.22
Vertebrae	3.00 ± 0.49	2.78 ± 0.21	4.10 ± 0.94
Tooth	1.70 ± 0.27	1.30 ± 0.33	1.17 ± 0.41

[a] Standard error of mean.

* $P < 0.05$ (0.6 vs. 2.4 per cent Ca).

** $P < 0.01$ (0.6 vs. 2.4 per cent Ca).

TABLE XI

PERCENTAGE MO-99 DOSE IN TOTAL TISSUE

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
	Per cent dose X 10^{-1} (Trial II only)		
Heart	0.23 ±0.09[a]	0.17 ± 0.004	0.30 ± 0.01
Liver	9.00 ± 1.50	14.33 ± 2.00	19.67 ± 1.40[b,c]
Lung	1.03 ± 0.45	0.40 ± 2.06	0.30 ± 0.25
Spleen	0.18 ± 0.03	0.23 ± 0.03	0.31 ± 0.08
Kidney	6.00 ± 1.71	3.00 ± 0.60	6.00 ± 1.00

[a]Standard error of mean.

[b]$P < 0.05$ (difference in treatment means).

[c]$P < 0.01$ (difference in means of 0.6 vs. 2.4 per cent Ca).

of Mo-99 in the liver may be due to higher xanthine oxidase activity, since this enzyme has been found to contain molybdenum (Richert and Westerfield, 1953; and De Renzo et al., 1953).

The concentration of Mo-99 on per gram basis in the seventeen tissues sampled were as follows: kidney > liver > vertebrae > small intestine wall > rib > hair > femur > lung > large intestine wall > spleen > tooth heart > bile > large intestine contents > muscle > skin > small intestine contents.

Cu-64 Results and Discussion

Cu-64 concentration in blood and plasma is shown in Figures 6 and 7, respectively. There was no effect of excessive calcium on the absorption of Cu-64. The concentration in blood and plasma increased from time of dosing for about 16 hours at which time the level in the plasma plateaued and remained relatively constant through 72 hours. The level of Cu-64 in the blood did not rise greatly after 2 hours post-dosing. The blood concentration of Cu-64 was relatively constant throughout the study. The points at 72 and 96 hours for the blood and 96 hours for plasma cannot be taken as real due to the short half-life of copper and the simultaneous dosing with Fe-59, making the variation high since the counts per minute were very low. As shown by the data, copper was found primarily in the plasma portion of the blood. These data are in accordance with results reported by Buescher et al. (1961), studying copper availability, in which approximately the same levels of Cu-64 was found in the blood and plasma even though dietary copper was higher in this experiment.

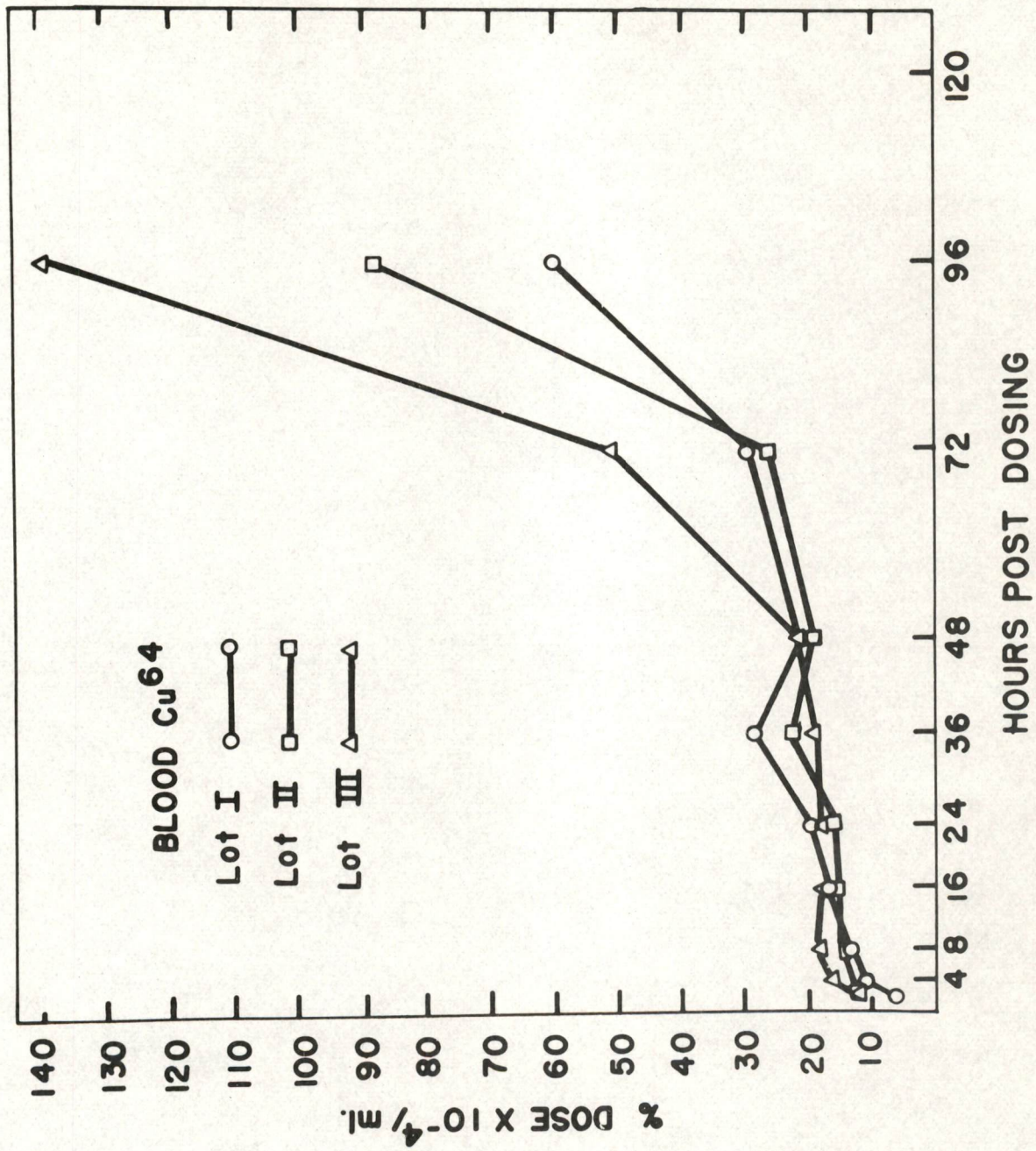


Figure 6. Copper-64 Concentration in Blood.

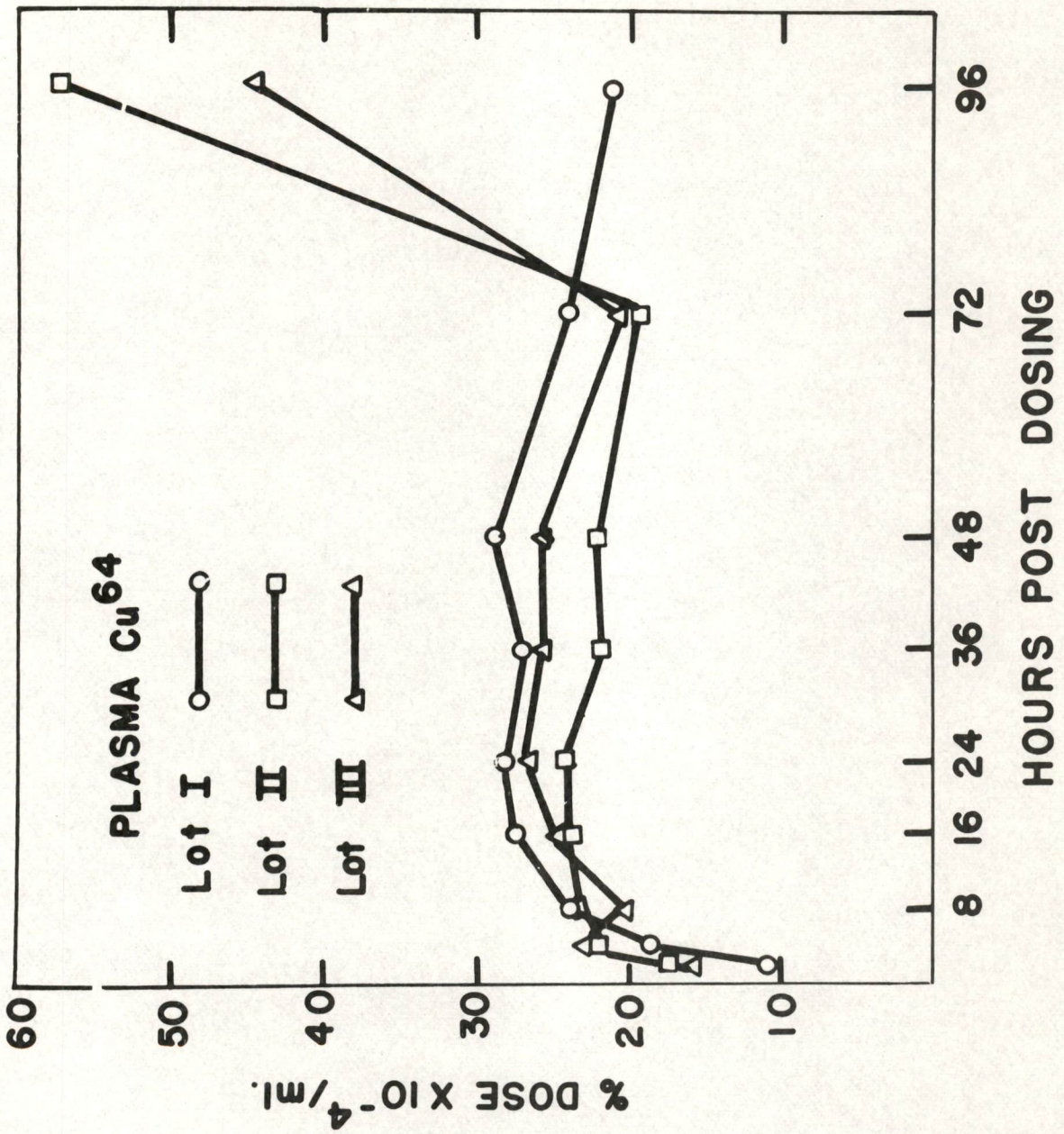


Figure 7. Copper Concentration in Plasma.

These data are in accordance with work by Miller et al. (1959) in swine who found that 1.25 per cent calcium, 3 p.p.m. copper and 0.4 per cent phosphorus had no effect on blood, muscle or rib copper concentration.

Tompsett (1940), indicated the absorption of copper in mice was influenced by the gastric acidity and the calcium content of the diet. Large amounts of calcium carbonate in sheep rations was stated by Dick (1954) to reduce the assimilation of copper. Investigations by Hardison et al. (1958) with dairy calves indicate copper fed at low levels in the presence of calcium has a depressing effect on copper storage.

Copper has been known for many years to be excreted into the gut and eliminated via the feces. This was corroborated in this experiment as shown in Table XII and Figure 8. There was no effect of excessive dietary calcium on the fecal excretion of Cu-64. Total fecal excretions of Cu-64 for 120 hours amounted to 72.6, 73.5 and 69.4 per cent for pigs in Lots I, II and III, respectively.

Copper-64 fecal excretion data reported by Buescher et al. (1961) averaged about 63 per cent for copper oxide, sulfate and carbonate in swine on the same ration as above without added copper. The 8-10 per cent more fecal Cu-64 in this study may be due to the added dietary copper or to the form of copper administered, since this was given as copper nitrate, although Lassiter and Bell (1960) found copper nitrate to be equally as available as copper sulfate and chloride in sheep.

Cumulative excretion of Cu-64 via the urine is shown in Figure 8. Excessive calcium (2.4 per cent) increased ($P < 0.01$) the output of

TABLE XII

EXCRETION OF CU-64 AND TOTAL VOLUME
AND WEIGHT OF URINE AND FECES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Number of pigs	6	6	6
Feces, kg.	4.57	4.81	2.67**
Urine, l.	14.07	14.99	28.82**
Per cent of Cu-64 dose			
Feces	72.60	73.48	69.36
Urine	2.02	2.68	3.83
Retained	25.38	23.84	26.81

** $P < 0.01$

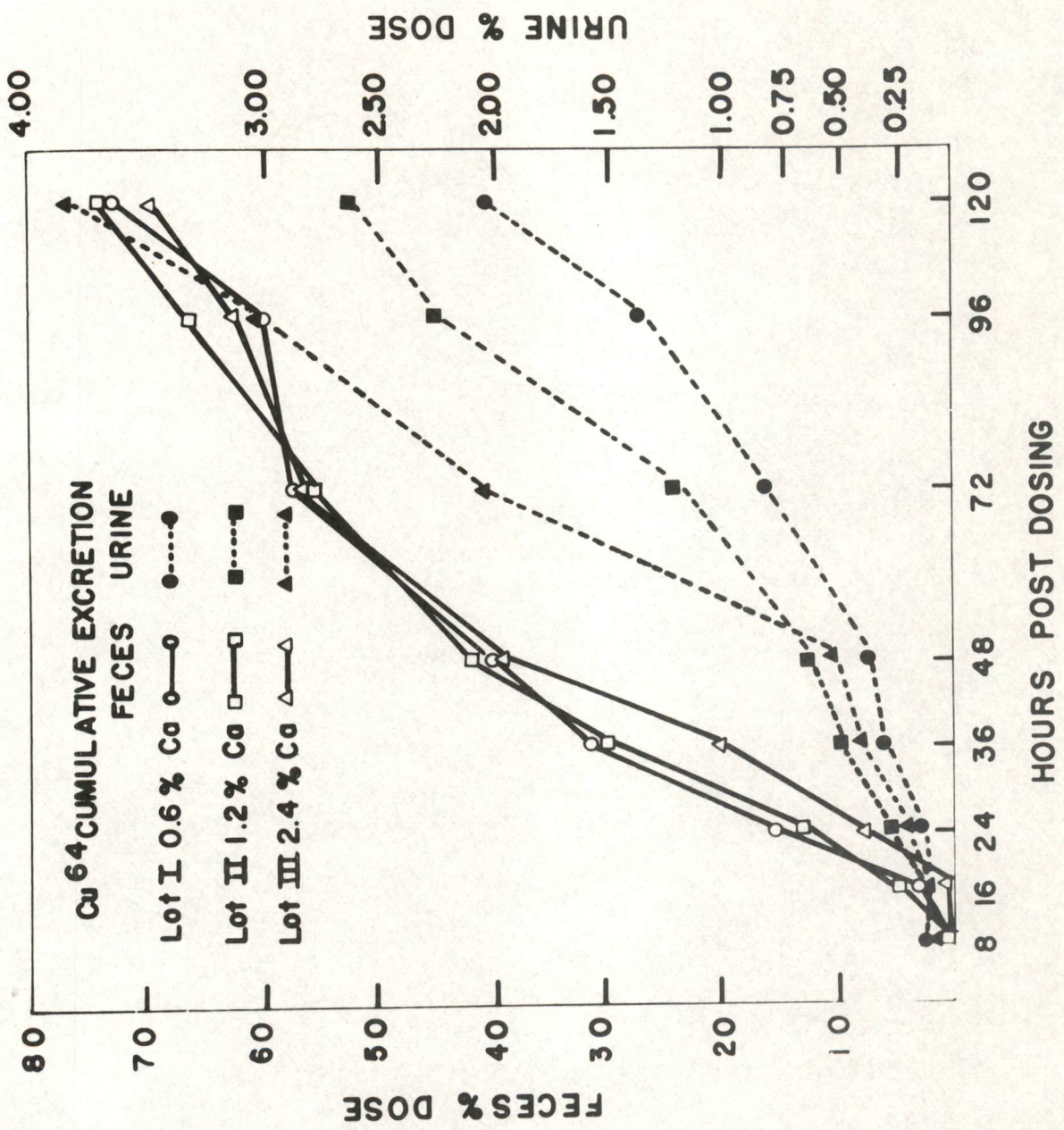


Figure 8. Cumulative Copper-64 in Feces and Urine.

Cu-64 in the urine over the basal (0.6 per cent). The total excretions of Cu-64 in the urine by 120 hours was 2.0, 2.7 and 3.8 per cent for pigs in Lots I, II and III, respectively. This may be attributed to significant increase in the volume of urine excreted by the pigs of the excessive calcium rations.

The total urinary Cu-64 excretion values compare with urine Cu-64 values by Buescher et al. (1961) in swine. Previous data on excretions of Cu-64 as affected by excessive calcium were unavailable. The urine values are comparable to those reported by Comar (1950) in cattle which were given oral doses of Cu-64. Lassiter and Bell (1960) found 3.1 per cent of Cu-64 dose excreted via the urine in sheep when the Cu-64 was given as copper carbonate but only 0.1 to 1.0 per cent when given as copper oxide, sulfate, nitrate or chloride.

The total excretion of Cu-64 amounted to 74.62, 76.16 and 73.19 per cent in 5 days for pigs in Lots I, II and III, respectively. The Cu-64 retained in the tissues and fluids of the body therefore was relatively high. The high retention of Cu-64 may be explained by the rapid growth rate of swine as compared to sheep and cattle.

Fe-59 Results and Discussion

Blood and plasma concentrations of Fe-59 are shown in Figures 9 and 10, respectively. The plasma levels of Fe-59 reached a peak at about 4 hours and then decreased to very low levels by 36 hours post-dosing. The blood levels of Fe-59, however, were low for the first 24 hours and then increased until termination of the experiment. The plasma portion of the blood amounted to 35-40 per cent of the blood. This

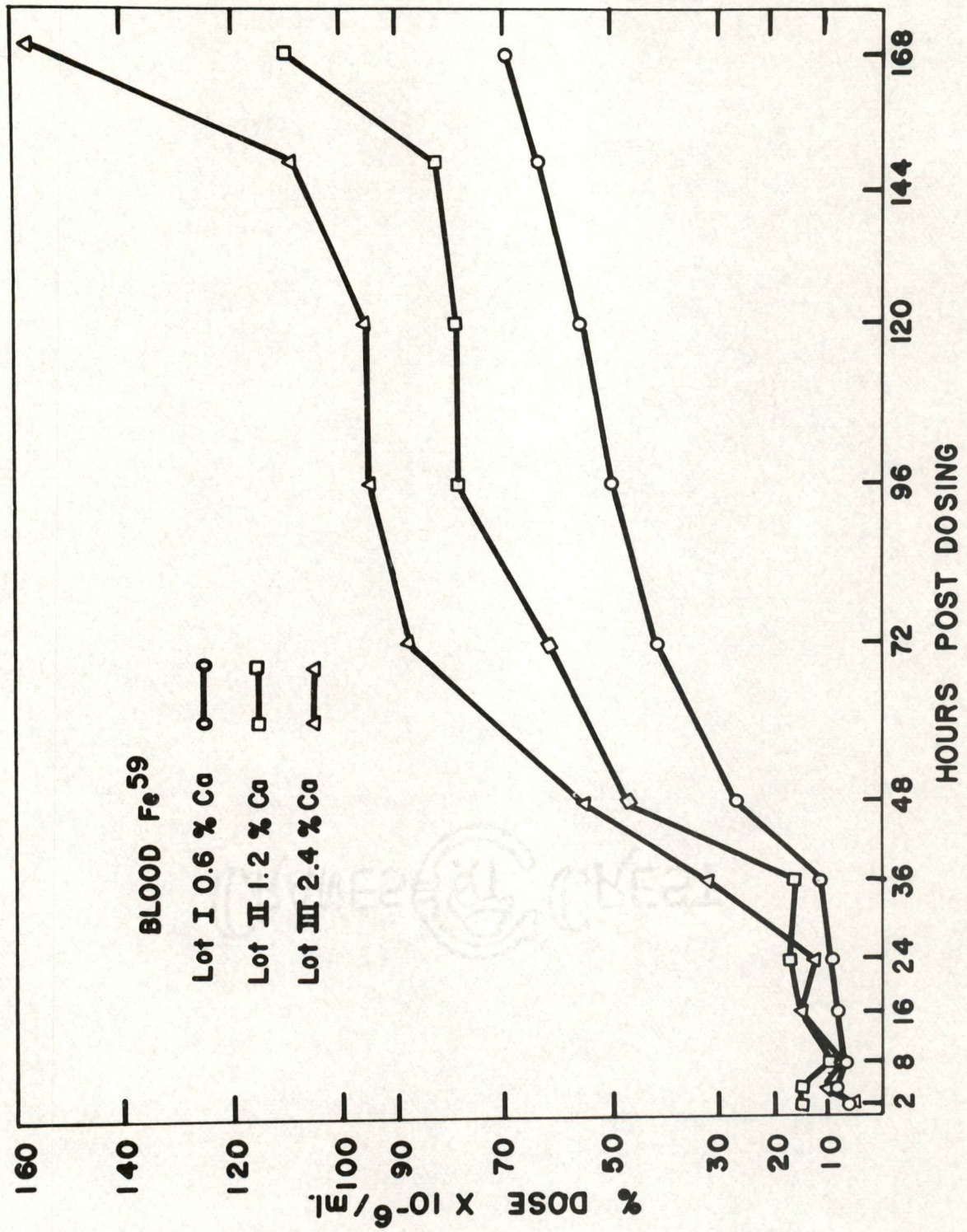


Figure 9. Iron-59 Concentration in Blood.

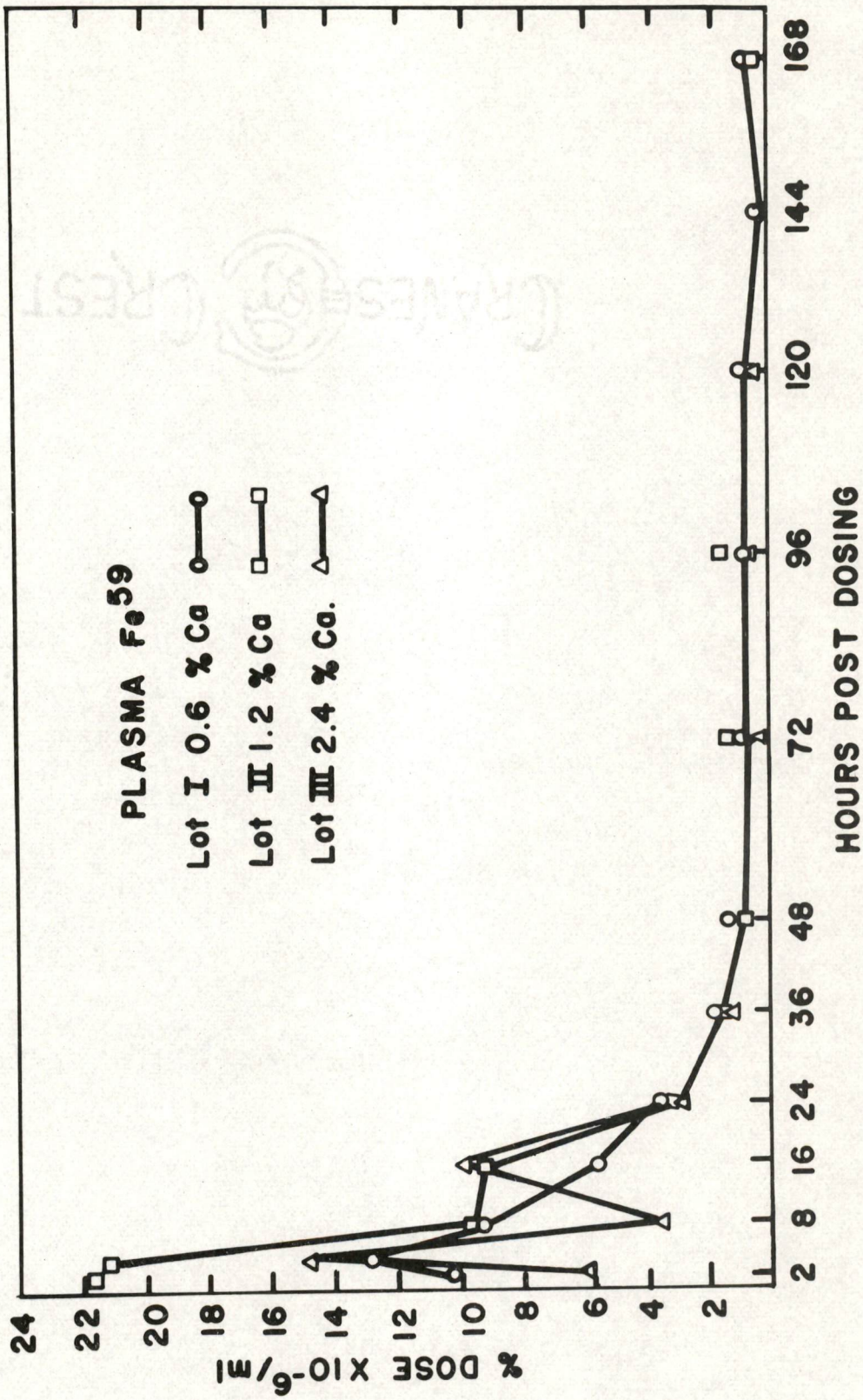


Figure 10. Iron-59 Concentration in Plasma.

increase with time indicates a hematopoetic effect in that the iron was in transition from the plasma to the red blood cells. Excessive calcium increased the level of Fe-59 per milliliter in the blood; however, excessive calcium depressed the body weight of the pigs and thereby lowered the volume of blood in the pigs on the high calcium rations. If these differences in concentration were not due to blood volume depression a difference in hemoglobin levels should have been apparent. This was not the case since hemoglobin levels were similar for all pigs on the three levels of calcium (Table XIII). However, Darby et al. (1947) and Hahn et al. (1947) have shown the efficiency of iron utilization to parallel the need for this element in growing children. If this were true for growing swine, the excessive calcium in this study increased the requirement for iron as indicated by the higher levels of iron per milliliter of blood in the pigs on the higher calcium levels.

Kletzien (1940) and Freeman and Ivy (1942) observed that high calcium (3.0 per cent) reduced iron retention. However, they were working with anemic rats in which calcium may not have had an effect on iron retention if sufficient iron were present. Feeding high levels of bone meal to rats, Chapman and Campbell (1957a) found low hemoglobin levels when low iron was fed but no effect on hemoglobin or liver iron when higher levels of iron were fed. Liver iron did not decrease but kidney iron did decrease when Miller et al. (1959) fed 1.25 per cent calcium to swine.

Like copper, iron is excreted primarily in the feces. The cumulative excretion of Fe-59 is shown in Figure 11. The total Fe-59

TABLE XIII

HEMOGLOBIN VALUES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Trial I	11.34	11.11	10.59
Trial II	11.57	11.52	10.67
Average	11.46	11.31	10.63

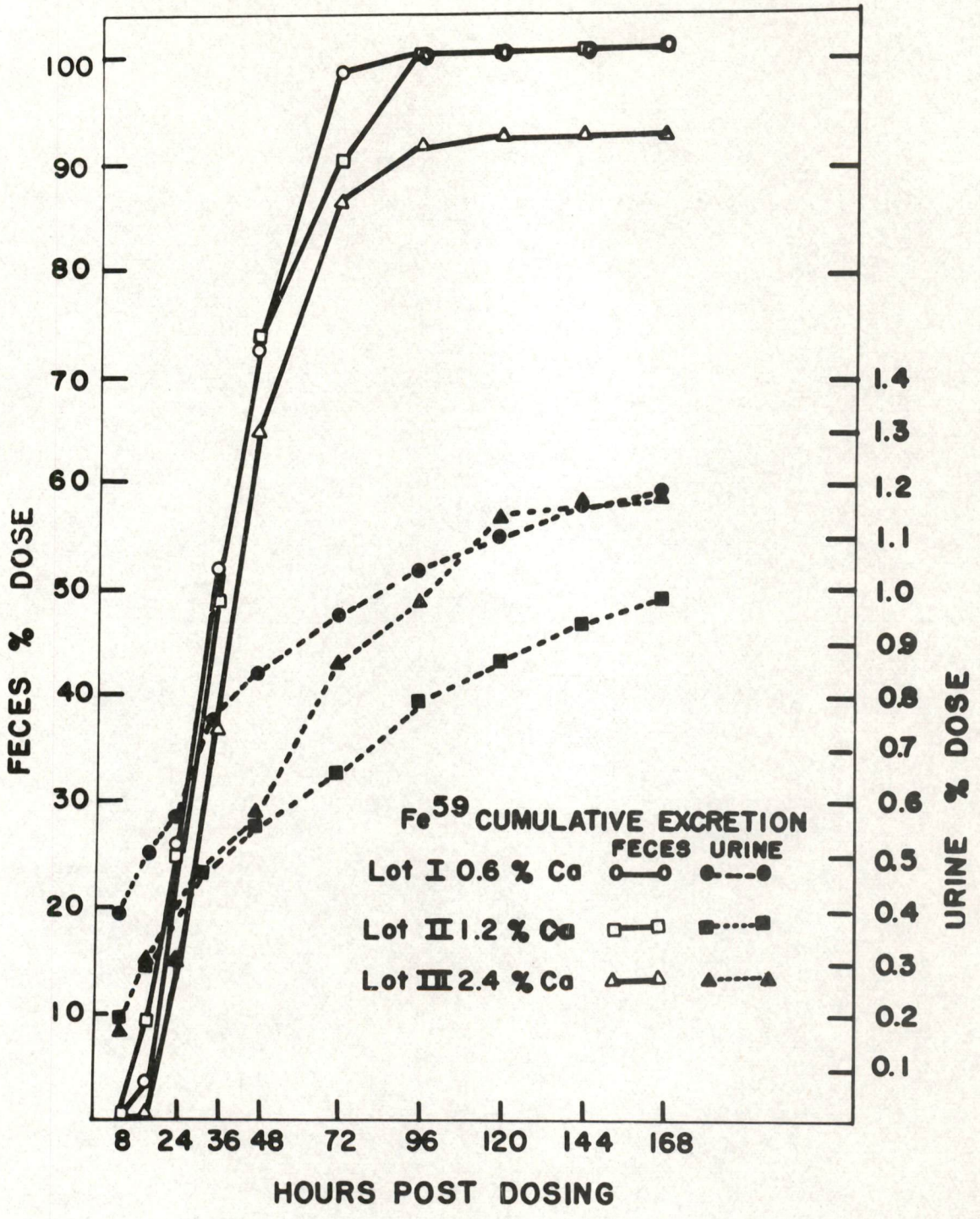


Figure 11. Cumulative Iron-59 in Feces and Urine.

excreted by pigs in Lots I, II and III averaged over 100 per cent of the dose. This may be accounted for by simultaneous dosing with Cu-64, variation in the amount used for standard counting and/or the biological variation of isotopes in general. Cu-64 has been reported to contain longer half-life contaminants (Frierson et al. 1952). The pigs in Lot III excreted an average of 93.2 per cent Fe-59; however, this was not significantly lower than the 103 per cent excreted by pigs in Lots I and II. The amount of Fe-59 excreted increased from 24 to 72 hours at which time the levels plateaued and only slight increases in excretions were noted through termination of the experiment at 168 hours.

Herndon et al. (1958) reported similar data from rats receiving Fe-59 and fed stock diets. Collecting for 5 days, these workers reported 96.8 (±2.29, std. error mean) per cent of the Fe-59 excreted in the feces. The radioactivity was not measured in the urine.

These data are not in agreement with that reported by Mayo et al. (1960), in that rats fed a semipurified type diet with 16 p.p.m. iron added, excreted only 48 per cent of the Fe-59 in the feces and a trace amount in the urine 90 hours after dosing. These disagreements may be due to dietary iron level and/or stage of growth of the animals.

Fe-59 excretion in the urine is shown in Table XIV. There were no differences due to excessive calcium in the rations. Total urinary excretion of Fe-59 amounted to 1.18, 0.99 and 1.18 per cent of the dose for pigs in Lots I, II and III, respectively.

TABLE XIV

EXCRETION OF FE-59 AND TOTAL VOLUME AND WEIGHT OF URINE AND FECES

Ration no. Per cent calcium	I 0.6	II 1.2	III 2.4
Number of pigs	6	6	6
Feces, kg.	4.57	4.81	2.67**
Urine, l.	14.07	14.99	28.82**
Per cent of Fe-59 dose			
Feces	103.29	103.49	93.15
Urine	1.18	0.99	1.18
Retained	00.00	00.00	5.67

** $P < 0.01$

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to study the effect of excessive calcium on the absorption and distribution of Se-75, Mo-99, Cu-64 and Fe-59 in swine. A total of 48 pigs were used in two replicate trials of each experiment. The pigs were allotted to dietary treatments with calcium the only variable, (0.6, 1.2 and 2.4 per cent Ca). Growth, feed consumption and feed conversion data were obtained weekly. After two weeks on experiment, 3 pigs from each lot were selected for experiment I and placed in metabolism crates. After a four day preliminary and adjustment period the 9 pigs were orally dosed simultaneously with 1 millicurie of Mo-99 and 250 microcuries of Se-75. Blood, urine and feces samples were obtained frequently for 5 days at which time the pigs were sacrificed for sampling of 17 tissues. Radiochemical determinations were made on all samples.

The pigs remaining after experiment I were maintained on their respective dietary treatments for a total of 4 weeks. Three pigs from each treatment were placed in metabolism crates and same procedures used as in experiment I, except that these pigs were orally dosed simultaneously with 2 millicuries of Cu-64 and 50 microcuries of Fe-59. Tissue data were not obtained from pigs in experiment II due to the short half-life of Cu-64 and low activity of the Fe-59.

The results of experiment I may be summarized as follows:

1. Excessive calcium increased ($P<0.01$) the concentration of

Se-75 per milliliter in blood and plasma.

2. Blood volume, calculated according to body weight, showed no differences in total Se-75.

3. Selenium was rapidly absorbed into the blood stream. The concentration reached a peak at 8 hours after dosing and then declined until 36-48 hours and plateaued to a constant level which remained through the 120 hour experiment. This constant blood level suggests a dietary need or a selenium-sulfur replacement.

4. Excessive calcium had no effect on Se-75 excretion.

5. Increasing calcium increased Se-75 concentration in the femur, large intestine contents ($P < 0.01$), large intestine wall, liver, skin, spleen and rib ($P < 0.05$).

6. Liver and kidney combined accounted for 8 per cent of the oral Se-75 dose.

7. Absorption and excretion of Mo-99 was rapid with excessive calcium showing no effect on either.

8. A significant difference in pathway of excretion of Mo-99 was found between trials. This was concluded to be due to extreme differences in the specific activity of the Mo-99 dosing solutions.

9. Excessive calcium increased the concentration of Mo-99 in the liver ($P < 0.01$) and spleen ($P < 0.05$) and the total Mo-99 in the liver ($P < 0.01$).

10. Increasing calcium increased ($P < 0.01$) the total urine volume and decreased ($P < 0.01$) the total fecal output.

The following observations were made from experiment II:

1. There was no effect of excessive calcium on the absorption of Cu-64.

2. The 2.4 per cent calcium ration increased ($P < 0.01$) the output of Cu-64 in the urine while the amount in the feces was unaffected.

3. Approximately 25 per cent of the Cu-64 was retained in the tissues and fluids of the body 5 days after administration.

4. Absorption of Fe-59 was relatively slow but increasing concentration was noted through the termination of the experiment.

5. Increasing calcium increased the concentration of Fe-59 per milliliter of blood.

6. Excretion of Fe-59 was not affected by excessive calcium.

Indications from growth data obtained are:

1. Excessive calcium (2.4 per cent) depressed ($P < 0.01$) average daily gains, feed consumption and increased the amount of feed required per pound of gain.

2. Differences between 0.6 and 1.2 per cent calcium fed pigs were not significant.

Precautions from simultaneous administration of doses are noted as follows:

1. Dosing with two gamma emitters simultaneously and differentiating by half-life decay seems undesirable with the technique employed in these experiments. The possibility of contaminants result in inaccurate determination. Separation by decay of direct counted samples allows error in concentration due to hydration or dehydration

from storing samples until the short half-life isotope has decayed out.

3. If equipment is available for separation by energy evolved, these procedures would seem more desirable.

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APPENDIX

APPENDIX TABLE I

BLOOD AND PLASMA - SE^{75} PER CENT DOSE X 10^{-4}/ML

		Blood					Plasma				
Hours		8	24	48	96	120	8	24	48	96	120
Ration	Pig no.										
I	274	116	99	74	67	66	165	117	99	87	86
	272	49	44	37	32	29	75	61	49	39	40
	257	93	69	49	46	44	123	97	71	61	59
	751	81	46	35	43	40	119	72	59	69	65
	749	75	55	44	43	44	136	--	65	67	68
	682	79	56	46	51	42	138	87	68	66	67
	Mean	82	62	48	47	44	126	87	69	65	64
II	255	127	90	67	65	62	181	122	97	81	81
	269	95	88	62	53	58	142	128	100	77	80
	260	127	97	77	64	63	178	135	101	87	89
	775	78	49	40	40	43	117	48	60	60	59
	758	95	62	48	47	45	132	81	72	70	68
	761	78	48	30	34	33	119	78	55	53	51
	Mean	100	72	54	51	51	165	100	81	71	71
III	259	134	97	78	70	71	186	130	108	90	92
	265	105	86	67	61	61	154	118	89	76	72
	254	153	114	93	86	87	212	144	137	118	124
	772	102	72	54	47	50	147	102	79	66	68
	710	64	52	39	32	35	108	72	56	46	49
	764	90	60	47	43	44	123	84	67	61	62
	Mean	108	80	63	57	56	155	108	89	73	78

APPENDIX TABLE II

FECES AND URINE - SE^{75} TOTAL PER CENT DOSE X 10^{-2}

Hours		Feces					Urine				
		24	48	72	96	120	24	48	72	96	120
Ration	Pig no.										
I	274	299	569	445	308	117	324	147	113	97	101
	272	184	269	111	229	169	107	63	26	81	38
	257	1223	1514	812	331	292	242	134	90	86	130
	751	619	483	245	138	120	271	91	110	74	68
	749	398	283	242	207	111	294	100	91	75	66
	682	436	269	212	208	91	453	106	95	75	67
	Mean	527	565	345	237	150	282	107	88	81	78
II	255	6	741	454	520	145	448	246	144	113	99
	269	238	866	207	170	97	214	163	107	159	175
	260	72	479	193	165	136	283	186	168	52	271
	775	253	747	90	294	152	378	106	97	77	69
	758	570	667	246	328	129	332	89	75	62	56
	761	357	585	196	323	142	309	91	88	72	58
	Mean	250	681	231	300	164	321	148	113	89	121
III	259	273	526	399	201	151	590	165	128	21	217
	265	188	606	1039	699	217	330	122	87	77	80
	254	70	1295	216	181	144	398	122	100	65	119
	772	743	604	215	162	48	331	114	108	53	128
	710	83	940	417	216	138	269	97	95	76	70
	764	762	625	179	182	96	328	103	78	69	58
	Mean	353	733	411	274	133	362	121	100	60	112

APPENDIX TABLE III

BLOOD AND PLASMA - MO^{99} PER CENT DOSE X 10^{-4}/ML

Hours		Blood					Plasma				
		2	4	8	16	24	2	4	8	16	24
Ration	Pig no.										
I	274	39	16	5	4	2	44	11	0	0	5
	272	19	28	18	14	1	26	39	24	5	2
	257	2	22	15	12	1	25	19	4	0	0
	751	51	31	6	1	1	61	26	0	0	0
	749	26	15	3	1	1	33	3	0	0	0
	682	26	15	2	1	1	24	0	0	0	0
	Mean	27	21	8	6	1	36	16	5	1	1
II	255	39	15	1	3	2	42	9	0	0	13
	269	7	11	12	2	1	10	16	2	4	0
	260	32	19	0	13	3	45	18	0	0	0
	775	24	14	3	1	1	33	10	0	0	1
	758	27	17	3	1	1	34	2	0	0	0
	761	24	20	5	2	1	32	19	0	0	0
	Mean	26	16	4	4	2	33	12	0	1	2
III	259	39	9	0	3	2	63	4	0	0	0
	265	24	15	4	0	1	32	11	0	0	0
	254	53	18	5	0	1	65	11	0	0	0
	772	55	29	5	1	1	65	22	0	0	0
	710	47	49	14	4	1	60	80	3	0	0
	764	27	20	2	2	1	34	12	0	0	0
	Mean	41	23	5	2	1	53	23	1	0	0

APPENDIX TABLE IV

FECES AND URINE - MO^{99} TOTAL PER CENT DOSE X 10^{-2}

Hours		Feces					Urine				
		24	48	72	96	120	24	48	72	96	120
Ration	Pig no.										
I	274	505	540	93	17	11	6657	452	211	111	105
	272	550	532	130	63	5	6226	626	182	403	137
	257	723	635	236	23	23	5610	476	215	73	156
	751	1543	1074	38	10	7	3482	233	192	102	74
	749	2483	1346	73	19	52	1817	236	140	75	52
	682	3025	2795	131	20	6	2700	185	117	74	51
	Mean	1471	1154	117	25	17	4415	368	176	140	96
II	255	3	1220	204	119	11	6228	377	235	132	108
	269	511	1186	43	10	5	4261	822	311	145	177
	260	255	930	70	17	6	3936	338	186	45	159
	775	748	5590	110	86	12	2833	206	99	68	52
	758	1852	3451	427	338	120	2454	159	92	64	61
	761	794	3461	126	33	8	3773	199	81	72	51
	Mean	694	2640	163	101	27	3914	350	167	88	101
III	259	264	281	56	11	6	6253	563	359	61	304
	265	506	833	427	206	27	2768	379	187	150	115
	254	39	147	21	12	7	6679	552	263	195	166
	772	1074	1106	102	25	10	3342	294	144	70	107
	710	191	1279	637	151	64	5190	518	227	145	118
	764	1123	2137	157	0	9	3108	270	115	75	59
	Mean	533	964	233	68	21	4557	429	216	116	145

APPENDIX TABLE V

SELENIUM AND MOLYBDENUM TISSUE DATA (PER CENT DOSE X 10^{-4}/GM)

		Bile		Femur		Hair		Heart		Kidney	
		Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}
Ration	Pig no.										
I	274	12.0	0.8	27	2.8	3.0	3.3	47	2.0	738	-
	272	4.0	3.2	11	2.7	1.0	1.6	25	2.2	392	-
	257	5.0	0.9	13	1.7	1.0	1.0	29	1.9	822	-
	751	10.0	1.0	21	3.1	2.0	0.8	28	1.7	155	92
	749	7.0	0.4	20	1.9	3.0	7.4	30	0.5	159	60
	682	7.0	0.9	22	1.7	2.0	1.3	30	1.2	144	34
	Mean	7.5	1.2	19	2.3	2.0	2.6	32	1.6	402	31
II	255	8.0	4.4	29	2.9	1.0	0.6	50	2.0	411	-
	269	5.0	0.6	17	2.0	1.0	0.9	49	2.4	676	-
	260	7.0	0.5	21	2.2	1.0	0.2	50	2.0	899	-
	775	7.0	1.2	18	2.3	2.0	2.4	26	0.8	109	29
	758	6.0	0.4	21	1.5	4.0	4.7	30	1.4	119	43
	761	7.0	2.2	20	2.4	2.0	-	24	0.3	139	25
	Mean	6.7	1.6	21	2.2	2.0	1.5	38	1.5	392	16
III	268	8.0	0.9	27	2.2	1.0	0.8	51	2.7	696	-
	263	12.0	1.7	21	2.1	1.0	0.4	45	3.7	568	-
	256	12.0	3.4	30	2.8	3.0	1.0	47	2.4	719	-
	771	9.0	1.0	39	1.3	4.0	24.9	31	1.1	173	54
	774	8.0	1.8	26	2.0	7.0	5.8	23	1.2	148	95
	763	7.0	0.5	29	1.5	16.0	108.5	32	4.7	145	55
	Mean	9.3	1.6	29	2.0	5.3	23.6	38	2.6	408	24

APPENDIX TABLE V (continued)

		L. int. con.		L. int. wall		Liver		Lung		Muscle	
		Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}
Ration	Pig no.										
I	274	21	0.6	62	1.3	206	14	82	2.0	12	0.7
	272	12	1.4	33	1.6	89	40	37	1.2	5	0.7
	257	30	0.6	50	1.4	136	18	48	1.0	9	0.7
	751	10	0.4	41	3.0	82	13	47	2.4	6	0.7
	749	11	0.4	41	2.8	83	8	47	4.9	5	0.4
	682	10	0.5	31	1.9	98	10	50	1.6	6	0.4
	Mean	16	0.6	43	2.0	116	17	52	2.2	7	0.6
II	255	21	2.2	71	3.0	199	35	81	1.8	15	0.7
	269	16	0.6	56	2.5	207	38	75	1.0	9	0.6
	260	18	-	63	1.2	188	19	86	0.9	12	0.5
	775	13	0.9	36	1.9	76	14	41	1.0	6	1.6
	758	10	0.2	49	2.2	82	22	53	2.0	7	0.6
	761	13	1.1	43	1.9	71	12	50	0.8	5	0.0
	Mean	15	0.8	53	2.1	137	23	64	1.2	9	0.7
III	268	31	1.1	81	2.0	226	35	74	1.8	15	1.6
	263	36	0.1	64	2.0	187	39	83	1.5	10	0.8
	256	33	1.6	85	2.0	286	36	90	1.3	11	0.5
	771	23	1.7	49	8.4	105	28	48	3.6	7	0.8
	774	19	2.0	31	1.2	88	37	50	-	5	0.5
	763	17	0.7	41	2.1	94	34	52	0.4	7	0.7
	Mean	26	1.2	58	3.0	164	35	66	1.4	9	0.8

APPENDIX TABLE V (continued)

		Skin		S. int. con.		S. int. wall		Spleen		Rib	
		Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}	Se^{75}	Mo^{99}
Ration	Pig no.										
I	274	19	0.7	4	0.3	71	1.0	112	1.5	40	2.3
	272	9	0.5	2	0.8	47	1.6	45	1.0	14	2.2
	257	12	0.8	5	0.4	69	1.6	76	1.8	29	2.0
	751	6	0.5	7	0.5	62	3.7	58	2.6	21	4.0
	749	10	0.5	9	0.7	69	2.3	56	1.8	20	2.7
	682	7	0.5	7	-	115	7.6	56	2.9	22	3.9
	Mean	10	0.6	6	0.4	72	3.0	67	1.9	24	2.8
II	255	19	1.0	11	3.0	77	2.1	109	1.9	36	2.3
	269	14	0.5	3	0.3	66	2.4	100	2.4	36	1.1
	260	19	0.5	5	0.2	63	0.9	93	1.7	33	2.5
	775	10	0.3	6	0.5	68	1.0	59	2.7	18	2.8
	758	7	0.4	12	2.7	78	3.9	66	3.5	21	3.3
	761	7	0.3	2	0.2	69	3.7	54	3.4	20	2.6
	Mean	13	0.5	6	1.2	70	2.3	80	2.6	27	2.4
III	268	24	1.2	4	0.3	127	2.6	103	1.9	41	2.5
	263	19	1.2	7	0.4	80	4.1	152	1.9	30	2.6
	256	24	0.6	12	2.1	107	4.6	103	1.0	52	2.4
	771	11	1.0	7	0.4	72	2.7	70	6.2	39	4.2
	774	5	1.2	13	0.6	76	3.5	56	3.5	26	4.5
	763	11	1.2	29	2.0	74	3.8	63	5.4	29	3.2
	Mean	16	1.1	12	1.0	89	3.6	91	3.3	36	3.2

APPENDIX TABLE V (continued)

Ration	Pig no.	Tooth Se^{75}	Tooth Mo^{99}	Vertebrae Se^{75}	Vertebrae Mo^{99}
I	274	17	2.9	59	4.9
	272	9	1.2	23	2.3
	257	9	1.9	33	2.8
	751	30	1.4	30	3.9
	749	29	1.2	29	1.6
	682	31	1.6	31	2.5
	Mean	21	1.7	34	3.0
	255	20	1.5	51	3.1
	269	20	1.5	52	2.1
	260	15	2.5	44	2.2
	775	29	1.1	29	3.3
	758	31	1.2	31	2.9
	761	32	-	32	3.1
	Mean	25	1.3	40	2.8
	268	15	2.9	56	2.6
	263	10	1.5	44	1.6
	256	15	1.1	56	2.1
	771	45	1.1	45	5.0
	774	30	-	30	6.2
	763	36	0.4	36	7.1
	Mean	25	1.2	45	4.1

APPENDIX TABLE VI

BLOOD AND PLASMA - CU^{64} PER CENT DOSE X 10^{-4}/ML

Hours		Blood					Plasma				
		8	24	48	72	96	8	24	48	72	96
Ration	Pig no.										
I	262	17	19	17	13	17	27	28	28	22	7
	271	11	18	17	20	12	18	26	27	20	9
	280	25	53	54	55	35	47	74	72	55	54
	753	9	10	17	38	127	19	10	18	18	31
	765	7	7	9	19	78	14	14	11	12	9
	678	10	9	12	28	91	18	16	15	16	17
	Mean	13	19	21	29	60	24	28	29	24	21
II	266	20	22	21	12	-	31	34	32	24	96
	261	21	21	21	5	-	30	30	30	21	92
	267	22	23	22	5	15	32	32	31	30	107
	762	10	11	18	55	214	17	17	15	10	18
	770	9	11	20	47	180	18	18	13	15	12
	769	6	7	11	33	123	12	13	11	17	18
	Mean	15	16	19	26	89	23	24	22	20	57
III	268	20	28	21	51	30	29	33	34	37	31
	263	22	29	21	37	49	31	35	37	20	148
	256	29	24	17	10	2	11	27	26	12	12
	771	14	16	18	79	322	11	26	23	18	29
	774	8	10	16	46	170	14	17	14	17	26
	763	14	-	32	79	309	24	22	20	18	22
	Mean	18	18	21	50	147	20	27	26	20	45

APPENDIX TABLE VII

FECES AND URINE - Cu^{64}

Hours		Feces (per cent dose)					Urine (per cent dose X 10^{-2})				
		24	48	72	96	120	24	48	72	96	120
Ration	Pig no.										
I	262	0	2.2	49.2	6.2	34.3	9.3	26.8	23.9	69.3	44.0
	271	2.4	20.2	13.8	1.7	9.4	12.3	15.0	10.8	9.1	48.3
	280	3.8	12.4	8.0	0.7	18.4	24.0	30.0	21.5	23.7	--
	753	28.2	27.9	7.9	1.3	3.1	20.1	25.1	63.1	81.5	208.9
	765	20.7	48.2	19.6	7.9	6.6	12.2	9.2	81.2	169.7	51.3
	678	33.5	38.3	5.8	2.3	1.5	22.0	20.0	78.7	--	--
	Mean	14.8	24.9	17.4	3.3	12.2	16.7	21.0	46.6	58.9	58.8
II	266	0.0	24.2	24.9	6.5	10.6	44.4	23.8	18.8	119.2	10.3
	261	0.5	16.6	21.4	2.9	10.9	40.7	38.1	20.6	76.9	82.8
	267	0.0	1.9	14.2	38.2	16.5	20.7	21.7	11.8	378.4	43.3
	762	33.8	31.7	3.1	1.9	1.1	16.9	83.5	186.5	31.9	89.5
	770	25.9	37.6	12.7	4.3	3.1	11.4	18.6	68.8	--	--
	769	17.6	54.3	16.9	5.2	2.5	11.1	26.4	37.1	28.4	35.8
	Mean	13.0	27.7	15.5	9.8	7.4	24.2	37.1	57.3	105.8	43.6
III	268	0.0	33.4	24.9	15.3	12.2	25.9	36.3	101.9	96.9	82.3
	263	0.1	55.1	18.7	5.2	10.2	28.6	15.2	151.9	234.7	--
	256	1.5	33.8	9.1	2.4	--	20.7	52.6	188.5	172.0	119.2
	771	16.8	25.4	9.6	3.6	7.3	9.0	26.7	73.7	--	126.2
	774	9.8	49.0	30.1	5.9	10.8	9.5	57.2	271.5	--	--
	763	16.7	22.1	9.5	2.2	1.1	15.3	8.5	124.6	68.6	177.8
	Mean	7.5	36.5	17.0	5.8	6.9	18.2	32.7	152.0	95.4	84.2

APPENDIX TABLE VIII

BLOOD AND PLASMA - FE^{59} PER CENT DOSE X 10^{-6}/ML

Hours		Blood					Plasma				
		24	48	72	96	120	2	8	16	24	48
Ration	Pig no.										
I	262	15	53	70	74	77	23	6	2	1	--
	271	1	3	4	6	7	2	2	--	--	--
	280	14	35	81	95	101	7	13	7	8	4
	753	12	34	50	60	74	13	13	15	6	2
	765	4	16	20	29	38	7	11	6	1	1
	678	6	20	24	37	40	9	10	3	3	0
	Mean	9	27	42	50	56	10	9	6	3	1
II	266	36	76	109	149	136	22	17	4	3	2
	261	26	66	79	89	97	59	5	0	1	1
	267	2	2	7	9	10	9	3	2	2	0
	762	21	61	85	100	104	11	16	12	6	0
	770	8	49	55	77	75	6	9	22	4	1
	769	8	30	38	47	51	23	7	15	4	1
	Mean	17	47	62	79	79	22	10	9	3	1
III	268	15	57	75	80	93	21	5	0	-	0
	263	16	70	164	113	117	2	7	1	0	1
	256	13	33	45	48	46	1	2	1	0	0
	771	16	64	99	133	147	10	4	33	8	3
	774	10	36	52	70	77	0	1	17	5	2
	763	--	71	92	128	96	1	2	7	3	2
	Mean	12	55	88	95	96	6	4	10	3	1

APPENDIX TABLE IX

FECES AND URINE - Fe^{59}

Hours		Feces (per cent dose)					Urine (per cent dose X 10^{-2})				
		24	48	72	96	120	24	48	72	96	120
Ration	Pig no.										
I	262	0	6.3	102.5	9.4	0.9	2.7	2.6	3.4	6.1	5.4
	271	6.4	80.6	22.9	2.0	0.6	4.1	1.0	2.3	1.2	5.9
	280	9.7	31.4	10.1	2.0	2.1	5.9	2.1	2.2	3.8	6.5
	753	54.7	44.5	8.3	0.5	0.3	121.5	102.0	30.3	19.6	--
	765	35.2	66.2	11.2	1.8	0.3	141.9	16.6	3.0	--	--
	678	49.6	52.3	3.9	0.2	0.2	80.1	37.8	24.9	20.9	26.9
	Mean	25.9	46.9	26.5	2.7	0.7	59.4	27.0	11.0	8.6	7.5
II	266	0	54.9	26.3	7.4	3.2	2.9	2.7	0.7	1.0	2.7
	261	3.9	79.3	23.3	0.4	0.2	6.1	2.0	1.8	9.1	5.1
	267	0	2.1	37.1	59.2	0.8	3.2	1.1	1.2	0.9	0.5
	762	64.1	37.4	1.5	0.5	0.3	93.1	60.4	35.6	23.1	23.0
	770	46.4	51.4	4.0	0.1	0.1	45.0	31.3	--	45.7	--
	769	33.9	72.3	7.5	0.7	0.0	66.4	16.8	21.0	3.5	7.9
	Mean	24.7	49.6	16.6	11.4	0.8	36.1	19.0	10.0	13.9	6.5
III	268	0	19.0	52.1	13.6	0.8	1.9	1.3	--	0.9	5.8
	263	0	45.2	38.8	7.9	0.8	0.8	5.8	--	--	3.9
	256	2.1	63.6	7.9	8.6	0.2	4.9	6.0	12.6	4.3	4.0
	771	39.1	59.8	4.7	0.6	0.3	87.9	10.7	25.0	25.9	4.4
	774	15.4	59.1	19.1	0.9	0.1	64.6	73.4	10.0	31.8	64.8
	963	34.5	54.0	7.5	0.5	0.1	92.4	23.0	17.8	13.3	9.3
	Mean	15.2	50.1	21.7	5.4	0.4	42.1	20.0	27.6	12.7	15.3