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# Placental transfer and maternal-fetal utilization of manganese and copper by swine and sheep

Charles T. Gamble

To the Graduate Council:

I am submitting herewith a dissertation written by Charles T. Gamble entitled "Placental transfer and maternal-fetal utilization of manganese and copper by swine and sheep." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

S.L. Hansard, Major Professor

We have read this dissertation and recommend its acceptance:

R.L. Murphee, R.R. Shrode, S.A. Griffin, C.S. Hobbs, D.O. Richardson, R.S. Dotson

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Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 24, 1969

To the Graduate Council:

I am submitting herewith a dissertation written by Charles T. Gamble entitled "Placental Transfer and Maternal-Fetal Utilization of Manganese and Copper by Swine and Sheep." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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Vice Chancellor for
Graduate Studies and Research

# PLACENTAL TRANSFER AND MATERNAL-FETAL UTILIZATION OF MANGANESE AND COPPER BY SWINE AND SHEEP

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A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Charles T. Gamble

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ABSTRACT

Both manganese and copper have been shown to be necessary for the normal functioning of the animal body, and although there is a vast quantity of information available from laboratory animals, little or no factual information is readily available on placental transfer or maternal-fetal utilization of these two minerals. For these reasons this study, utilizing 15 yearling gilts (73 fetuses) and 15 yearling ewes (13 fetuses), was initiated during the spring of 1968. Pregnant gilts and ewes were dosed with a single tracer level of radio-manganese and radio-copper for blood balance and subsequent tissue-organ distribution and placental transfer studies. Results indicated that, within species, stage of pregnancy did not influence blood disappearance rate, tissue-organ distribution or excretion of manganese and copper. Species did influence fecal excretion of manganese, with sheep retaining approximately one-fourth the intravenously injected manganese as did swine. Amount and rate of manganese transfer was also influenced by species. During the third trimester and at 168 hr. post-dose administration, an 8,000-gm. swine litter contained 22.8 percent of the intravenously injected $^{54}Mn$; while a 5,000 gm. lamb fetus contained 3.0 percent. Fetal weight within species was the main factor in determining total percent $^{54}Mn$ transfer. However, 24 hr. post-dosing, an 8,000-gm. swine litter contained 2.4 percent and a 5,000-gm. lamb fetus contained 2.2 percent of the $^{64}Cu$ dose, indicating that fetal weight, rather than species, influenced the total percent transferred. Total fetal analysis indicated that radio-manganese

transferred during the 168-hr. time study was in relation to manganese content, but this relationship was not reached for radio-copper and copper by 24 hr. post-dosing. Many factors have been shown to influence placental transfer, and this study further indicated that numerous interrelated factors contribute to the determination of what passes from dam to fetus.

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# CHAPTER I

## INTRODUCTION

Maternal-fetal nutrition has been a point of interest since the earliest written history, but it has only been during the last century that this interest has been applied to determine nutritional relationships between dam and fetus.

During the last 30 years, it has been established that the long-accepted maxim that "a pregnant mother must eat for two" is not completely true since it is now known that the demands of pregnancy are not excessive, and, for the most part, nutritional needs of the fetus take precedence over those of the mother. However, with the increasing number of swine being kept in confinement and with the reduction in the amount of feed that gestational sows are now receiving, the ratios and amounts of microminerals have become increasingly important.

Although numerous studies have been conducted to determine the roles of manganese and copper in animal nutrition, there is a lack of information on the regulatory mechanisms of mineral absorption, metabolism and utilization. However, it has been clearly demonstrated that these minerals are widely distributed in nature, that they have many essential functions in the animal and that their consideration is of practical importance in animal nutrition.

Since these early studies were conducted, radioisotope tracer techniques have been introduced into the field of nutrition and

physiology, and their use has been valuable in the study of mineral interrelationships and fetal nutrition. In fact, this has become the primary manner by which specific functions and metabolic pathways are assigned to different nutrient elements in the fetus, particularly with respect to the placental transfer of these elements.

To contribute to the understanding of maternal-fetal nutrition, this study was conducted to determine the absorption, excretion and placental transfer of copper and manganese and their radioisotopes in swine and sheep.

# CHAPTER II

# LITERATURE REVIEW

## I. MANGANESE

History. Before manganese was first isolated by Schecle (1742-86) it was recognized by the Romans as possessing "female" magnetic properties. However, at that time the term magnesia was applied interchangeably to the ores of both magnesium and manganese.

Von Oettinger (1935) and Cotzias (1958) presented evidence that manganese is essential to life, and a growing number of functions is being assigned to it. However, with improvement in analytical methods, trace amounts of manganese in biological material tend to become smaller and smaller. Although manganese shows a wide range of functions *in vitro*, it is impossible at present to assign any specific function *in vivo*.

Methods of analysis. It is understood that the determination of manganese content of a substance is difficult under conditions known today. Many methods of manganese analysis have been proposed. In these methods the difficulty lies in handling the sample, during which losses of manganese and contamination with external manganese are likely to occur. Cotzias (1962) outlined methods and estimated sensitivities of various procedures used for manganese determinations.

Functions in metabolism. It has been shown (Mohammed and Greenberg, 1945) that arginase preparations are markedly stimulated by divalent

metals, the extent of activation depending upon the kind of metal, its concentration and the period and temperature of incubation. Dialyzed yeast arginase was shown to lose its activity, but to become reactivated by divalent metal additives. In both cases, manganese was only one of several divalent metals which would initiate these reactions. However, Boyer et al. (1942) and Shils and McCollum (1943) showed that arginase activity was lower in liver from manganese-deficient rats than in liver from normal rats. Shils and McCollum (1943) were able to raise arginase activity to control levels by manganese additives in vitro. Cotzias (1962) expressed doubt that these experiments showed the exclusive site of manganese action since birds, which lack urea-synthesizing systems, develop a manganese deficiency similar to that of mammals. Many workers have noted manganese activation of other enzymes: cysteine desulfhydase, Binkley (1943); thiaminase, Reddy et al. (1948); deoxyribonuclease, McCarty (1946); carnosinase, Hanson and Smith (1949); enolase, Malmstrom et al. (1958); intestinal prolinase, Smith and Bergmann (1944); glycl-L-leucine dipeptidose, Smith (1948b,c) and prolidase, Smith (1948a).

Oxidative phosphorylation is thought to require manganese ions. Lindberg and Ernster (1954) considered these ions to be a necessary co-factor for the reaction. This view is supported by the work of Cotzias and Maynard (1955), who found rapid accumulation of injected radio-manganese in the mitochondria and Bronk and Kielly (1957a,b), who found that although several metals activated this reaction, manganese was the most effective among them.

Manganese is considered to play a major role in the metabolism

of fatty acids in the intact organism. This statement is supported by the work of Amdur et al. (1946), who found lipotropic action of manganese on the liver of the intact rat, and by Curran (1954, 1955), who indicated that the rat liver incorporation of acetate into fatty acids is markedly stimulated by $Mn^{++}$; Tietz (1957) reported that manganese was more effective than the magnesium in the enzymatic conversion of mevalonic acid to squalene, a cholesterol precursor.

Requirement in normal nutrition. Underwood (1956) and Cotzias (1958) have stated that manganese is considered essential in nutrition for the following reasons:

1. It is always present in foodstuffs.
2. Its concentration in mammalial tissues is steady; fairly characteristic of each organ and not species-linked.
3. It shows numerous impressive biochemical functions in vitro.
4. When diets deficient in manganese are eaten, specific symptoms result.
5. When given to severely manganese-deficient animals, it specifically and reproducibly relieves a major part of their deficiency.
6. When given as a dietary supplement, it prevents symptoms of deficiency from appearing.

Even with this knowledge, the actual dietary requirement for manganese can be only surmised since nutritional experiments have been carried out using supplements of inorganic manganese; while manganese

Various other abnormalities have been associated with a manganese deficiency. Shils and McCollum (1943) used a diet which provided only 3 µg. Mn/day per adult rat. The young born to deficient mothers displayed striking manifestations of apparent central nervous involvement consisting of backward gait, ataxia, loss of balance and a severe postural reaction to external stimuli. However, deficient mothers were able to support young born to normal mothers. Later, Hill et al. (1950) who raised successive generations of rats on a diet offering ten times as much manganese as used by Shils and McCollum, observed the same symptoms.

Hurley et al. (1958) discovered that ataxia in offspring of manganese-deficient rats could be prevented by giving manganese supplements to the mothers during the first half of pregnancy, but not during the last half. Later, Hurley and Everson (1959) localized ataxia in the central nervous system.

In 1943 Wachtel et al. reported that when rations containing high calcium to phosphorus ratios were incorporated into the diet of rats receiving 5 µg. manganese per rat per day, the manganese deficiency was aggravated. Comparable results have been obtained with mice (Kemmerer et al., 1931) and rabbits (Smith et al., 1944; Ellis et al., 1947). In the manganese-deficient rabbit, Rudra (1941) observed a vicarious appetite termed "manganese hunger."

Later, Smith et al. (1944) found that the addition of manganese to a powdered milk diet increased the growth of rabbits, but this rate did not reach that of animals raised normally. Bone weight, density,

length, breaking strength and ash content were significantly below those of control animals. Although these animals were anemic, their bone marrow was abnormally overcellular. In these deficient animals, manganese and arginase contents of the liver were diminished.

An almost linear relations was observed between liver or bone manganese and dietary manganese in the study by Smith and Ellis (1947). This relation held until the manganese intake reached 4 µg. per rabbit per day, after which point the tissue concentration fell significantly, probably due to homeostatic responses.

In cattle, manganese deficiency has been reported to occur on low-manganese pastures (Grashuis et al., 1962). Also, Munro (1957) reported that manganese supplementation improved the fertility of dairy cows in England. Bentley and Phillips (1951) reported the same deficiency symptoms in dairy cattle as have been observed in swine and laboratory animals.

Wilgus et al. (1936, 1937) were the first workers to link perosis with a manganese deficiency in chicks. Perosis is characterized by shortening and thickening of the bones accompanied by slipping of the epiphysis and of the Achilles tendon. Wilgus and Patton (1939) reported that high amounts of calcium, phosphorus and ferrous citrate tended to aggravate the disease in deficient birds and induced it in normal chickens fed normal amounts of manganese.

The bodies of manganese-deficient chicks showed reduced manganese content in both the bones and the soft tissues. Caskey et al. (1939, 1946) reported that normal chick bone contained about 2 µg. manganese

per gram, and bone from deficient chicks contained one-third to one-fourth that amount. Similar results were obtained for some of the soft tissues of the body, while eggs from deficient birds contained reduced levels of manganese. Gallup and Norris (1939a,b) and Wilgus et al. (1939) reported that hatchability was poor, that mortality of the young was high and that surviving young were often ataxic.

Concentration in feedstuffs. The varying content of plant materials reflects the ambient manganese concentration during plant growth. Of the cereal grains used in animal feeds, corn is low (11 ppm), barley (22 ppm) and wheat (31 ppm) are intermediate and oats (66 ppm) are highest in manganese content. Soybean meal was found to contain 30 ppm manganese; while protein supplements of animal origin were low in manganese content (Cotzias, 1962). Bentley and Phillips (1951) indicated that forages in the United States contained 50 to 150 ppm manganese.

Concentration in animal tissues. It has been well established that the manganese content of given tissues is relatively constant between species (Zondek, 1939; Kehoe et al., 1940; Von Oettingen, 1942; Fore and Morton, 1952; Butt et al., 1954, 1956; Griffith et al., 1954; Underwood, 1956; and Cotzias, 1958). Liver has been considered to be a possible site of manganese storage, but bone may also serve this function in the animal body since it is the site of highest concentration (Cotzias, 1962).

Absorption and excretion. The precise location of manganese absorption in the gastrointestinal tract is not known. However, Emmel

(1944), Gallup et al. (1951), Gessert et al. (1952) and Hobbs and Hansard (1952) have reported that the amount of metal absorbed is proportional to that presented for absorption.

Manganese is excreted primarily in the feces, with less than 1 percent excreted in the urine. Cotzias (1962) indicated that the gastrointestinal contents are both the source of and the sewer for manganese. This makes it very difficult to differentiate between the ions being absorbed and those being excreted. He also indicated that bile-bound manganese is probably reabsorbed so that each metal ion finally eliminated may have entered a given tissue several times. This is one reason for the confusion in trying to determine absorption and excretion of this metal.

Using an isotope of low specific activity, Greenberg and Campbell (1940) found that the rat excreted 97 percent of orally administered $^{54}Mn$, while 90 percent was excreted when the isotope was given parenterally. In later work, Greenberg et al. (1943) found that 3 to 4 percent of oral manganese was absorbed.

The biliary excretion of manganese was further investigated by Bertinchamps and Cotzias (1958). They indicated that the concentration in the bile paralleled that of the blood by a ratio of 10:1. This would account for the rapid disappearance of the injected radio-isotope from the circulating blood stream and its rapid increase in the digestive tract. These workers also noted a second wave in the bile after one hour in rats receiving rations more than adequate in manganese. They postulated that each wave indicated an individual

excretory pathway, possibly one each for two predominant valence states of the metal. Burnett et al. (1952) found the pancreatic juice to be a secondary route which contributes to fecal excretion of manganese. Here, radio-manganese excretion paralleled that of protein.

It has been observed also that the excretion of manganese is influenced by food intake, drugs and body load of stable manganese (Cotzias, 1962).

Interrelationships. Cotzias (1962) reviewed studies of various workers who have noted that many factors affect manganese metabolism.

Jukes (1940), Hogan et al. (1941), Jukes and Welch (1942) and Evans et al. (1943) indicated that choline deficiency aggravated perosis in birds and that manganese and choline acting together produced more than the sum of the two effects. Various workers have questioned also the relationship of thiamine and manganese.

Hormonal levels are though to affect manganese metabolism. Estradiol increased the manganese level in blood (Bolton, 1955). Ray and Deysach (1942) reported that the thyroid gland accumulated the metal and when manganese was given in large amounts, the basal metabolic rate was depressed. Glucocorticoids caused manganese to be moved from liver to muscle tissue in rats (Hughes and Cotzias, 1960).

Manganese metabolism apparently is coupled to amino acid metabolism. It served as an activator of some hydrolyzing enzymes in that it formed chelates with amino acids (Christensen et al., 1956; Christensen, 1957).

Wakil et al. (1957) and Tietz (1957) demonstrated that manganese was an activator in the synthesis of fatty acids in vitro. Later work by Lassiter (1966) indicated that there was a significant interaction between fat level in the ration and net manganese absorption in sheep.

Antibodies, antibiotics and certain diseases were thought to affect manganese metabolism (Cotzias, 1962).

Placental transfer. Orent and McCollum (1931) were the first to indicate placental transfer of manganese in rats. Later, Johnson (1943, 1944) reported that manganese was present in the newborn pig. Thus, placental transfer of this mineral must occur.

Newland and Davis (1961) used radio-manganese to study fetal development and placental transfer at various stages of gestation in sows fed rations of natural foodstuffs containing high (100 ppm) and relatively low (6 ppm) levels of manganese. They found no difference in fetal weights from sows maintained on the two diets, nor did it appear that the level of manganese in the maternal diet had an effect on the rate of passage of radio-manganese to the fetus. However, they observed higher total manganese concentrations in fetuses from sows on the higher manganese ration. In this study blood and urine levels of radioactive manganese were undetectable, thus giving no information on the rate at which the manganese isotope disappeared from the blood or urine. It was noted that 3 hr. post-dosing, sufficient radio-manganese had been transferred across the placenta to be detected in the fetus.

# II. COPPER

History. Copper was first identified in biological materials (plants) by Bucholtz (1816) and Meissner (1817). Its presence in animals was demonstrated by Sarzeau (1830) who found ox blood to contain 700 µg./l. Early investigators believed that the copper of plants and animals represented accidental contamination from the soil. But the demonstration of copper in colluscan blood (Harless, 1847) as a constituent of octopus hemocyanin (Fredericq, 1878) and in the tail feather pigment of the turaco (Church, 1869) indicated that the element was associated with specific biological substances. However, the element was not accepted as a definite physiological constituent until the twentieth century.

Results of studies of the copper content of biological materials were quite varied, and it was not until Bodansky (1921) reported reproducible figures on the copper content of the brain of humans that investigators realized the variation in biological materials. At approximately the same time, Guerithault (1920) and Fleurent and Levi (1920) suggested that this element participated in life processes as a catalyst.

It is now established that copper is an essential nutrient, the absence of which can lead to severe derangements in growth, physiology, and metabolism.

Method of analysis. Fister (1950) and Henry (1964) outline methods of sample preparation and copper determination in biological materials.

Functions in metabolism. Maynard and Loosli (1962) stated that copper is an activator of several enzymes, notably ascorbic acid

oxidase, tyrosinase, cytochrome oxidase and catalase.

However, copper metabolism is not completely understood, and in general, biochemical studies have been limited to the effects of copper deprivation on enzyme activities. Gallagher et al. (1956a,b) studied the biochemical activities in copper-deficient rat tissues. They reported that a moderate-to-advanced depletion markedly reduced both cytochrome oxidase and heme. Mitochondria from deficient animals were very susceptible to aging, and this susceptibility was reversed by pyridine nucleotides, glutathione and manganese ions. Adelstein and Vallee (1962) and Maynard and Loosli (1962) indicated that in a severe copper deficiency the oxidative capacity of mitochondria was lost. This loss has been ascribed to a decrease of cytochrome oxidase activity and aging. Also a depression of mitochondrial phospholipid synthesis in the liver, but not in the brain, has been reported by Adelstine and Vallee (1962). In addition, in severely deficient animals, the tricarboxylic acid cycle is upset, since acetyl CoA does not condense with glycerophosphate to form phosphotides.

Requirement in normal nutrition. In 1928, Hart et al. demonstrated that young rats fed on milk alone failed to gain in weight and developed anemia. When iron alone was added, weight gain and erythrocyte hemoglobin formation were not restored, but the addition of iron plus copper restored both.

Anemia has been demonstrated in rats, rabbits, chickens, pigs, dogs, sheep, goats and cattle on copper-deficient diets. Thus, copper is needed in the diets of most farm animals.

Maynard and Loosli (1962) reported the daily requirement to be about 50 mg. for cattle, 5 mg. for sheep and 5 mg./kg. in the dry matter of swine rations. The National Research Council indicated that 4.5 mg./lb. feed was adequate for baby pigs.

Deficiency symptoms. In addition to preventing anemia, copper is needed for normal bone development. Copper-deficient sheep and cattle exhibited osteoporosis and developed spontaneous fractures; young calves developed a ricket-like disease (Adelstein and Vallee, 1962). Teague and Carpenter (1951), Baxter and VanWyk (1953a,b) and Follis et al. (1955) reported that pigs deprived of copper showed deformities of the limbs, swayed backs and crooked forelegs. However, serum calcium and phosphorus concentrations were normal. It was suggested that the abnormalities resulted from excess resorption of bone or decreased deposition of bone matrix or both.

An incoordination of gait, known as anzootic ataxia or swayback, has been noted in lambs born to ewes grazed on copper-deficient pastures. A similar disease has been observed in calves and pigs (Cunningham, 1950; Underwood, 1956). Examination of the central nervous system showed a diffused symmetrical cerebral demyelination with secondary degeneration of the motor tracts of the cord. The histological changes resembled those present in multiple sclerosis. However, this disease can be prevented by the administration of copper to ewes during pregnancy.

Copper is needed also for normal wool and hair growth. Adelstein and Vallee (1962) reported that rabbits deprived of copper developed

alopecia and dermatosis, that dogs showed toughening and dullness of hair, that cattle developed a harsh coat and that wool lost pigment and crimp. Copper-deficient wool contained more N-terminal glycine and alanine and sometimes more N-terminal serine and glutamate groups than normal wool (Burley and deKock, 1957).

Severe copper deprivation in the bovine leads to fibrosis of the myocardium and a seasonal incidence of sudden death known as "falling disease." Pathological examination of the heart shows small cell infiltration and large, widely distributed areas of atrophy leading to collagen displacements. Blood and liver concentrations are extremely low, and profound anemia is present. Anemia has been suggested as an etiological factor leading to myocardial lesions (Underwood, 1956). Gubler et al. (1957a) reported that cardiac hypertrophy was a prominent feature of copper deficiency in pigs. They have suggested that the reduction of cytochrome oxidase activity found in copper deprivation may lead to the observed cardiac hypertrophy in an effort to compensate for the reduction in respiratory activity.

A mild transient diarrhea, observed in cattle, occasionally progresses to a persistent and debilitating scouring and is associated with low copper content of blood and tissue. Although no relationship of this disease to low copper content of the forage has been shown, the disease responds to oral or intramuscular administration. It has been suggested that this copper deficiency may be a result of an excess of molybdenum in the forage (Adelstein and Vallee, 1962).

Concentration in feedstuffs. Baumeister (1958) indicated that the copper content of higher plants, cereals, molds and vegetables is relatively uniform, but depends to some degree on the content and availability of copper in the soil and is of the order of 1 to 30 µg./gm. This work confirmed early work by Guerithault (1920) and Fluerent and Levi (1920), who found 3 to 40 mg./kg. copper in all plant materials analysed. Spector (1956), Underwood (1956) and Ruhland (1958) reported exhaustive summaries of the copper concentrations contained in plant and animal material.

Concentration in animal tissue. A wide variation has been reported both in the copper concentration between species and in the distribution within the body of any given species. Chou and Adolph (1935) estimated that of the 100 to 150 mg. copper found in the adult human body, 65 mg. is found in the muscle mass, 23 mg. in the bones and 18 mg. in the liver. In the normal adult rat, 21 percent of the total body copper has been found in the muscles, 23 percent in the bones, 13 percent in the liver and 36 percent in the skin (Lindow et al., 1929). Rusoff (1941) found marked differences in the copper distribution in two newborn calves. He found 18 and 25 percent in the muscles; 54 and 7 percent in the bones; 12 and 12 percent in the liver; and 2 and 17 percent in the skin. Later, Dick (1955) found more comparable results in his analysis of two adult sheep (72 and 79 percent in the liver, 8 and 12 percent in the muscles, 9 percent in the skin and wool and 2 percent in the skeleton).

Although highly variable concentrations of copper have been

observed in the tissues and organs of all animal species, certain tissues consistently have higher concentrations of this element than others. The susceptibility of individual tissues to variations in dietary copper intake varies considerably. The endocrine glands, muscles, heart, brain and skin are the most resistant to change; whereas, copper concentrations in the liver, kidney, spleen and lungs can be greatly increased by high copper intakes, and those of the liver, kidney, spleen and blood readily reduced under conditions of copper deficiency (Underwood, 1962).

It is evident that many factors affect the copper concentrations in the tissues, but under a given set of circumstances, the concentration will be in a relatively constant range.

Absorption and excretion. Adelstein and Vallee (1962) indicated that little is known about the mechanisms of copper absorption in higher animals and that precise definition of the sites of absorption is lacking. All work reviewed indicated copper to be absorbed from the upper alimentary tract. Tompsett (1940) concluded that the stomach of the mouse was the site of absorption. Additional evidence was presented by Sach et al. (1943), who found that when the element was placed in an isolated loop of the upper jejunum, the serum copper concentration increased slightly; however, this was not observed in the distal or middle segments. Results with the oral administration of radio-copper have confirmed conclusions that copper is absorbed in the upper tract (Earl et al., 1954b; Bearn and Kunkel, 1955).

There is no evidence of any mechanism for the regulation of the absorption of copper from the gastrointestinal tract according to

demand (Adelstein and Vallee, 1962). Scheinberg and Morrell (1957) suggested that the level of copper absorption is set by the concentration of diffusible copper in intestinal plasma in equilibrium with the bound copper of the ceruloplasmin present. A more logical hypothesis may be that presented by Gubler (1957a), who stated that copper homeostasis is accomplished by adjusting the rate of excretion to that of absorption.

Comar et al. (1948) estimated the absorption of this element in the ruminant. Seventy-five percent of an oral dose of $^{64}Cu$ was found in the feces and 5 percent in the urine; while 3 percent of an intravenous dose was found in the feces and 3 percent in the urine. Therefore, an estimated 28 percent was absorbed.

Bile is the major pathway of copper excretion, as first suggested by the studies of Flinn and Inovye (1929) in the cat and Van Ravenstevn (1944) in the human. Thus, information on the excretion of copper is more complete than that concerning its absorption. The absolute value of copper excreted in human bile has been estimated to be 35 to 205 µg./100 ml., and in urine 2.7 to 29.9 µg./l. or 3.9 to 29.6 µg./day (Butler and Newman, 1956). Mahoney et al. (1955) indicated that in the dog, 7 to 10 percent of the intravenously-administered radio-activity was excreted in the bile, about 1.5 percent was passed directly through the intestinal wall and about 0.6 percent was excreted in the urine. The rate of urinary copper excretion can be correlated with serum concentration of nonceruloplasmin copper. The normal clearance of plasma nonglobulin $^{64}Cu$ varies from 0.2 to 0.3 ml./min. (Jensen and

Kamin, 1957). The close relationship between the nonglobulin $^{64}Cu$ concentration and urinary excretion of $^{64}Cu$ suggests that copper, loosely bound to albumin, is the main source of urinary copper.

Placental transfer. Although copper may be transferred to the fetuses of many species, a direct study of this in sheep or swine was not found.

General review. A comprehensive review of the placental transfer of various nutrients has been presented by Hoskins (1963), and Hansard (1966) reported additional discussion of the rate and the factors affecting placental transfer.

In view of these findings and the lack of knowledge concerning the placental transfer of manganese and copper in swine and sheep, it was of interest to compare the relative manganese and copper transfer values using radiochemical procedures to measure absorption, movement and deposition during the second and third trimesters of pregnancy in swine and sheep.

# CHAPTER III

# PROCEDURE

## I. GENERAL

Pregnant and control animals of three species, representing three of the five types of placentas currently recognized (Nalbandov, 1964) were utilized in this study of the mode and kinetics of manganese transport across the placental membranes. In addition, two species were used for concurrent studies of the transport of copper. Preliminary data were collected from members of a closed line of Spraque-Dawley rats (hemoendothelial placenta) during the final trimester of pregnancy. Placental transfer of manganese and copper as a function of stage of gestation and time after dosing was studied during the second and third trimesters of pregnancy in yearling Duroc gilts (epitheliochorial placenta) and in yearling Suffolk ewes (syndesmochorial placenta).

Until three weeks prior to dosing, gravid sows and ewes were maintained on pasture and rations of known composition (Table I), and rats were maintained on a commercially prepared laboratory chow containing 25 percent protein and 70 ppm manganese. Table II outlines the procedures for handling the experimental animals. For an adjustment period prior to dosing and sacrifice, all animals were placed into individual metabolism units equipped for the quantitative separate collection of urine and feces. Dosing was either oral, intravenous or intraperitoneal with a single tracer level of carrier-free $^{54}Mn$ or $^{64}CuSo_4$

TABLE I. COMPOSITION AND ANALYSIS OF EXPERIMENTAL RATIONS FED SWINE AND SHEEP

| Ration Ingredients | Swine[a] | Sheep[b] |
|---|---|---|
| Ground yellow corn | 77.4 | 50.5 |
| Soybean oil meal (44%) | 20.0 | 11.0 |
| Cotton seed hulls | ---- | 24.0 |
| Molasses, dry | ---- | 8.0 |
| Alfalfa meal | ---- | 5.0 |
| Limestone | 1.0 | 0.5 |
| Dicalcium phosphate | 1.0 | ---- |
| Iodized salt | 0.5 | 1.0 |
| Trace mineral premix[c] | 0.1 | ---- |
| Vitamin premix[d] | 0.05 | ---- |

[a]As fed basis: 15.5% protein, 3.1% ether extracts, 2.9% fiber, 11.5% moisture, 4.2% ash, 52 ppm manganese and 13 ppm copper.

[b]As fed basis: 12.1% protein, 2.7% fat, 12.0% fiber, 8.9% moisture, 4.4% ash, 19 ppm manganese and 5.7 ppm copper.

[c]Provides an addition to the ration of 2.5 gm. Mn, 0.07 gm. I, 0.05 gm. Co, 4.5 gm. Fe, 0.5 gm. Cu, 9.0 gm. Zn and 0.7 gm. Ca.

[d]Provides an addition to the ration of 0.2 gm. riboflavin, 0.4 gm. pantothenic acid, 0.9 gm. niacin, 1.0 gm. choline, 1.0 mg. $B_{12}$, 50,000 I.U. Vitamin A and 25,000 I.U. Vitamin D.

TABLE II. DISTRIBUTION OF EXPERIMENTAL ANIMALS

| | Rats | Swine | Sheep |
|---|---|---|---|
| Placental Type | Hemoendo-thelial | Epithelio-chorial | Syndesmo-chorial |
| No. of Animals | | | |
| Mn | 17 | 15 | 15 |
| Cu | -- | 10 | 9 |
| No. of Fetuses | | | |
| Mn | 124 | 73 | 13 |
| Cu | --- | 45 | 9 |
| Route Dosing[a] | | | |
| Mn | O, IP. | O, IV. | O, IV. |
| Cu | --- | O, IV. | O, IV. |
| Trimester Pregnancy[b] | | | |
| Mn | 3 | 2, 3 | 2, 3 |
| Cu | --- | 2, 3 | 2, 3 |
| Sacrifice Time | | | |
| Mn | 5 min. to 48 hr. | 3 to 168 hr. | 12 to 168 hr. |
| Cu | --- | 3 to 48 hr. | 2 to 24 hr. |

[a] O = Oral, IP. = Intraperitoneal, IV. = Intravenous.

[b] 2 = 70 and 95 days of gestation swine and sheep, respectively; 3 = 105, 135, 20 days of gestation swine, sheep and rats, respectively.

for blood balance and subsequent placental transfer studies at sacrifice after 168 and 24 hr. for animals in midpregnancy and at specified times after dose administration during the final trimester. Daily feed intake of swine and sheep was measured for calculation of total manganese and copper balance. Feces were quantitatively collected separately (usually at 12-hour intervals), weighed and thoroughly mixed; and aliquots were taken and ashed for 36 hours at 550°C. for radio-chemical manganese and copper analyses. Short-term urine samples (5 min. to 1 hr.) were collected during the first 12 hr. post-dosing (and thereafter at 12-hour intervals). Urine samples were weighed, and aliquots were taken and dried at 100°C. for 12 hr. prior to ashing for 24 hr. at 550°C. Ashed samples were taken into solution with 2 to 5 ml. concentrated HCl, transferred to graduated tubes and made to appropriate dilution with deionized water for radio-chemical manganese and copper analyses. Periodic blood samples were taken either from the jugular vein or by cardiac puncture by means of a needle attached to a heparinized syringe. Radioactivity was measured in a Nuclear Chicago automatic gamma counter unit. All samples were then centrifuged, hemocrits read and plasma removed for radioisotope and for total manganese and copper analyses. From these data, plasma $^{54}Mn$ and $^{64}Cu$ clearances were calculated.

Total plasma and whole blood manganese and copper determinations were made from composite samples after drying in an oven at 100°C. for 12 hr. and ashing for 24 hr. at 550°C. All samples were taken into solution with 2 to 3 ml. 6 N HCl and diluted with deionized water to a

definite volume, and aliquots were taken for reading on a Perkin Elmer 303 atomic absorption spectrophotometer. Plasma and whole blood total manganese and copper were then calculated in mg./gm. by the formula

$$\frac{(\text{dilution})(\text{sample abs.})(\text{concentration of standard})}{(\text{fresh weight})(\text{standard abs.})}$$

At sacrifice the following organs, tissues and fluids were taken from both dam and fetus: whole blood, bile, brain, pituitary, thyroid, thymus, heart, aorta, spleen, liver, kidney, adrenal, muscle, skin, mandible, sternum, rib shaft, rib epiphysis, femur shaft and femur epiphysis. Samples approximating 1 gm. were placed in tared flat-bottom counting tubes for radio-manganese and copper determinations, and additional tissue samples were taken in tared crucibles for ashing and chemical analysis. All samples were ashed 24 hr. at 550°C. Ashed weights were recorded; and the ash was taken into solution with 2 to 3 ml. 6 N HCl, transferred to graduated tubes and made to volume with deionized water for total manganese and copper analyses. Samples, such as the pituitary and some fetal tissues of limited size, were first counted and then wet-ashed with 2 ml. of a 2:1 70 percent perchloric concentrated nitric acid mixture, transferred to graduated tubes and diluted with deionized water to desired concentration for total manganese and copper measurements.

Following sampling, swine and sheep fetal remains were ground in a commercial blender, and aliquots were taken for radioactivity radio-chemical manganese and copper determinations.

Since this study was conducted to supply a basic understanding of the placental transfer of manganese and copper, only appropriate

# CHAPTER IV

# RESULTS AND DISCUSSION

## I. MANGANESE

### Results with Rats

Absorption and excretion. Accumulative total percent dose of $^{54}Mn$ appearing in the feces as a function of time after intraperitoneal and oral dose administration was comparable to that observed in swine. Oral data suggested that manganese moves very rapidly through the body, since by 6 hr. post-dosing, radioactivity could be detected in the feces, the largest percent excretion being detected in the 12- to 24-hr. fecal samples. However, calculated total percent excretion (67.5) did not agree with the results of Greenberg and Campbell (1940), who reported 97 percent of an oral $^{54}Mn$ dose to be eliminated in the feces. This, in part, may be explained by differences in animal rations and experimental procedures.

Tissue-organ distribution. Radio-manganese content and specific activities of selected maternal organs and tissues of rats as a function of time after intraperitoneal injection of $^{54}Mn$ are summarized in Table III. As expected, on the basis of previously reported research, radio-manganese disappeared rapidly from the total blood. In the present study, blood and muscle reached equilibrium 2 hr. post-dose administration. Liver $^{54}Mn$ concentration peaked at 30 min. and followed a linear decline to 48 hr. post-dosing. Maximum concentration occurred

TABLE III. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF MATERNAL ORGANS AND TISSUES IN RATS AS FUNCTIONS OF TIME AFTER INTRAPERITONEAL DOSE ADMINISTRATION

| Tissue | µg./gm. | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 8 | | 16 | | 48 | |
| | | %[a] | S.A.[b] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 1.36 | 0.13 | 0.1 | 0.03 | 0.02 | 0.03 | 0.02 | 0.02 | 0.01 |
| Liver | 2.95 | 21.1 | 7.2 | 15.3 | 5.4 | 10.7 | 3.6 | 6.7 | 2.3 |
| Kidney | 5.57 | 16.8 | 3.0 | 11.5 | 2.1 | 16.6 | 3.0 | 13.1 | 2.4 |
| Spleen | 3.00 | 5.4 | 1.8 | 6.0 | 2.0 | 5.1 | 1.7 | 3.3 | 1.1 |
| Heart | 6.23 | 5.7 | 0.9 | 7.4 | 1.2 | 4.0 | 0.6 | 1.9 | 0.3 |
| Pancreas | 11.10 | 23.0 | 2.1 | 2.9 | 0.3 | 2.7 | 0.2 | 2.4 | 0.2 |
| Muscle | 2.73 | 0.5 | 0.2 | 0.5 | 0.2 | 0.5 | 0.2 | 0.4 | 0.1 |
| Femur Shaft | 20.40 | 1.4 | 0.7 | 1.4 | 0.1 | 1.2 | 0.6 | 1.2 | 0.6 |
| Femur Epiphysis | 13.88 | 1.2 | 0.1 | 2.6 | 0.2 | 1.4 | 0.1 | 1.5 | 0.1 |
| Uterus and Placenta | 1.63 | 5.1 | 3.1 | 3.0 | 1.8 | 2.5 | 1.5 | 1.3 | 0.8 |

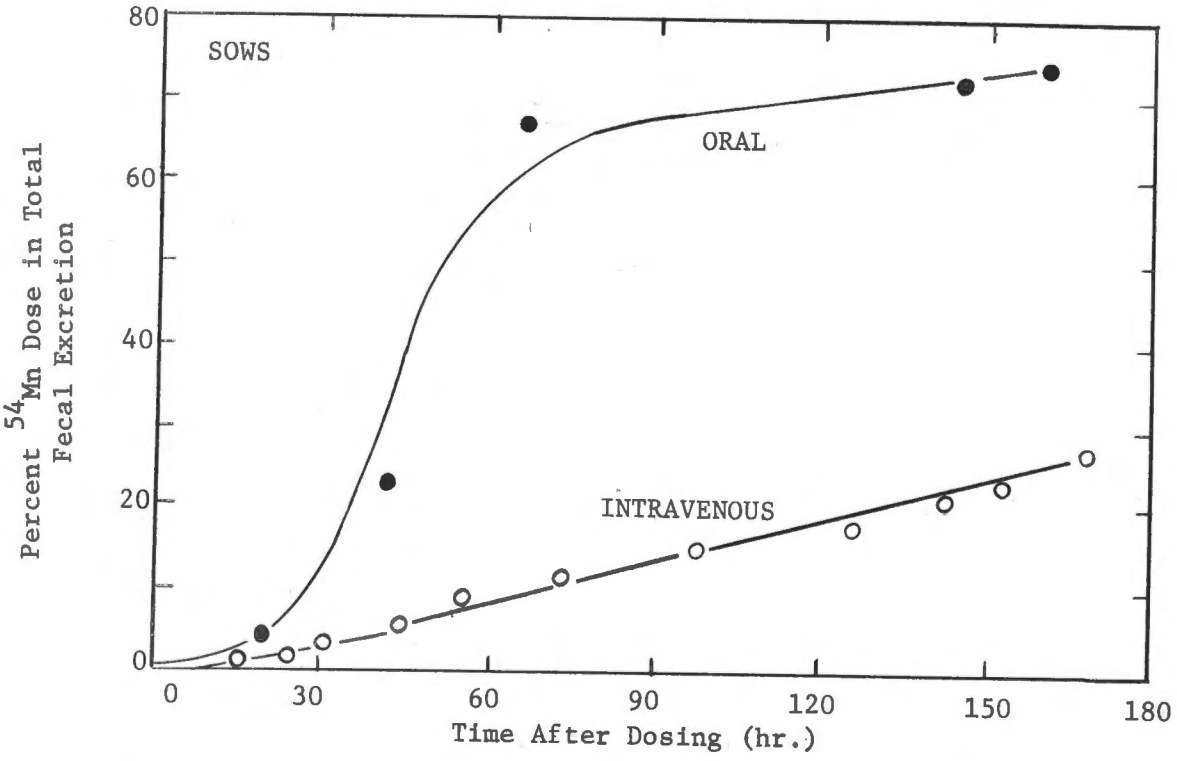


Figure 2. Accumulative fecal excretion of $^{54}$Mn by swine as a function of time after intravenous and oral dose administration.

cleared manganese at the rate of 0.33 ml. plasma per minute. Plasma clearance of radio-manganese varied with time, but by 48 hr. post-dosing, this clearance was equal to the clearance for stable manganese.

Total organs. Cotzias (1962) considered liver to be one of the major metabolic pools for manganese. Results of the present study (Table IV), suggested that at any time post-dosing, liver contained 80 to 85 percent of the total organ radio-manganese in liver, kidney, spleen and heart.

Tissue-organ distribution. Percent $^{54}Mn$ and specific activity in maternal organs and tissues of swine as a function of time after dose administration are summarized in Table V. Since stage of pregnancy had no apparent influence on tissue concentration or turnover rates in maternal tissues, emphasis will be given primarily to the effects of time post-dosing on tissue distribution and turnover rates. Maximum turnover rates were observed in the major organs--liver, kidney, spleen and heart--6 hours post-dosing with relatively stable percentages being reached at 48 hr. Highest specific activities were observed in the spleen and kidney, with liver being intermediate and heart lowest. However, turnover rates at 168 hr. post-dosing indicated that liver and kidney were maintaining higher rates than either spleen or heart. Pancreas too reached maximum concentrations 6 hr. post-dosing, but only a slight decline was noted to 125 hours, after which time there was a rapid decline. Pancreas concentration of $^{54}Mn$ 168 hours post-dosing was slightly higher in second-trimester than in non-pregnant or third-trimester animals.

TABLE IV. PARTITION OF RADIO-MANGANESE IN MATERNAL SWINE ORGANS

| Tissue | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | | 6 | | 120 | | 168 | |
| | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total | 15.3 | 100 | 33.3 | 100 | 14.0 | 100 | 15.4 | 100 |
| Liver | 12.5 | 81.7 | 27.7 | 83.2 | 11.3 | 80.7 | 13.1 | 85.0 |
| Kidney | 1.7 | 11.1 | 3.4 | 10.2 | 2.1 | 15.0 | 1.7 | 11.0 |
| Heart | 0.5 | 3.3 | 0.8 | 2.4 | 0.2 | 1.4 | 0.3 | 2.0 |
| Spleen | 0.6 | 3.9 | 1.4 | 4.2 | 0.4 | 2.9 | 0.3 | 2.0 |

[a]Total percent dose $^{54}Mn$ contained in each organ, corrected to 150 kg. gilt.

[b]Percent of the total.

TABLE V. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF MATERNAL ORGANS AND TISSUES IN SWINE AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION, THIRD TRIMESTER

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 0.40 ± 0.2 | 2.8 | 7.0 | 0.9 | 2.2 | 0.2 | 0.5 | 0.7 | 1.8 |
| Bile | 0.54 ± 0.3 | 45.9 | 85.0 | 65.7 | 121.7 | 6.6 | 12.2 | 14.7 | 27.2 |
| Liver | 1.81 ± 0.4 | 66.4 | 36.7 | 141.1 | 78.0 | 60.5 | 33.4 | 66.2 | 36.6 |
| Kidney | 1.29 ± 0.2 | 94.7 | 73.4 | 244.4 | 189.4 | 118.3 | 91.7 | 97.7 | 75.7 |
| Spleen | 0.74 ± 0.3 | 51.0 | 68.9 | 95.2 | 128.6 | 21.0 | 28.4 | 14.2 | 19.2 |
| Heart | 1.19 ± 0.1 | 12.2 | 10.2 | 19.6 | 16.5 | 5.1 | 4.3 | 5.9 | 5.0 |
| Pancreas | 1.49 ± 0.4 | 106.3 | 71.3 | 146.1 | 98.0 | 121.8 | 81.7 | 85.2 | 57.2 |
| Muscle | 0.46 ± 0.3 | 0.4 | 0.9 | 1.2 | 2.6 | 1.6 | 3.5 | 2.0 | 4.3 |
| Brain | 0.98 ± 0.7 | 0.4 | 0.4 | 2.3 | 2.3 | 1.8 | 1.8 | 1.0 | 1.9 |
| Pituitary | 3.73 ± 1.6 | 33.3 | 8.9 | 70.9 | 19.0 | 33.2 | 8.9 | 15.0 | 4.0 |
| Thyroid | 1.02 ± 0.6 | 24.1 | 23.6 | 47.2 | 46.3 | 8.9 | 8.7 | 10.9 | 10.7 |
| Adrenal | 1.07 ± 0.7 | 48.4 | 45.2 | 133.2 | 124.5 | 41.9 | 39.2 | 41.5 | 38.8 |
| Aorta | 1.43 ± 0.9 | 1.6 | 1.1 | 2.0 | 1.4 | 2.4 | 1.7 | 2.5 | 1.7 |

TABLE V (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Bone Marrow | 0.51 ± 0.2 | 0.9 | 1.8 | 0.9 | 1.8 | 0.9 | 1.8 | 0.6 | 1.2 |
| Sternum | 1.89 ± 0.6 | 7.1 | 3.8 | 13.2 | 7.0 | 5.1 | 2.7 | 4.8 | 2.5 |
| Mandible | 1.94 ± 1.3 | 1.6 | 0.8 | 3.7 | 1.9 | 1.2 | 0.6 | 1.7 | 0.9 |
| Rib Shaft | 1.83 ± 0.4 | 6.0 | 3.3 | 7.6 | 4.2 | 4.0 | 2.2 | 5.0 | 2.7 |
| Rib Epiphysis | 2.48 ± 0.7 | 6.6 | 2.6 | 15.3 | 5.9 | 10.0 | 3.9 | 17.9 | 6.9 |
| Femur Shaft | 1.25 ± 0.8 | 1.1 | 0.9 | 1.9 | 1.5 | 1.1 | 0.9 | 1.3 | 1.0 |
| Femur Epiphysis | 1.16 ± 0.4 | 3.2 | 2.8 | 4.1 | 3.5 | 3.0 | 2.6 | 3.0 | 2.6 |
| Placenta and Uterus | 0.41 ± 0.2 | 33.1 | 80.7 | 28.0 | 68.3 | 8.1 | 19.8 | 7.9 | 19.3 |
| Placental Fluids | 0.53 ± 0.4 | 1.2 | 2.3 | 0.3 | 0.6 | 0.6 | 1.1 | 0.8 | 1.5 |
| Fetus | 0.80 ± 0.2 | 0.4 | 0.5 | 2.4 | 3.0 | 27.4 | 38.3 | 28.5 | 35.6 |

[a] Mean ± standard deviation.

[b] Percent dose $^{54}Mn/gm. \times 10^{-4}$, corrected to 150 kg. maternal weight.

[c] Calculated: $\frac{\% \ ^{54}Mn/gm. \times 10^{-4}}{Mg.\ Mn/gm.}$, fresh bases.

Muscle continued to accumulate a small percent of $^{54}Mn$ to 168 hr. post-dosing; however, differences in concentration and turnover were small. The data indicated that skeletal muscle contained little manganese. Although muscle concentrations of $^{54}Mn$ were lower in second-trimester animals than in open or third-trimester animals, differences were small.

Manganese has been hypothesized to affect the secretion of the pituitary gland (Orent and McCollum, 1931). Cotzias (1962) reported that the adrenal corticoids affect blood concentration of manganese. Both adrenal and pituitary concentrated radio-manganese rapidly, but due to the higher concentration of stable manganese, calculated turnover rates were lower in pituitary than in adrenal. Both reached peak concentrations of $^{54}Mn$ 6 hr. post-dosing, declined to 48 hr. and remained relatively constant to 168 hr. Radio-manganese uptake by brain was low, but peak concentration was reached 6 hr. after dose administration, indicating the brain barrier to manganese to be low.

Cotzias (1962) indicated that bone could possibly serve as a storehouse for manganese as it does for calcium; however, in the present study, mandible, sternum, rib shaft and femur shaft contained less total manganese (1.25 to 1.94 µg./gm.) than was reported by Cotzias (1962) (3.5 µg./gm.). Radio-manganese values were greater for sternum and rib shaft than for femur shaft and mandible. In all cases, except for mandible, highest concentrations of $^{54}Mn$ were observed 6 hr. post-dosing. Stage of gestation had no apparent effect on uptake of radio-manganese, except that mandibles from third-trimester

gilts contained less $^{54}Mn$ than did those of open and second-trimester gilts.

Tsai and Everson (1967) have suggested that manganese functions in cartilage matrix formation. Results of the present study indicated that rib epiphysis, high in cartilage, did concentrate and turn over more radio-manganese than did mandible, sternum, rib or femur shaft.

Placental complex. Manganese, radio-manganese and specific activities in the placental complex of third-trimester gilts are summarized in Table VI. Although concentrations of $^{54}Mn$ were small and irregular, placental fluids of third-trimester gilts were higher in radio-manganese than fluids of second-trimester gilts. Stable manganese concentrations did not differ between second- and third-trimester gilts; thus, specific activities or turnover rates were somewhat higher in the third trimester. Indications were that placental fluids had little or no function in the passage of manganese to or from the fetus. Radio-manganese uptake by placenta was greater in second- than in third-trimester gilts, with stable manganese remaining relatively constant. These data indicated a higher turnover rate in the placenta during the second trimester of pregnancy.

The whole fetus. A record of crown-rump length, age, weight, radio-manganese, manganese and specific activity in whole swine fetuses as functions of gestation age and of time after dose administration are listed in Table VII. Between the second and third trimesters of pregnancy, fetal length increased 1.5 times and weight 4 times. Although radio-manganese concentration 168 hr. post-dosing was

TABLE VI. SUMMARY OF MANGANESE, TOTAL RADIO-MANGANESE, AND SPECIFIC ACTIVITY IN THE PLACENTAL COMPLEX OF THIRD TRIMESTER GILTS

| Tissue | Manganese mg. | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | %[a] | S.A.[b] | % | S.A. | % | S.A. | % | S.A. |
| Total Complex | 8.4 | 12.56 | --- | 10.55 | --- | 25.08 | --- | 25.72 | --- |
| Placenta & Uterus | 1.4 | 12.10 | 80.7 | 8.60 | 68.3 | 3.10 | 19.8 | 2.80 | 19.3 |
| Placental Fluids | 0.6 | 0.12 | 2.3 | 0.03 | 0.6 | 0.08 | 1.1 | 0.12 | 1.5 |
| Fetuses (8) | 6.4 | 0.34 | 0.5 | 1.92 | 3.0 | 21.90 | 34.3 | 22.80 | 35.6 |

[a] Total percent dose $^{54}Mn$, corrected to 150 kg. gilt.

[b] Calculated: $\frac{\%\ ^{54}Mn/gm. \times 10^{-4}}{\mu g.\ Mn/gm.}$, fresh bases.

TABLE VII. SUMMARY OF LENGTH, AGE, WEIGHT, RADIO-MANGANESE, TOTAL MANGANESE, AND SPECIFIC ACTIVITY VALUES IN WHOLE SWINE FETUSES AS A FUNCTION OF GESTATION AGE AND TIME AFTER $^{54}Mn$ INJECTION

| Trimester | HAD | Length cm. | Age days | Weight gm. | Percent Dose gm. | Percent Dose Total[a] | Total Mn (Mg.) | S.A.[b] |
|---|---|---|---|---|---|---|---|---|
| II | 168 | 17.7 | 74 | 262 | 22.1 | 5.80 | 0.2 | 27.7 |
| III | 3 | 29.0 | 107 | 1,181 | 0.4 | 0.3 | 0.9 | 0.5 |
| III | 6 | 25.0 | 106 | 982 | 2.4 | 1.9 | 0.8 | 3.0 |
| III | 48 | 48.0 | 105 | 1,508 | 17.9 | 14.3 | 1.0 | 22.4 |
| III | 96 | 30.0 | 112 | 1,430 | 23.8 | 19.0 | 1.1 | 29.8 |
| III | 120 | 27.0 | 109 | 1,119 | 27.4 | 21.9 | 0.9 | 34.3 |
| III | 168 | 28.0 | 112 | 1,315 | 28.5 | 22.8 | 1.1 | 35.6 |

[a] Total percent dose $^{54}Mn$, 8 pig litter.

[b] Calculated: $\frac{\% \ ^{54}Mn/gm. \times 10^{-4}}{\mu g. \ Mn/gm.}$, fresh bases.

somewhat higher in third-trimester fetuses, data were not sufficient to indicate a significant difference. Only slight differences were noted in stable manganese concentration in fetuses at these two ages. Total radio- and stable manganese were higher in third-trimester fetuses due to differences in weight, and the results indicated that there were differences in the rate of transfer at these two stages of pregnancy. However, ratios of weight to percent transfer completely explained these differences. It was concluded that fetal weight and need were in direct relation to total percent transferred. Fetuses of third-trimester gilts increased in concentration of $^{54}Mn$ to 168 hr. post-dosing; however, only a slight difference in percent per fetus was noted 120 and 168 hr. after dose administration.

Fetal tissue distribution. Manganese, radio-manganese and specific activity values of third-trimester swine fetal organs and tissues are summarized in Table VIII. Uptake or concentration of radio-manganese by third-trimester fetal liver and spleen reached a peak 48 hr. post-dosing, decreased to 96 hours and remained relatively constant to 168 hr.; while in the heart and kidney $^{54}Mn$ did not reach maximum concentration until 120 and 168 hr., respectively. Both liver and spleen of second-trimester fetuses concentrated a higher percent $^{54}Mn$ than third-trimester fetuses, but there were no appreciable differences observed in kidney or heart values. The data suggested that liver and spleen first concentrated radio-manganese and that it was then distributed throughout the body. Muscle further demonstrated this trend by reaching equilibrium 96 hr. after dose administration. Fetal blood

TABLE VIII. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF FETAL ORGANS AND TISSUES IN SWINE AS FUNCTIONS OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION, THIRD TRIMESTER

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 0.48 ± 0.2 | 1.57 | 3.3 | 1.8 | 3.8 | 2.1 | 4.4 | 1.10 | 2.3 |
| Bile | 2.75 ± 1.5 | 16.50 | 6.0 | 129.2 | 47.0 | 212.5 | 77.3 | 192.4 | 70.0 |
| Liver | 1.73 ± 0.5 | 6.70 | 3.9 | 42.7 | 24.7 | 88.2 | 51.0 | 91.2 | 52.7 |
| Kidney | 1.23 ± 0.3 | 0.65 | 0.0 | 8.85 | 7.2 | 62.2 | 50.6 | 58.5 | 47.6 |
| Spleen | 2.66 ± 1.3 | 0.70 | 0.3 | 6.0 | 2.3 | 14.7 | 5.5 | 12.18 | 4.6 |
| Heart | 0.64 ± 0.2 | 0.60 | 0.9 | 4.8 | 6.6 | 10.5 | 16.4 | 11.64 | 18.2 |
| Pancreas | 7.40 ± 3.7 | 0.70 | 0.1 | 10.3 | 1.4 | 408.7 | 55.2 | 282.8 | 38.2 |
| Muscle | 0.81 ± 0.5 | 0.10 | 0.1 | 0.8 | 1.0 | 7.2 | 8.9 | 5.65 | 7.0 |
| Brain | 0.53 ± 0.2 | --- | --- | 0.2 | 0.4 | 4.9 | 0.2 | 5.2 | 9.8 |
| Pituitary | 1.37 ± 0.2 | --- | --- | 6.5 | 4.7 | 197.9 | 144.4 | 171.9 | 125.5 |
| Thyroid | 1.99 ± 0.2 | 0.61 | 0.3 | 9.4 | 5.0 | 65.7 | 34.9 | 39.7 | 21.1 |
| Adrenal | 1.85 ± 1.2 | 1.2 | 0.7 | 6.8 | 3.4 | 52.7 | 28.5 | 30.7 | 16.6 |
| Aorta | 2.00 ± 1.0 | 0.2 | 0.1 | 2.2 | 1.1 | 10.25 | 5.1 | 9.1 | 4.6 |

TABLE VIII (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | % | S.A. | % | S.A. | % | S.A. | % | S.A. |
| Sternum | 5.3 ± 2.8 | 0.36 | 0.07 | 9.0 | 1.7 | 34.0 | 6.4 | 44.5 | 9.3 |
| Mandible | 3.49 ± 1.0 | 0.10 | 0.03 | 2.2 | 0.6 | 50.2 | 14.4 | 69.37 | 19.9 |
| Rib Shaft | 8.93 ± 4.0 | 0.22 | 0.02 | 2.2 | 0.2 | 60.05 | 6.7 | 76.60 | 8.6 |
| Rib Epiphysis | 12.88 ± 3.6 | --- | --- | 5.2 | 0.4 | 94.95 | 7.4 | 88.69 | 6.9 |
| Femur Shaft | 7.74 ± 2.2 | 0.30 | 0.04 | 1.9 | 0.2 | 39.4 | 5.1 | 47.50 | 6.1 |
| Femur Epiphysis | 4.35 ± 1.3 | 0.20 | 0.05 | 2.1 | 0.5 | 71.54 | 1.64 | 93.50 | 21.5 |

[a] Mean ± standard deviation.

[b] Percent dose $^{54}$Mn/gm. x $10^{-4}$.

[c] Calculated: $\frac{\%\ ^{54}\text{Mn/gm.} \times 10^{-4}}{\mu\text{g. Mn/gm.}}$, fresh bases.

was very irregular in its concentration of radio-manganese, indicating (in agreement with Cotzias, 1962) that manganese ions were being passed to and from the blood stream. Ratio of bile to blood $^{54}Mn$ 120 hr. post-dosing (100:1) was higher in the fetus than in the mother; however, by 168 hr., this ratio had dropped to 30:1, approximately one-half that observed in the mother. No appreciable differences were observed in blood or muscle when comparing second- and third-trimester fetuses.

A high concentration of radio-manganese was noted in fetal pancreas, and it was not until 120 hr. post-dosing that maximum concentration occurred. Percent $^{54}Mn$ at 168 hr. post-dosing was higher in second-trimester fetuses; however, differences were not great.

At 168 hr. post-dosing, pituitary of third-trimester fetuses was two times as high in percent $^{54}Mn$ as pituitary of second-trimester fetuses; however, the opposite was observed for the adrenal. When analyzing these results, consideration should be given to the difficulty of comparable sampling of such small organs. Third-trimester fetal thyroid concentrated one-half the percent $^{54}Mn$ as did those of second-trimester, and at 168 hr. post-dosing, concentration in thyroid of second-trimester fetuses was eight times that of maternal thyroid. Fetal brain tended to concentrate $^{54}Mn$ slower, but to concentrate a higher percent than maternal brain.

Fetal aorta contained the same concentration of stable manganese and tended to concentrate radio-manganese in the same pattern as

maternal aorta. As was observed for many fetal organs and tissues, fetal aorta was higher in percent radio-manganese than was the corresponding maternal organ or tissue.

Fetal bone increased in concentration of $^{54}Mn$ up to 168 hr. after dosing, with rib epiphysis being the only exception. Differences in uptake by shaft and epiphysis were not as great as in maternal bone.

Partition of $^{54}Mn$ in fetal organs. The partition of $^{54}Mn$ in whole organs of swine litters, standardized to eight pigs, is summarized in Table IX. Illustrated is the relative magnitude of $^{54}Mn$ transferred to various fetal organs with respect to total litter $^{54}Mn$ at different times after dose administration. A slight difference in percent fetal liver manganese was noted when comparing second- and third-trimester fetuses. This trend indicated that manganese was being moved from body pools faster in third- than in second-trimester fetuses. The time study clearly indicated liver to be the initial body pool since at 6 hr. post-dosing, 48.8 percent of the transferred dose was contained in liver.

Partition of $^{54}Mn$ in dam and litters. The pregnant sow must supply nutrients not only to the litter but also to the uterus, placental membranes and placental fluids. Partition of radio-manganese in dams and litters of swine as a function of time after dose administration of $^{54}Mn$ is illustrated in Table X. At 168 hr. after dose administration, placenta and uterus in second-trimester gilts contained 47.2 percent of the available radio-manganese, but during the third trimester,

TABLE IX. PARTITION OF RADIO-MANGANESE IN FETAL ORGANS OF SWINE LITTERS

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Litter | III | 0.34 | 100 | 1.92 | 100 | 21.9 | 100 | 22.8 | 100 |
| Liver | | 0.17 | 49.4 | 0.93 | 48.8 | 2.11 | 9.6 | 2.68 | 11.8 |
| Kidney | | 0.002 | 0.7 | 0.034 | 1.8 | 0.22 | 1.0 | 0.25 | 1.1 |
| Spleen | | 0.0006 | 0.2 | 0.0064 | 0.3 | 0.02 | 0.1 | 0.02 | 0.1 |
| Heart | | 0.0048 | 1.4 | 0.0272 | 1.4 | 0.054 | 0.2 | 0.09 | 0.4 |

[a] Total percent $^{54}Mn$ dose.

[b] Percent of the transferred dose.

TABLE X. PARTITION OF RADIO-MANGANESE IN THE DAM AND LITTERS OF SWINE

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 120 | | 168 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total Complex | III | 12.56 | 100 | 10.55 | 100 | 25.08 | 100 | 25.72 | 100 |
| Placenta & Uterus | | 12.1 | 96.3 | 8.6 | 81.5 | 3.1 | 12.4 | 2.8 | 10.9 |
| Placental Fluids | | 0.12 | 1.0 | 0.03 | 0.3 | 0.08 | 0.3 | 0.12 | 0.5 |
| Fetuses (8) | | 0.34 | 2.7 | 1.92 | 18.2 | 21.9 | 87.3 | 22.8 | 88.6 |

[a]Total percent $^{54}Mn$ dose, corrected to 150 kg. gilt.

[b]Percent of the total complex.

this percentage had dropped to 10.9, or approximately one-fourth. During both trimesters, only traces of $^{54}Mn$ were contained in the placental fluids.

Summary. In this study sows retained 24.9 mg. dietary manganese (28.5 percent of the intake) per day, and 26.1 percent orally administered $^{54}Mn$ was retained during the 7-day study. A third-trimester litter contained 6.4 mg. manganese; uterus and placenta, 1.4 mg.; and placental fluids, 0.6 mg.; for a total of 8.4 mg. manganese contained in the placental complex. The data suggested that gilts fed rations containing 50 ppm would deposit 73.7 µg. manganese per day in the products of conception. If absorption did not change with pregnancy, sows would need to increase daily feed intake by only 1 mg. per day to meet the needs of pregnancy. However, the additional 73.7 µg. manganese needed to support the products of conception represents less than 0.1 percent of the average daily intake. This small percentage would indicate the need for a large number of animals to estimate differences with confidence.

## Results With Sheep

Blood $^{54}Mn$. Immediately after dose administration, there was observed an extremely rapid decline in the slope of the disappearance curve, during which time radio-manganese was being removed from the circulating plasma. Clearances indicated that red cell and plasma $^{54}Mn$ reached equilibrium 24 hr. post-dosing and continued to 168 hr.

Disappearance rates in sheep were faster than those reported in the rat and human.

Absorption and excretion. Before any mineral can be assimilated or utilized in the metabolic pathways within an organism, it must first be made available to the animal. Absorption has little meaning and value unless adequate manganese is available from dietary sources to allow normal functioning of the animal body. Careful measurements of feed intake and fecal excretion of manganese facilitated calculations of absorption and total manganese balance. Examination of these data showed that 1 of the 14 ewes was in a state of negative manganese balance, caused by failure to consume adequate feed. Other ewes consumed an average of 23.03 ± 3.79 mg. manganese per day, excreted 21.2 ± 4.96 mg. and were in a positive balance by 1.83 mg. per day.

The accumulative fecal excretion of $^{54}Mn$ in sheep as a function of time after oral and intravenous dose administration is shown in Figure 3. Manganese is excreted almost exclusively in the feces, and it is noteworthy that up to 100 hr. post-dosing, intravenously injected radio-manganese was excreted faster than orally administered $^{54}Mn$. Bile excretion of radio-manganese appeared to follow the same pattern as that observed in swine; however, since sheep were not sacrificed at 3 and 6 hr. post-dosing, it was impossible to present the exact pattern. Considering the pattern to be the same, percent excretion of $^{54}Mn$ per ml. bile was two to four times as high in sheep as in swine. The movement of radio-manganese by way of liver and bile to the gastrointestinal tract was faster than its movement through the

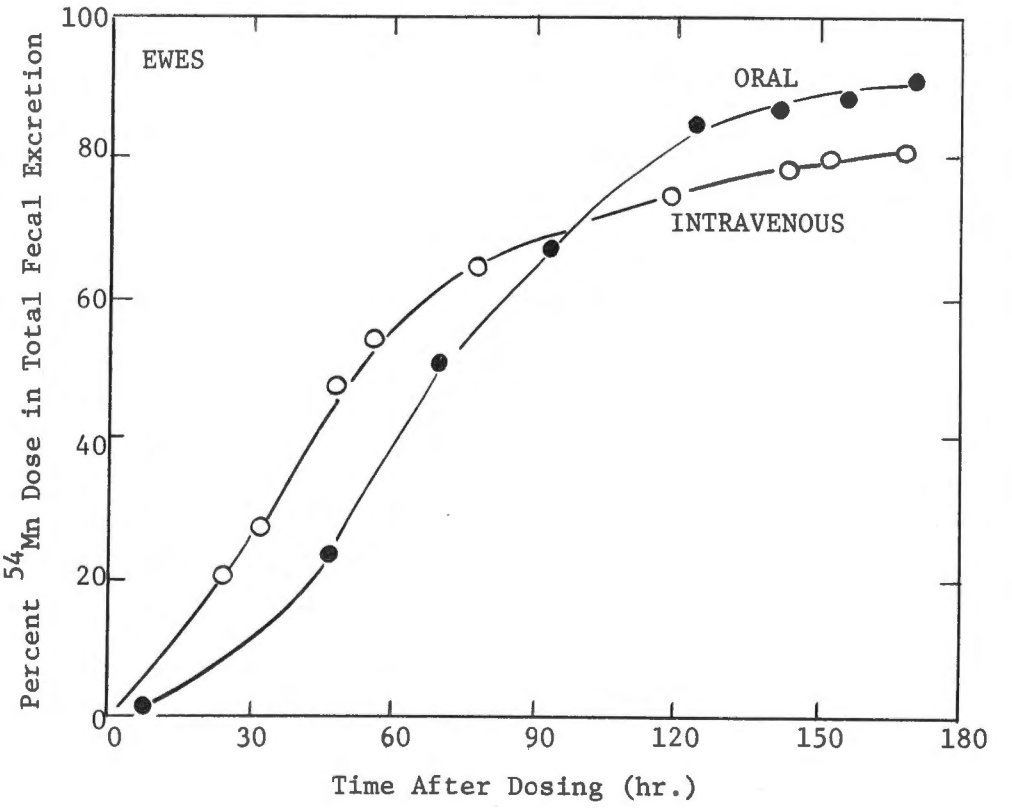


Figure 3. Accumulative fecal excretion of $^{54}$Mn by sheep as a function of time after intravenous and oral dose administration.

rumen, reticulum, omasum and abomasum to the intestinal tract. At 168 hr. post-dosing, accumulative total percent oral dose excretion (90.0) was 8.9 percent higher than for intravenously administered radio-manganese (81.1 percent). Chemical balances indicated that ewes retained 7.9 percent of the stable manganese intake, but for 200-hr. oral radio-manganese balance, ewes retained 7 percent, or approximately the same percentage as for chemical balances.

Urinary excretion of radio-manganese represented less than 0.1 percent of the injected dose, with the highest percent dose per ml. observed 45 min. after dose administration. Chemical urine balances indicated that ewes excreted 1.06 ± 0.36 ml. urine per minute containing 0.2 µg./ml., a total of 0.32 ± 0.09 mg. manganese per day. As in swine, these data indicated the limited capacity of the kidney to excrete manganese. However, the kidney clears stable manganese from 0.32 ml. plasma per minute. Plasma clearance of radio-manganese varied with time, but 32 hr. post-dosing, this clearance was equal to the clearance for stable manganese.

Total organs. Data showed that in swine, 168 hr. post-dosing, 85 percent of the radio-manganese contained in the major organs was in liver; however, in sheep, 168 hr. post-dosing, liver contained only 54 percent (Table XI). This may be explained in part by the difference in total percent contained in these organs (15.4 percent in swine and 3.7 percent in sheep).

Tissue-organ distribution. Percent $^{54}Mn$ and the specific activity in maternal organs and tissues of sheep as functions of time are shown

TABLE XI. PARTITION OF RADIO-MANGANESE IN MATERNAL SHEEP ORGANS

| Tissue | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 144 | | 168 | |
| | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total | 31.7 | 100 | 18.0 | 100 | 4.9 | 100 | 3.7 | 100 |
| Liver | 22.6 | 71.3 | 13.8 | 76.7 | 2.7 | 55.1 | 2.0 | 54.1 |
| Kidney | 5.0 | 15.8 | 2.6 | 14.4 | 1.5 | 30.6 | 1.2 | 32.4 |
| Heart | 0.9 | 2.8 | 0.4 | 2.2 | 0.2 | 4.1 | 0.2 | 5.4 |
| Spleen | 3.2 | 10.1 | 1.2 | 6.7 | 0.5 | 10.2 | 0.3 | 8.1 |

[a] Total percent $^{54}Mn$ dose contained in each organ, corrected to 50 kg. ewe.

[b] Percent of the total.

in Table XII. Stage of pregnancy did not affect tissue concentration or turnover rates. Hence, this discussion will concentrate primarily on the effects of time post-dosing on tissue distribution and turnover rates. Twelve hours post-dosing, liver, kidney, spleen and heart had reached maximum concentration of $^{54}Mn$. At this time, as was observed in swine, spleen and kidney turnover rates were higher than those of either liver or heart. However, the data suggested that radio-manganese was removed from these metabolic pools faster in sheep than in swine. Although the differences were not great, trends indicated that $^{54}Mn$ was removed from liver faster in pregnant than in nonpregnant animals. Radio-manganese concentrations in pancreas (highest concentration per gram of fresh tissue) was at a peak 12 hr. post-dosing, was maintained to 24 hr., declined to 144 hr. and remained relatively constant to 168 hr. post-dosing. In contrast to swine, sheep muscle was at peak radio-manganese concentration 12 hr. post-dosing and declined curvilinearly thereafter.

Endocrine glands (pituitary, adrenal and thyroid) concentrated radio-manganese rapidly, with peak concentrations occurring 12 hr. post-dosing. As in swine, sheep adrenal concentrated and maintained a high percentage radio-manganese per gram of fresh tissue.

The pattern of radio-manganese uptake and turnover by sheep was comparable to that in swine, except for the time needed to obtain peak concentrations.

Placental complex. Manganese, radio-manganese and specific activities in the placental complex of third-trimester ewes are

TABLE XII. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF MATERNAL ORGANS AND TISSUES IN SHEEP AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION, THIRD TRIMESTER

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 0.38 ± 0.24 | 1.1 | 2.9 | 1.0 | 2.6 | 1.0 | 2.6 | 0.7 | 1.8 |
| Bile | 2.13 ± 0.71 | 390.0 | 183.1 | 125.0 | 58.7 | 9.0 | 4.2 | 26.2 | 12.3 |
| Liver | 1.56 ± 0.44 | 369.9 | 237.1 | 204.0 | 130.8 | 48.1 | 30.8 | 30.0 | 19.2 |
| Kidney | 0.92 ± 0.31 | 545.3 | 592.7 | 294.9 | 320.5 | 139.7 | 151.8 | 107.9 | 117.3 |
| Spleen | 0.63 ± 0.22 | 316.5 | 502.4 | 165.4 | 262.5 | 56.2 | 89.2 | 37.3 | 59.2 |
| Heart | 0.53 ± 0.18 | 42.8 | 80.8 | 15.5 | 29.2 | 11.5 | 21.7 | 13.1 | 24.7 |
| Pancreas | 1.50 ± 0.35 | 590.8 | 393.9 | 566.4 | 377.6 | 288.4 | 192.3 | 260.4 | 173.6 |
| Muscle | 0.48 ± 0.36 | 12.3 | 25.6 | 7.5 | 15.6 | 4.5 | 9.4 | 3.9 | 8.1 |
| Brain | 0.45 ± 0.09 | 8.0 | 17.8 | 7.0 | 15.6 | 6.9 | 15.3 | 7.4 | 16.4 |
| Pituitary | 3.13 ± 1.40 | 198.7 | 63.5 | 152.6 | 48.8 | 126.3 | 40.4 | 87.3 | 27.9 |
| Thyroid | 1.80 ± 0.80 | 483.0 | 268.3 | 280.0 | 155.6 | 48.3 | 26.8 | 34.7 | 19.3 |
| Adrenal | 2.00 ± 0.87 | 508.5 | 254.2 | 315.7 | 157.8 | 209.8 | 104.9 | 107.2 | 53.6 |
| Ovaries | 3.59 ± 1.20 | 24.1 | 6.7 | 32.5 | 9.1 | --- | --- | 21.0 | 5.8 |

TABLE XII (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Aorta | 1.66 ± 0.99 | 6.7 | 4.0 | 4.8 | 2.9 | 5.0 | 3.0 | 2.7 | 1.6 |
| Bone Marrow | 1.32 ± 0.79 | 3.6 | 2.7 | 2.2 | 1.7 | 0.5 | 0.4 | 0.7 | 0.5 |
| Sternum | 1.86 ± 0.68 | 37.0 | 19.9 | 22.6 | 12.2 | 15.0 | 8.1 | 6.1 | 3.3 |
| Mandible | 1.74 ± 0.67 | 9.2 | 5.3 | 8.5 | 4.9 | 14.3 | 8.2 | 15.9 | 9.1 |
| Rib Shaft | 2.34 ± 1.31 | 21.1 | 9.0 | 11.9 | 5.1 | 10.0 | 4.3 | 5.8 | 2.5 |
| Rib Epiphysis | 3.04 ± 1.59 | 48.0 | 15.8 | 31.6 | 10.4 | 36.2 | 11.9 | 17.8 | 5.9 |
| Femur Shaft | 1.12 ± 0.29 | 3.1 | 2.8 | 5.7 | 5.1 | 4.0 | 3.6 | 2.2 | 2.0 |
| Femur Epiphysis | 1.78 ± 0.94 | 11.5 | 6.5 | 17.3 | 9.7 | 3.9 | 2.2 | 7.3 | 4.1 |
| Cotyledons | 0.70 ± 0.20 | 71.1 | 101.6 | 67.0 | 95.7 | 22.0 | 31.4 | 26.1 | 37.3 |
| Uterus & Placenta | 0.63 ± 0.10 | 47.6 | 75.6 | 63.3 | 100.5 | 32.0 | 50.8 | 25.6 | 40.6 |
| Placental Fluids | 0.20 ± 0.17 | 0.4 | 0.2 | --- | --- | 0.5 | --- | 0.6 | --- |
| Fetus | 0.48 ± 0.09 | 3.9 | 8.1 | 6.3 | 13.1 | 5.2 | 10.8 | 6.0 | 12.5 |

[a] Mean ± standard deviation.

[b] Percent dose $^{54}Mn/gm. \times 10^{-4}$, corrected to 50 kg. maternal weight.

[c] Calculated: $\frac{\% \ ^{54}Mn/gm. \times 10^{-4}}{\mu g. \ Mn/gm.}$, fresh bases.

summarized in Table XIII. Placental fluids of third-trimester ewes were higher in radio-manganese than fluids of second-trimester ewes. However, concentrations in both were very low. No appreciable differences were noted in placental and uterine uptake or turnover when comparing second- and third-trimester ewes, but manganese and total radio-manganese were higher in the third trimester due to increased weight. These data were in contrast to those observed in swine, indicating a difference in the manganese uptake and turnover in the two types of placenta.

The whole fetus. A record of crown-rump length, age, weight, radio-manganese, manganese and specific activity in whole sheep fetuses as functions of age and time after dose administration are listed in Table XIV. Fetal length increased approximately 1.7 times, and weight approximately 5 times during the period 90 to 135 days of gestation in sheep. At 168 hr. post-dosing, percentage uptake of radio-manganese per gram of fresh tissue was higher in second- ($8.7 \times 10^{-4}$) than in third-trimester fetuses ($6.0 \times 10^{-4}$). However, the difference was not great, and with the rapid increase in fetal weight, dilution could have been a factor. Concentration of stable manganese also was greater in second-trimester fetuses; thus, turnover rates were relatively equal. As was true for swine, there were appreciable differences in the transfer rate during these stages of pregnancy, but ratios of fetal weight to transfer rate explained these differences. In sheep, maximum transfer as indicated by percent dose per fetus was reached by 24 hr. post-dosing. Maximum transfer to the sheep fetus was faster

TABLE XIII. SUMMARY OF MANGANESE, TOTAL RADIO-MANGANESE, AND SPECIFIC ACTIVITY IN THE PLACENTAL COMPLEX OF THIRD TRIMESTER EWES

| Tissue | Manganese mg. | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | Dose[a] | S.A.[b] | Dose | S.A. | Dose | S.A. | Dose | S.A. |
| Total Complex | 3.0 | 9.20 | --- | 6.84 | --- | 5.36 | --- | 5.89 | --- |
| Placenta & Uterus | 0.6 | 7.18 | 75.6 | 4.00 | 100.5 | 2.54 | 50.8 | 2.82 | 40.6 |
| Placental Fluids | 0.2 | 0.04 | 0.2 | --- | --- | 0.06 | 2.5 | 0.08 | 3.0 |
| Fetus (5,000 gm.) | 2.2 | 1.98 | 8.1 | 2.84 | 13.1 | 2.76 | 10.8 | 2.99 | 12.5 |

[a] Total percent $^{54}Mn$ dose, corrected to 50 kg. ewe.

[b] Calculated: $\frac{\%\ ^{54}Mn/gm. \times 10^{-4}}{\mu g.\ Mn/gm.}$, fresh bases.

TABLE XIV. SUMMARY OF LENGTH, AGE, WEIGHT, RADIO-MANGANESE, TOTAL MANGANESE, AND SPECIFIC ACTIVITY VALUES IN WHOLE SHEEP FETUSES AS A FUNCTION OF GESTATION AGE AND TIME AFTER $^{54}Mn$ INJECTION

| Trimester | HAD | Length cm. | Age days | Weight gm. | Percent Dose | | Total Mn (Mg.) | S.A.[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | | gm. | Total[a] | | |
| II | 168 | 25 | 91 | 877 | 8.70 | 0.76 | 0.91 | 0.84 |
| III | 12 | 42 | 133 | 7,117 | 3.85 | 1.98 | 3.42 | 0.81 |
| III | 24 | 42 | 131 | 3,441 | 6.32 | 2.84 | 1.65 | 1.31 |
| III | 96 | 42 | 134 | 3,471 | 6.43 | 3.60 | 1.67 | 1.32 |
| III | 144 | 43 | 143 | 4,262 | 5.18 | 2.76 | 1.18 | 1.08 |
| III | 168 | 45 | 137 | 3,920 | 5.98 | 2.99 | 1.88 | 1.25 |

[a] Total percent dose $^{54}Mn$, 5,000 gm. fetus.

[b] Calculated: $\frac{\%\ ^{54}Mn/gm. \times 10^{-4}}{Mg.\ Mn/gm.}$, fresh bases.

than to the swine fetus. Several factors contributed to this rate:

1. Differences in the placenta (syndomoschorial, 5 layers vs. epitheliochorial, 6 layers).
2. Total percent transfer 168 hr. post-dosing (5.9 percent to a 5,000 gm. fetus vs. 22.8 percent to an 8,000 gm. litter).
3. Need for manganese (0.48 µg./gm. fresh tissue vs. 0.80 µg./gm. fresh tissue).
4. Less available for transfer (0.2 percent x $10^{-4}$ per ml. circulating plasma vs. 0.8 percent x $10^{-4}$ per ml. circulating plasma).

Transfer rates indicated that there could be a trend toward a slightly higher transfer per gram of fetus in the second trimester; however, differences were small. The conclusion drawn from these data was as in swine: fetal weight and need are in direct relation to manganese transferred across the placental membrane.

Fetal tissue distribution. Manganese, radio-manganese and specific activity values of third-trimester sheep fetal organs and tissues are summarized in Table XV. Gestation age did not affect the uptake of radio-manganese by liver, kidney, spleen or heart in the sheep fetus, and in all these organs except heart, radio-manganese continued to increase up to 168 hr. after dose administration. As was noted in swine and maternal sheep, pancreas and bile concentrated a high percent $^{54}Mn$, with highest bile turnover rate occurring at 24 hr. post-dosing. Indications were that the same metabolic paths are followed in the fetus as in the mother.

TABLE XV. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF FETAL ORGANS AND TISSUES IN SHEEP AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION, THIRD TRIMESTER

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 0.39 ± 0.18 | 0.3 | 0.7 | 0.2 | 0.5 | 1.8 | 4.62 | 1.3 | 0.3 |
| Bile | 3.46 ± 2.37 | 19.6 | 5.6 | 259.0 | 74.8 | --- | --- | 23.8 | 6.8 |
| Liver | 3.30 ± 1.09 | 76.3 | 23.1 | 57.1 | 17.3 | 140.2 | 42.49 | 187.2 | 56.7 |
| Kidney | 0.50 ± 0.08 | 5.1 | 10.2 | 6.4 | 12.8 | 10.2 | 20.40 | 12.3 | 24.6 |
| Spleen | 0.69 ± 0.18 | 3.6 | 5.2 | 4.7 | 6.8 | 7.1 | 10.29 | 8.0 | 11.5 |
| Heart | 0.47 ± 0.19 | 1.5 | 3.1 | 1.5 | 3.1 | 3.8 | 8.09 | 3.0 | 6.3 |
| Pancreas | 2.17 ± 1.40 | 1.5 | 6.9 | 20.3 | 9.3 | 56.0 | 25.81 | 77.6 | 35.7 |
| Muscle | 0.28 ± 0.04 | 0.4 | 1.4 | 0.9 | 3.2 | 2.9 | 10.36 | 3.1 | 11.0 |
| Brain | 0.50 ± 0.22 | 0.3 | 0.6 | 0.4 | 0.8 | 2.5 | 5.00 | 3.6 | 7.2 |
| Pituitary | 5.15 ± 2.40 | 1.3 | 0.2 | 12.5 | 2.4 | 46.3 | 8.99 | 50.4 | 9.7 |
| Thyroid | 7.48 ± 2.83 | 16.2 | 2.1 | 24.0 | 3.2 | 127.2 | 17.00 | 138.9 | 18.5 |
| Adrenal | 5.73 ± 1.43 | 8.2 | 1.4 | 17.5 | 3.0 | 29.1 | 5.08 | 45.0 | 7.8 |
| Aorta | 3.12 ± 1.58 | 0.9 | 0.2 | 1.8 | 0.5 | 5.0 | 1.60 | 7.0 | 2.2 |
| Sternum | 4.55 ± 2.14 | 2.2 | 0.4 | 1.6 | 0.3 | 7.5 | 1.65 | 8.5 | 1.8 |

TABLE XV (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Mandible | 2.14 ± 1.39 | 1.6 | 0.7 | 1.5 | 0.7 | 13.6 | 6.36 | 10.9 | 5.0 |
| Rib Shaft | 3.03 ± 0.72 | 2.8 | 0.9 | 1.1 | 0.3 | 5.6 | 1.49 | 12.1 | 3.9 |
| Rib Epiphysis | 7.84 ± 3.27 | 3.9 | 0.4 | 5.1 | 0.6 | 18.9 | 2.41 | 19.7 | 2.5 |
| Femur Shaft | 4.56 ± 1.99 | 1.3 | 0.2 | 1.3 | 0.2 | 6.8 | 1.59 | 12.6 | 2.7 |
| Femur Epiphysis | 2.12 ± 0.88 | 2.8 | 1.3 | 2.1 | 0.9 | 16.7 | 7.88 | 21.9 | 10.3 |

[a] Mean ± standard deviation.

[b] Percent dose $^{54}Mn/gm. \times 10^{-4}$.

[c] Calculated: $\frac{\% \ ^{54}Mn/gm. \times 10^{-4}}{\mu g \ Mn/gm.}$, fresh bases.

Endocrine glands (pituitary, thyroid and adrenal) readily concentrated radio-manganese, and each continued to accumulate $^{54}Mn$ to 168 hr. post-dosing. In contrast to that observed in maternal sheep, highest turnover rates of these glands were observed in the thyroid of the fetus, possibly indicating a more active thyroid but a less active adrenal.

Concentration of radio-manganese by fetal bone was irregular, with cartilage-containing parts (epiphysis) reaching maximum concentrations prior to attainment of maximum concentrations in the shaft. Variations were noted in stable manganese, but as was true in the dam, rib epiphysis contained the highest concentration per gram of bone tissue. Small differences in concentration of $^{54}Mn$ were noted in the second compared to the third trimester for mandible, rib shaft and rib epiphysis; however, no appreciable difference was noted in sternum, femur shaft or femur epiphysis. Stable manganese remained relatively constant through these two periods of gestation.

Third-trimester fetal muscle continued to concentrate radio-manganese to 168 hr. post-dosing, but as was observed in the dam, these percentages were low. At 168 hr. post-dosing, radio-manganese uptake by second-trimester fetal muscle was approximately two times as great as in third-trimester fetuses. No appreciable difference was noted in stable manganese, indicating a higher turnover rate in muscle of second-trimester fetuses.

Partition of $^{54}Mn$ in fetal organs. Partition of $^{54}Mn$ in total organs of sheep fetuses is shown in Table XVI. Illustrated is the

TABLE XVI. PARTITION OF RADIO-MANGANESE IN FETAL ORGANS OF SHEEP FETUSES

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Fetus | III | 1.98 | 100 | 2.84 | 100 | 2.76 | 100 | 2.99 | 100 |
| Liver | III | 0.12 | 6.06 | 0.10 | 3.52 | 1.02 | 36.95 | 1.64 | 54.8 |
| Kidney | III | 0.002 | 0.10 | 0.002 | 0.07 | 0.008 | 0.28 | 0.01 | 0.33 |
| Spleen | III | 0.0002 | 0.01 | 0.0004 | 0.01 | 0.002 | 0.07 | 0.006 | 0.20 |
| Heart | III | 0.002 | 0.10 | 0.002 | 0.07 | 0.006 | 0.21 | 0.01 | 0.33 |

[a]Total percent $^{54}Mn$ dose.

[b]Percent of the transferred dose.

relative magnitude of radio-manganese transferred to various fetal organs with respect to total transfer at different stages of pregnancy and different times after dose administration. Liver of second-trimester fetuses tended to contain a higher percent of the radio-manganese transferred than did liver of third-trimester fetuses. The time study indicated that fetal sheep liver does not concentrate radio-manganese to the same extent as does fetal swine liver; however, with the extreme differences in the total percent transferred, this comparison may not be valid.

Partition of $^{54}Mn$ in dam and fetus. The pregnant ewe must supply nutrients not only to the fetus but also to the uterus and placental membranes and fluids. The partition of radio-manganese in the dam and fetus of sheep as a function of time after dose administration of $^{54}Mn$ is shown in Table XVII. At 168 hr. after dose administration, the placenta and uterus of second-trimester ewes contained 72.2 percent of the available radio-manganese, but during the third trimester, this percentage had dropped to 47.8, or almost one-half; while the percent contained in the fetus increased two-fold. At all times studied, placental fluids contained only a small percentage of $^{54}Mn$ in the total complex.

Summary. In this study ewes retained 1.83 mg. manganese (7.9 percent of the intake) per day, and the data indicated that 7 percent of the orally administered $^{54}Mn$ was retained by ewes. Third-trimester lamb fetuses contained 2.2 mg. stable manganese; uterus and placenta, 0.6 mg. and placental fluids, 0.2 mg., for a total of 3.0 mg.

TABLE XVII. PARTITION OF RADIO-MANGANESE IN THE DAM AND FETUS OF SHEEP

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 | | 24 | | 144 | | 168 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total Complex | III | 9.20 | 100 | 6.84 | 100 | 5.36 | 100 | 5.89 | 100 |
| Placenta & Uterus | | 7.18 | 78.1 | 4.00 | 58.5 | 2.54 | 47.4 | 2.82 | 47.8 |
| Placental Fluids | | 0.04 | 0.4 | --- | --- | 0.06 | 1.1 | 0.08 | 1.4 |
| Fetus (5,000 gm.) | | 1.98 | 21.5 | 2.84 | 41.5 | 2.76 | 51.5 | 2.99 | 50.8 |

[a] Total percent $^{54}Mn$ dose, corrected to 50 kg. ewe.

[b] Percent of the total complex.

manganese contained in the placental complex of yearling ewes. In this study, gravid ewes deposited in the products of conception an average of 20.7 µg. manganese per day.

Results of this study indicated that the total amount of elemental manganese transferred to a 5,000 gm.-sheep fetus (135 days of age) is approximately 2.2 mg. Balance and absorption studies showed no appreciable differences among open, second- or third-trimester ewe groups. However, the additional 20.6 µg. manganese per day needed to support the products of conception represents only 0.09 percent of the average daily intake. As was observed in swine, this small percentage would dictate the need for an extremely large number of animals to demonstrate differences to be significant.

## II. COPPER

### Results with Swine

Blood $^{64}Cu$. The total percent dose of $^{64}Cu$ appearing in the whole blood of swine as a function of time following intravenous dose administration is shown in Figure 4. Immediately after intravenous dosing, there was a rapid decline in the slope of the curve indicating that radio-copper was actively being removed from the circulating plasma. However, the rate of copper disappearance from the circulating blood was slower than that observed for manganese. Plasma and red cell clearances indicated that equilibrium was not reached within the 24-hr. study, although they did parallel in concentration of $^{64}Cu$ from 6 hr. post-dosing.

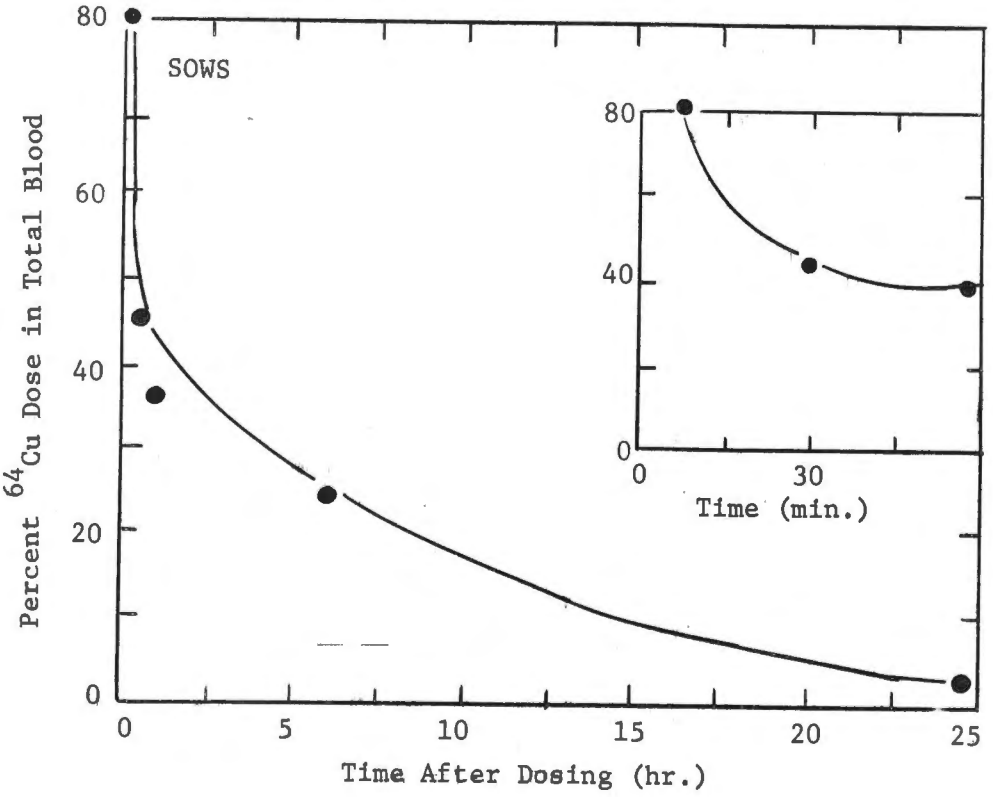


Figure 4. Percent $^{64}$Cu in the total circulating blood of swine as a function of time after intravenous dose administration.

Absorption and excretion. During this study, total copper balances (chemical) were calculated for each gilt to determine the general body status with respect to this element. Dietary copper intake averaged 21.7 ± 3.77 mg. per day, and excretion averaged 16.0 ± 3.82 mg. Copper balances were calculted to be a positive 5.7 mg. (26.8 percent) per day per gilt. Since these gilts were in a positive balance, they were assumed to be in a satisfactory overall status in regard to copper balance. Results of this study indicate that pregnancy exerted no marked effect on the pattern of fecal excretion of copper in swine; consequently, excretion values were grouped together for all animals.

Fecal excretion of $^{64}Cu$ was comparable to that observed for manganese (Figure 2, page 38). Bile is considered to be the major route for copper, as well as manganese, excretion. Mahoney et al. (1955) indicated that 7 to 10 percent intravenously administered radio-copper was excreted in the bile. Data from the present study suggested that bile contained twice as high a concentration of copper as manganese. However, rate of increase in bile concentration of radio-copper was slower than for radio-manganese, and ratios of bile $^{64}Cu$ to blood $^{64}Cu$ were also lower than were observed for $^{54}Mn$.

Mahoney et al. (1955) indicated that dogs excreted 0.6 percent of the $^{64}Cu$ dose by way of the urine, and Comar et al. (1948) estimated that in the ruminant, urine excretion accounted for 3 percent of the radio-copper dose. Short-term urine collections (24 hr.) accounted for 0.2 percent of the radio-copper dose. Chemical urine balances indicated that gilts excreted 0.72 ± 0.15 ml. urine per minute, containing 0.46 µg. Cu/ml., a total excretion of 0.5 ± 0.05 mg. copper

per day. Calculated clearances indicated that the kidneys cleared copper at the rate of 0.24 ml. plasma per minute. This value (0.2 to 0.3 ml./min.) agrees closely with the range reported by Jensen and Kamin (1957). Kidney clearance rates were comparable for copper and manganese, but due to differences in plasma content of these two minerals, total daily clearance from plasma was approximately twice as great for copper as for manganese.

Total organs. In swine, liver serves as the main metabolic pool for manganese. Data from the present study indicated that swine liver also served as the main metabolic pool for radio-copper (Table XVIII). At 48 hr. post-dosing, 89.4 percent of the radio-copper contained in liver, kidney, spleen and heart was in liver.

Tissue-organ distribution. Percent radio-copper and specific activity in maternal organs and tissues of swine as functions of time after dose administration are summarized in Table XIX. Stage of pregnancy appeared not to influence tissue concentration or turnover rates in maternal tissues. Thus, this discussion will concentrate primarily on the effect of time post-dosing on tissue distribution and turnover rates. Highest concentrations of radio-copper were observed in kidney and liver, with both reaching maximum or near maximum concentration 6 hr. post-dose administration. This concentration decreased in liver at 24 hr., but kidney remained relatively constant in its concentration of radio-copper throughout the 48-hr. study. Other organs and tissues concentrated only a small percentage of the radio-copper, and of these heart attained the highest concentration.

TABLE XVIII. PARTITION OF RADIO-COPPER IN MATERNAL SWINE ORGANS

| Tissue | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | | 6 | | 24 | | 48 | |
| | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total | 10.0 | 100 | 33.7 | 100 | 23.3 | 100 | 28.4 | 100 |
| Liver | 7.5 | 75.0 | 30.3 | 89.9 | 20.4 | 87.6 | 25.4 | 89.4 |
| Kidney | 2.1 | 21.0 | 3.0 | 8.9 | 2.5 | 10.7 | 2.5 | 8.8 |
| Heart | 0.3 | 3.0 | 0.3 | 0.9 | 0.3 | 1.3 | 0.4 | 1.4 |
| Spleen | 0.1 | 1.0 | 0.1 | 0.3 | 0.1 | 0.4 | 0.1 | 0.4 |

[a]Total percent $^{64}Cu$ dose contained in each organ, corrected to 50 kg. ewe.

[b]Percent of the total.

TABLE XIX. SUMMARY OF COPPER, RADIO-COPPER, AND SPECIFIC ACTIVITY VALUES OF SELECTED MATERNAL ORGANS OF SWINE AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 24 | | 48 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 1.50 ± 0.30 | 15.2 | 10.1 | 21.0 | 14.0 | 16.7 | 11.1 | 3.5 | 2.3 |
| Bile | 0.96 ± 0.94 | 2.7 | 2.8 | 25.6 | 26.7 | 39.7 | 62.2 | 14.0 | 14.6 |
| Liver | 4.33 ± 1.75 | 40.3 | 9.3 | 144.6 | 33.4 | 114.1 | 26.4 | 121.2 | 28.0 |
| Kidney | 4.01 ± 0.80 | 119.4 | 27.0 | 153.7 | 34.8 | 157.1 | 35.5 | 144.1 | 32.6 |
| Spleen | 3.17 ± 1.81 | 4.3 | 1.4 | 3.6 | 1.1 | 5.4 | 1.7 | 5.9 | 1.9 |
| Heart | 3.57 ± 0.77 | 6.6 | 1.9 | 7.8 | 2.2 | 5.6 | 1.6 | 9.7 | 2.7 |
| Pancreas | 2.03 ± 1.70 | 4.5 | 2.2 | 3.0 | 1.5 | 6.4 | 3.2 | 7.3 | 3.6 |
| Muscle | 1.88 ± 1.09 | 1.8 | 1.0 | 4.0 | 2.1 | 2.0 | 1.1 | 1.8 | 1.0 |
| Brain | 1.67 ± 0.24 | 0.9 | 0.5 | 0.9 | 0.5 | 1.4 | 0.8 | 3.1 | 1.9 |
| Pituitary | 4.95 ± 1.09 | 12.6 | 2.6 | 12.1 | 2.4 | 14.8 | 3.0 | 18.1 | 3.7 |
| Thyroid | 0.95 ± 0.05 | 3.0 | 3.2 | 3.1 | 3.3 | 4.1 | 4.3 | 5.2 | 5.5 |
| Adrenal | 3.63 ± 0.83 | 10.2 | 2.8 | 16.8 | 4.6 | 19.6 | 5.4 | 20.5 | 5.7 |
| Aorta | 1.63 ± 0.84 | 3.0 | 1.8 | 7.2 | 4.4 | 5.0 | 3.1 | 4.3 | 2.6 |
| Bone Marrow | 0.83 ± 0.26 | 1.5 | 1.8 | 2.3 | 2.8 | 1.5 | 1.8 | 2.2 | 2.7 |

TABLE XIX (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 24 | | 48 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Sternum | 2.13 ± 0.48 | 5.1 | 2.4 | 9.8 | 4.6 | 9.1 | 4.3 | 19.1 | 9.0 |
| Mandible | 1.78 ± 0.84 | 1.7 | 1.0 | 5.4 | 3.0 | 4.1 | 2.3 | 1.5 | 0.8 |
| Rib Shaft | 1.99 ± 0.60 | 4.3 | 2.2 | 7.6 | 3.8 | 9.7 | 4.9 | 10.5 | 5.3 |
| Rib Epiphysis | 3.14 ± 1.16 | 10.0 | 3.7 | 17.6 | 5.6 | 17.1 | 5.5 | 49.6 | 15.8 |
| Femur Shaft | 1.28 ± 0.78 | 0.8 | 0.6 | 1.7 | 1.3 | 1.7 | 1.3 | 0.6 | 0.5 |
| Femur Epiphysis | 1.26 ± 0.36 | 3.2 | 2.5 | 5.7 | 4.5 | 5.5 | 4.4 | 6.1 | 4.8 |

[a] Mean ± standard deviation.

[b] Percent dose $^{64}Cu/gm. \times 10^{-4}$, corrected to 150 kg. gilt.

[c] Calculated: $\frac{\% \ ^{64}Cu/gm. \times 10^{-4}}{\mu g. \ Cu/gm.}$, fresh bases.

In most cases, uptake of radio-copper was proportional to the concentration of stable copper. Three hours post-dosing, endocrine glands (adrenal, pituitary and thyroid) concentrated radio-copper in direct relation to stable copper concentration; however, at 48 hr., adrenal and pituitary were approximately equal and approximately four times as high as thyroid in percent dose per gram of fresh tissue. Bone concentration of radio-copper was low, except for the epiphysis area. As was noted for manganese, rib epiphysis concentrated a higher percent radio-copper than did other bone tissue.

Placental complex. Copper, radio-copper and specific activities in the placental complex of third-trimester gilts are summarized in Table XX. Radio-copper uptake by uterus and placenta was not greatly different (4.7 percent for the second trimester; 5.5 percent for the third), nor were there appreciable differences in the concentration in the placental fluids. Stable copper content was almost equal; thus, turnover rates in these products of conception during the second trimester were assumed to be equal.

The whole fetus. A record of crown-rump length, age, weight, radio-copper and specific activity values in whole swine fetuses as functions of age and time after dose administration of $^{64}Cu$ are tabulated in Table XXI. A trend toward higher concentration of both radio- and stable copper in second-trimester fetuses indicated that transfer rates were higher during the second than the third trimester of pregnancy. However, data were not sufficient to indicate significant differences. Total radio- and stable copper were higher in the third

TABLE XX. SUMMARY OF COPPER, TOTAL RADIO-COPPER, AND SPECIFIC ACTIVITY IN THE PLACENTAL COMPLEX OF THIRD TRIMESTER GILTS

| Tissue | Trimester | Copper (Mg.) | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | | 6 | | 24 | | 48 | |
| | | | %[a] | S.A.[b] | % | S.A. | % | S.A. | % | S.A. |
| Total Complex | III | 32.2 | 4.15 | --- | 7.30 | --- | 9.25 | --- | 9.98 | --- |
| Uterus and Placental Membranes | | 6.0 | 4.14 | 7.40 | 5.61 | 8.49 | 5.00 | 7.33 | 3.10 | 6.23 |
| Placental Fluids | | 1.2 | 0.01 | 0.16 | 0.01 | 0.16 | 0.01 | 0.16 | --- | --- |
| Fetuses (8) | | 26.0 | --- | --- | 1.68 | 1.10 | 4.24 | 1.90 | 6.88 | 4.45 |

[a] Total percent dose $^{64}Cu$, corrected to 150 kg. gilt.

[b] Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10^{-4}}{\mu g.\ Cu/gm.}$, fresh bases.

TABLE XXI. SUMMARY OF LENGTH, AGE, WEIGHT, RADIO-COPPER, TOTAL COPPER, AND SPECIFIC ACTIVITY VALUES IN WHOLE SWINE FETUSES AS A FUNCTION OF GESTATION AGE AND TIME AFTER $^{64}Cu$ INJECTION

| Trimester | HAD | Length cm. | Age Days | Weight gm. | Percent Dose gm. | Percent Dose Total[a] | Total Cu (Mg.) | S.A.[b] |
|---|---|---|---|---|---|---|---|---|
| II | 24 | 18 | 72 | 273 | 10.2 | 2.24 | 7.2 | 3.09 |
| III | 3 | 27 | 109 | 1,119 | bk. | bk. | 22.0 | --- |
| III | 6 | 28 | 105 | 1,308 | 2.2 | 1.72 | 26.0 | 1.11 |
| III | 24 | 29 | 107 | 1,408 | 3,71 | 2.98 | 28.0 | 1.87 |
| III | 48 | 30 | 111 | 1,430 | 8.6 | 6.89 | 28.0 | 4.34 |

[a]Total percent dose $^{64}Cu$, 8 pig litter.

[b]Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10\text{-}4}{\mu g.\ Cu/gm.}$, fresh bases.

trimester, but in contrast to manganese, it was not in direct relation to weight.

Fetal tissue distribution. Copper, radio-copper and specific activity values of third-trimester fetal organs and tissues are summarized in Table XXII. Fetal liver and kidney contained the highest percent $^{64}Cu$; however, liver concentrated a much higher percent than kidney. This was opposite to the observation in the mother, but stable concentration in liver was approximately six times that observed in the mother. Other organs and tissues followed the trend observed in maternal tissues and organs, except for fetal pancreas, which continued to accumulate radio-copper throughout the 48-hr. study. Fetal blood reached maximum concentrations of radio-copper 6 hr. post-dosing, but it was not until 24 hr. post-dosing that fetal muscle contained the highest percent radio-copper. Other fetal tissues and organs concentrated only trace amounts of $^{64}Cu$.

Partition of $^{64}Cu$ in fetal organs. The magnitude of $^{64}Cu$ transferred to various fetal organs relative to total litter $^{64}Cu$ transferred at different times after dose administration is illustrated in Table XXIII. Liver of third-trimester pigs contained twice the percent of the transferred dose as did liver of second-trimester pigs; however, if data were available, a more valid comparison could possibly have been made 48 hr. post-dosing. As was observed for manganese, liver was the main fetal metabolic pool for copper.

Partition of $^{64}Cu$ in dam and litters. The pregnant sow must supply nutrients not only to the litter but also to the uterus and

TABLE XXII. SUMMARY OF COPPER, RADIO-COPPER, AND SPECIFIC ACTIVITY VALUES OF FETAL SWINE AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 24 | | 48 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 1.80 ± 0.33 | 0.4 | 0.2 | 130.2 | 72.3 | 0.8 | 0.4 | 7.8 | 4.3 |
| Bile | 2.75 ± 1.15 | 1.1 | 0.4 | 6.3 | 2.3 | 0.3 | 0.1 | 34.8 | 12.7 |
| Liver | 29.50 ± 12.09 | 6.5 | 0.2 | 5.9 | 0.2 | 31.0 | 1.1 | 123.5 | 4.2 |
| Kidney | 3.62 ± 0.85 | 1.0 | 0.3 | 1.8 | 0.5 | 4.3 | 1.2 | 21.9 | 6.0 |
| Spleen | 5.42 ± 1.91 | 0.5 | 0.1 | --- | --- | 4.4 | 0.8 | 8.7 | 1.6 |
| Heart | 3.74 ± 0.72 | 0.2 | 0.1 | --- | --- | 2.0 | 0.5 | 3.1 | 0.8 |
| Pancreas | 3.18 ± 1.09 | --- | --- | 2.2 | 0.7 | 6.5 | 2.1 | 6.8 | 2.1 |
| Muscle | 1.20 ± 0.38 | 0.3 | 0.2 | 0.6 | 0.5 | 0.9 | 0.8 | --- | --- |
| Brain | 2.76 ± 1.18 | 0.1 | --- | 0.2 | 0.1 | 1.6 | 0.6 | 5.4 | 2.0 |
| Pituitary | 13.50 ± 3.34 | --- | --- | 3.5 | 0.3 | --- | --- | --- | --- |
| Thyroid | 1.70 ± 0.56 | --- | --- | --- | --- | 2.5 | 1.5 | 8.0 | 4.7 |
| Adrenal | 3.2 ± 0.17 | --- | --- | 2.9 | 0.9 | 5.1 | 1.6 | --- | --- |
| Aorta | 2.26 ± 1.16 | 0.2 | 0.1 | 3.0 | 1.3 | 0.4 | 0.2 | 2.7 | 1.2 |
| Sternum | 5.33 ± 2.21 | --- | --- | --- | --- | 3.4 | 0.6 | --- | --- |

TABLE XXII (continued)

| Tissue | µg./gm.[a] | | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | | 6 | | 24 | | 48 | |
| | | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Mandible | 2.94 | 1.01 | --- | --- | 0.6 | 0.2 | 3.3 | 1.1 | 3.2 | 1.1 |
| Rib Shaft | 4.95 | 2.00 | 0.7 | 0.1 | 1.4 | 0.3 | 1.0 | 0.2 | --- | --- |
| Rib Epiphysis | 8.8 | 3.3 | 0.4 | 0.1 | 2.5 | 0.3 | 4.8 | 0.6 | 7.0 | 0.8 |
| Femur Shaft | 3.59 | 1.76 | 0.6 | 0.2 | 1.5 | 0.4 | 4.6 | 1.3 | --- | --- |
| Femur Epiphysis | 2.63 | 0.84 | --- | --- | 1.8 | 0.7 | 8.9 | 3.4 | --- | --- |

[a] Mean ± standard deviation.

[b] Percent dose $^{64}Cu/gm. \times 10^{-4}$.

[c] Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10^{-4}}{\mu g.\ Cu/gm.}$, fresh bases.

TABLE XXIII. PARTITION OF RADIO-COPPER IN FETAL ORGANS OF SWINE LITTERS

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 6 | | 24 | | 48 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Litter | III | bkg. | --- | 1.72 | 100 | 2.98 | 100 | 6.89 | 100 |
| Liver | | --- | --- | 0.16 | 9.30 | 0.89 | 39.86 | 4.4 | 63.86 |
| Kidney | | --- | --- | 0.008 | 0.46 | 0.02 | 0.67 | 0.08 | 1.16 |
| Spleen | | --- | --- | --- | --- | 0.005 | 0.16 | 0.008 | 0.11 |
| Heart | | --- | --- | --- | --- | 0.02 | 0.67 | 0.02 | 0.29 |

[a] Total percent $^{64}Cu$ dose.

[b] Percent of the transferred dose.

placental membranes and fluids. Table XXIV partitions the radio-copper in the dam and litters of swine as functions of time after dose administration of $^{64}Cu$. Placenta and uterus of second- and third-trimester gilts contained nearly equal percentages of the available radio-copper, further illustrating the differences in transfer rates during these trimesters of pregnancy. The 48-hr. sample indicated that copper passed the placenta slower than manganese, but because of the short half-life of $^{64}Cu$, longer time studies needed to determine the exact rate were not conducted.

Summary. Radiochemical studies indicated that gilts retained 5.7 mg. (26 8 percent) copper per day and that a third-trimester litter contained 26.0 mg. copper; uterus and placenta, 6.0 mg., and placental fluids, 1.2 mg. A pregnant gilt maintained on a ration similar to the one used in this experiment would deposit 282 µg. copper per day in the products of conception. Absorption of copper was not appreciably affected by stage of pregnancy; however, the 282 µg. copper deposited in these products of conception represents only 1.3 percent of the average daily consumption.

## Results with Sheep

Blood $^{64}Cu$. Total percent dose $^{64}Cu$ appearing in the whole blood of sheep as a function of time following intravenous administration was comparable to that observed for swine. Immediately after intravenous dose administration, there was a rapid decline observed in the slope of the curve. However, $^{64}Cu$ disappearance was slower than $^{54}Mn$ in

the extent of this metabolic pool for copper. Trends in total organ radio-copper concentration are illustrated in Table XXV.

Tissue-organ distribution. Percent radio-copper and the specific activity in maternal organs and tissues of sheep as functions of time after dose administration are listed in Table XXVI. Stage of pregnancy appeared not to influence tissue concentration or turnover rates in maternal tissues. Highest concentrations of radio-copper were observed in kidney and liver, with both at maximum concentration 24 hr. post-dose administration. Uptake of $^{64}Cu$ by spleen and heart was similar, and both of these organs continued to accumulate radio-copper throughout this study. In agreement with observations in swine, radio-copper uptake paralleled total copper concentration in most soft tissues, organs and bones. Excluding heart, liver, kidney and spleen, highest percentages per gram of fresh tissue were noted in pituitary, adrenal, rib epiphysis and sternum, respectively.

Placental complex. Copper, radio-copper and specific activities in the placental complex of third-trimester ewes are summarized in Table XXVII. Radio-copper was undetectable in the placental fluids of both second- and third-trimester ewes, and although stable copper content varied, the average concentration was 0.74 µg. Cu/ml. Uterus and placenta of third-trimester ewes contained approximately three times the percent $^{64}Cu$/gm. contained by uterus and placenta of second-trimester ewes, but no appreciable differences were noted in stable copper content, making turnover rates higher in the third

TABLE XXV. PARTITION OF RADIO-COPPER IN MATERNAL SHEEP ORGANS

| Tissue | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 3 | | 12 | | 24 | |
| | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total | 60.4 | 100 | 73.7 | 100 | 85.0 | 100 | 97.0 | 100 |
| Liver | 59.5 | 98.5 | 72.4 | 98.2 | 93.4 | 98.1 | 95.0 | 98.0 |
| Kidney | 0.6 | 1.0 | 0.7 | 1.0 | 1.0 | 1.2 | 0.9 | 0.9 |
| Heart | 0.3 | 0.5 | 0.5 | 0.7 | 0.5 | 0.6 | 0.8 | 0.8 |
| Spleen | bkg. | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.3 |

[a]Total percent dose $^{64}Cu$ contained in each organ, corrected to 50 kg. ewe.

[b]Percent of the total.

TABLE XXVI. SUMMARY OF COPPER, RADIO-COPPER, AND SPECIFIC ACTIVITY VALUES OF SELECTED MATERNAL ORGANS AND TISSUES IN SHEEP AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 2.00 ± 0.59 | 16.6 | 8.3 | 15.2 | 7.6 | 12.2 | 6.1 | 19.4 | 9.7 |
| Bile | 2.98 ± 2.56 | 45.4 | 15.2 | 1.0 | 0.3 | 2.9 | 1.0 | 8.0 | 2.7 |
| Liver | 36.48 ± 9.10 | 1,170.9 | 32.1 | 1,069.4 | 29.3 | 1,328.3 | 36.4 | 1,860.0 | 51.0 |
| Kidney | 3.46 ± 0.81 | 134.2 | 38.8 | 138.4 | 40.0 | 122.9 | 35.5 | 153.1 | 44.3 |
| Spleen | 2.52 ± 1.15 | --- | --- | 14.3 | 5.7 | 12.7 | 5.0 | 29.2 | 11.6 |
| Heart | 3.46 ± 0.48 | 13.6 | 3.9 | 19.4 | 5.6 | 21.7 | 6.3 | 30.3 | 8.8 |
| Pancreas | 3.25 ± 1.79 | --- | --- | 56.5 | 17.4 | 16.5 | 5.1 | 18.2 | 5.6 |
| Muscle | 5.44 ± 1.68 | 2.0 | 0.4 | 2.2 | 0.4 | 4.4 | 0.8 | 5.2 | 1.0 |
| Brain | 3.10 ± 0.83 | 2.0 | 0.7 | 2.2 | 0.7 | 3.9 | 1.3 | 3.6 | 1.2 |
| Pituitary | 7.8 ± 1.05 | 61.0 | 0.8 | 81.8 | 1.1 | 84.4 | 1.1 | 127.7 | 1.6 |
| Thyroid | 22.63 ± 2.90 | 25.3 | 1.1 | 25.0 | 1.1 | 23.3 | 1.0 | 18.6 | 0.8 |
| Adrenal | 6.0 ± 1.29 | 51.2 | 0.9 | 46.7 | 0.8 | 56.1 | 0.9 | 51.5 | 0.9 |
| Aorta | 3.07 ± 1.22 | 14.6 | 4.8 | 12.0 | 3.9 | 13.5 | 4.4 | 18.5 | 6.0 |
| Bone Marrow | 1.30 ± 0.25 | 2.2 | 1.7 | 4.4 | 3.4 | 1.0 | 0.8 | 2.8 | 2.2 |

TABLE XXVI (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Sternum | 3.43 ± 1.47 | 13.0 | 3.8 | 19.9 | 5.8 | 21.1 | 6.2 | 22.0 | 6.4 |
| Mandible | 1.86 ± 0.84 | 11.9 | 6.4 | 12.0 | 6.5 | 15.2 | 8.2 | 6.3 | 3.4 |
| Rib Shaft | 3.10 ± 1.37 | 6.0 | 1.9 | 7.1 | 2.3 | 9.2 | 3.0 | 14.9 | 4.8 |
| Rib Epiphysis | 4.64 ± 1.40 | 21.6 | 4.7 | 37.7 | 8.1 | 25.4 | 5.5 | 38.7 | 8.3 |
| Femur Shaft | 1.78 ± 0.58 | 3.1 | 1.7 | 4.9 | 2.8 | 2.9 | 1.6 | 2.1 | 1.2 |
| Femur Epiphysis | 2.52 ± 0.64 | 11.7 | 4.6 | 16.8 | 6.7 | 8.1 | 3.2 | 15.1 | 6.0 |

[a] Mean ± standard deviation.

[b] Percent dose $^{64}Cu/gm. \times 10^{-4}$, corrected to 50 kg. ewe.

[c] Calculated: $\frac{\% \ ^{64}Cu/gm. \times 10^{-4}}{\mu g./gm.}$, fresh bases.

TABLE XXVII. SUMMARY OF COPPER, RADIO-COPPER, AND SPECIFIC ACTIVITY IN THE PLACENTAL COMPLEX OF THIRD TRIMESTER EWES

| Tissue | Trimester | Copper (mg.) | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | | 3 | | 12 | | 24 | |
| | | | %[a] | S.A.[b] | % | S.A. | % | S.A. | % | S.A. |
| Total Complex | III | 10.7 | 3.9 | --- | 3.1 | --- | 7.8 | --- | 5.4 | --- |
| Uterus and Placenta | | 1.1 | 3.9 | 43.9 | 3.1 | 42.4 | 7.3 | 39.7 | 3.9 | 42.7 |
| Placenta Fluids | | 0.8 | --- | --- | --- | --- | --- | --- | --- | --- |
| Fetus (5,000 gm.) | | 8.8 | 0.02 | 0.023 | 0.03 | 0.028 | 0.5 | 0.51 | 1.5 | 2.32 |

[a] Total percent dose $^{64}Cu$, corrected to 50 kg. ewe.

[b] Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10^{-4}}{\mu g.\ Cu/gm.}$, fresh bases.

trimester. These differences in turnover rates were greater than those observed in swine.

The whole fetus. A record of crown-rump length, age, weight, radio-copper and specific activity values in whole sheep fetuses as functions of time after dose administration is listed in Table XXVIII. A trend toward higher transfer rates was noted in the second trimester in swine; however, this difference was more pronounced in sheep, indicating a markedly higher transfer rate during the second trimester. Total stable and radio-copper were higher in the third trimester fetus, but not by just a weight factor as was observed for manganese.

Fetal tissue distribution. Copper, radio-copper and specific activity values of third-trimester fetal organs and tissues are summarized in Table XXIX. Liver tended to concentrate the highest percent radio-copper, with kidney being second in total percent $^{64}Cu$. Other organs and tissues accumulated radio-copper relative to stable copper content. However, 24 hr. post-dosing, low concentrations of $^{64}Cu$ were observed in the fetal thyroid and brain. Fetal bone was irregular in its concentration of $^{64}Cu$, indicating that a further time study would be needed to obtain the exact trends.

Partition of $^{64}Cu$ in fetal organs. Partition of $^{64}Cu$ in total organs of sheep fetuses is shown in Table XXX. Amount of radio-copper transferred to various fetal organs relative to total fetal $^{64}Cu$ transferred at different times after dose administration is illustrated. Two hours post-dosing, third-trimester fetal liver contained

TABLE XXVIII. SUMMARY OF LENGTH, AGE, WEIGHT, RADIO-COPPER, TOTAL COPPER AND SPECIFIC ACTIVITY VALUES IN WHOLE SHEEP FETUSES AS A FUNCTION OF GESTATION AGE AND TIME AFTER $^{64}Cu$ INJECTION

| Trimester | HAD | Length cm. | Age days | Weight gm. | Percent Dose | | Total Cu (Mg.) | S.A.[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | | gm. | Total[a] | | |
| II | 24 | 25 | 91 | 877 | 14.4 | 1.25 | 1.5 | 8.2 |
| III | 2 | 44 | 137 | 4,139 | 0.04 | 0.02 | 7.3 | 0.02 |
| III | 3 | 42 | 131 | 3,441 | 0.05 | 0.03 | 6.1 | 0.03 |
| III | 12 | 42 | 133 | 5,281 | 0.9 | 0.5 | 9.3 | 0.51 |
| III | 24 | 42 | 134 | 3,471 | 4.1 | 2.2 | 6.1 | 2.32 |

[a] Total percent dose $^{64}Cu$, 5,000 gm. fetus.

[b] Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10^{-4}}{\mu g.\ Cu/gm.}$, fresh bases.

TABLE XXIX. SUMMARY OF COPPER, RADIO-COPPER, AND SPECIFIC ACTIVITY VALUES SELECTED FETAL TISSUES IN SHEEP AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Blood | 2.0 ± 1.77 | 0.2 | 1.1 | 0.4 | 0.2 | 0.5 | 0.3 | 1.5 | 0.8 |
| Bile | 3.46 ± 2.09 | 20.2 | 5.8 | 4.9 | 1.4 | 4.7 | 1.4 | 6.5 | 1.9 |
| Liver | 28.38 ± 7.03 | 2.9 | 0.1 | 5.4 | 0.2 | 14.0 | 0.5 | 81.7 | 2.9 |
| Kidney | 2.36 ± 0.54 | --- | --- | 5.4 | 2.3 | 1.6 | 0.7 | 9.1 | 3.9 |
| Spleen | 2.56 ± 0.70 | --- | --- | --- | --- | 0.8 | 0.3 | 1.4 | 0.6 |
| Heart | 3.15 ± 0.28 | --- | --- | --- | --- | 0.9 | 0.3 | 2.8 | 0.9 |
| Pancreas | 5.05 ± 1.59 | --- | --- | 1.7 | 0.3 | 1.1 | 0.2 | 6.0 | 1.2 |
| Muscle | 0.88 ± 0.24 | --- | --- | 0.2 | 0.2 | 0.2 | 0.2 | 1.4 | 1.6 |
| Brain | 2.02 ± 0.62 | 1.0 | 0.5 | 0.3 | 0.2 | 0.6 | 0.3 | 0.5 | 0.3 |
| Pituitary | 14.4 ± 3.19 | --- | --- | --- | --- | --- | --- | 14.4 | 1.0 |
| Thyroid | 6.20 ± 1.33 | 8.5 | 1.4 | 5.0 | 0.8 | 1.4 | 0.2 | --- | --- |
| Adrenal | 8.50 ± 0.96 | --- | --- | 1.8 | 0.2 | 1.7 | 0.2 | 3.3 | 4.0 |
| Aorta | 4.54 ± 1.34 | --- | --- | 1.1 | 0.2 | 5.3 | 1.2 | 3.6 | 0.8 |
| Sternum | 5.52 ± 2.91 | 3.6 | 0.7 | 2.3 | 0.4 | 0.7 | 0.1 | 3.2 | 0.6 |

TABLE XXIX (continued)

| Tissue | µg./gm.[a] | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | %[b] | S.A.[c] | % | S.A. | % | S.A. | % | S.A. |
| Mandible | 3.9 ± 2.78 | --- | --- | 0.9 | 0.2 | 1.2 | 0.3 | 1.6 | 0.2 |
| Rib Shaft | 4.83 ± 1.73 | --- | --- | 1.3 | 0.3 | 2.7 | 0.6 | 1.6 | 0.3 |
| Rib Epiphysis | 32.9 ± 13.20 | --- | --- | 3.1 | 0.1 | 0.8 | --- | 1.9 | 0.1 |
| Femur Shaft | 3.85 ± 2.34 | --- | --- | 1.0 | 0.3 | 0.3 | 0.1 | 14.7 | 3.8 |
| Femur Epiphysis | 4.20 ± 1.26 | 1.0 | 0.2 | --- | --- | 0.3 | 0.1 | 1.5 | 0.4 |

[a] Mean ± standard deviation.

[b] Percent dose $^{64}Cu/gm. \times 10^{-4}$.

[c] Calculated: $\frac{\%\ ^{64}Cu/gm. \times 10^{-4}}{\mu g.\ Cu/gm.}$, fresh bases.

TABLE XXX. PARTITION OF RADIO-COPPER IN FETAL ORGANS OF SHEEP FETUSES

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Fetus (5,000 gm.) | III | 0.02 | 100 | 0.03 | 100 | 0.50 | 100 | 2.20 | 100 |
| Liver | | 0.02 | 100 | 0.02 | 80.0 | 0.12 | 24.0 | 0.71 | 32.3 |
| Kidney | | bkg. | --- | 0.004 | 13.3 | 0.002 | 0.40 | 0.007 | 0.3 |
| Heart | | bkg. | --- | bkg. | --- | 0.001 | 0.20 | 0.007 | 0.3 |
| Spleen | | bkg. | --- | bkg. | --- | 0.001 | 0.20 | 0.001 | 0.04 |

[a] Total percent $^{64}Cu$ dose.

[b] Percent of the transferred dose.

approximately 100 percent of $^{64}Cu$ transferred, but by 24 hr. after dose administration, this percentage had dropped to 32.3. Although a higher transfer rate was observed during the second trimester, the percentage of the transferred radio-copper contained in second-trimester fetal liver (23.1) was comparable to that in the liver of third-trimester fetuses. Other organs contained only trace amounts and percentages.

Partition of $^{64}Cu$ in dam and fetus. The pregnant ewe must supply nutrients not only to the fetus, but also to uterus and placental membranes and fluids. Partition of radio-copper in the dam and fetuses of sheep as a function of time after dose administration of radio-copper is illustrated in Table XXXI. Stage of pregnancy did not affect the percent of the available copper contained in second- or third-trimester uterus and placenta. The time study indicated that it was 24 hr. post-dose administration before noticeable amount of the $^{64}Cu$ made its way to the developing fetus; thus, a longer time study would be needed for estimation of transfer rates.

Summary. Radiochemical studies suggested that ewes retained 1.39 mg. copper per day and that a third-trimester fetus (5,000 gm.) contained 8.8 mg. copper; uterus and placenta, 1.1 mg., and placental fluids, 0.8 mg. A pregnant yearling ewe maintained on a ration similar to the one used in this experiment would deposit 74 µg. copper per day in the products of conception. Although stage of pregnancy did not appear to affect copper absorption, the amount of copper deposited in the products of conception represented only 5.3 percent of the calculated average daily copper intake.

TABLE XXXI. PARTITION OF RADIO-COPPER IN THE DAM AND FETUSES OF SHEEP

| Tissue | Trimester | Hours After Dose Administration | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | 3 | | 12 | | 24 | |
| | | Dose[a] | %[b] | Dose | % | Dose | % | Dose | % |
| Total Complex | III | 3.9 | 100 | 3.1 | 100 | 7.8 | 100 | 6.1 | --- |
| Placenta & Uterus | | 3.9 | 99.5 | 3.1 | 99.0 | 7.3 | 93.6 | 3.9 | 63.9 |
| Placental Fluids | | bkg. | --- | bkg. | --- | bkg. | --- | bkg. | --- |
| Fetuses (5,000 gm.) | | 0.02 | 0.5 | 0.03 | 1.0 | 0.5 | 6.4 | 2.2 | 36.1 |

[a]Total percent $^{64}Cu$ dose, corrected to 50 kg. ewe.

[b]Percent of the total complex.

General Discussion

Interest in the effects of nutrition during pregnancy upon the growth and performance of the offspring has stimulated investigation of the basic physiological relationships between the dam and her fetus. Laboratory animals have been employed most extensively for placental transfer and fetal nutrition studies because they offer the added advantage of total fetal-dam analyses, and permit effective use of sufficient numbers for reliable estimation. These investigations have been especially valuable for perfecting methods of procedure, techniques and criteria for evaluating the dynamic nature of the naternal-fetal relationships. The large metabolic pools in farm animals however, has permitted intensive investigations of membrane permeability, ion transport mechanisms and fetal utilization of absorbed minerals. The peculiar position of manganese and of copper in animal physiology and nutrition, the economic importance of farm animals and the lack of factual information concerning utilization of these metals by the fetus has emphasized the need for quantitative data on the maternal source, utilization efficiency and progressive requirements for fetal growth and development during gestation.

Relationships of manganese to histological and biochemical lesions in bone, skeletal development, enzyme levels, reproduction, hypoglycemia and impaired vestibular function (Cotzias, 1962), much like that of copper deficiency in lambs (Follis et al., 1955; Adelstein and Vallee, 1962), where brain and spinal lesions are involved, suggest a need for comparison of the metabolism of these two minerals. In

addition, manganese and copper deficiency disorders in grazing animals occur in many parts of the world on rations differing widely in copper and manganese content (Adelstein and Vallee, 1962; Cotzias, 1962), suggesting utilization to be markedly influenced by dietary composition. Specific roles of these minerals in animal metabolism are also of interest because of their relationships to metabolic reactions and to other minerals.

Since blood serves as the main metabolic pool through which all nutrients must pass prior to utilization by the animal's body, a comparison of disappearance rates between species was of interest. Data indicated that sheep removed both radio-manganese and radio-copper faster than did swine. When $^{54}Mn$ and $^{64}Cu$ were removed from the blood, liver served as the primary metabolic pool, subsequent to their passage to the maternal and fetal tissues, reflecting the movement of stable manganese and copper.

One week after dose administration ewes had excreted four times as much of the intravenous $^{54}Mn$ as had swine; however, since there was not apparent differences observed with respect to kidney clearance in these two species, a distinct difference is suggested for manganese need. Radio-manganese concentration of maternal and fetal tissues supported this assumption.

Both manganese and copper have been reported in the newborn of different animal species (Orent and McCollum, 1931; Newland and Davis, 1961) indicating placental transfer. Only limited studies have indicated that the placentas of swine and rats are permeable to

manganese, and no information was readily available on rate and quantity of transfer in swine and sheep. The placental transfer of manganese and copper as a function of placental type and time after dose administration is shown in Table XXXII. Illustrated is the percent transferred per kg. of fetus. Species are listed in order of placental complexity as described in the literature and reviewed earlier in the present dissertation. Placental structure, when comparing rats with either sheep or swine, affected total percent and rate of transfer; however, other factors were involved in the ultimate determination of what passed from the mother to the fetus. Within species, weight was the major determining factor in total percent transfer, and in both swine and sheep fetal need for copper (as determined by whole body analysis) was greater than for manganese, but percent copper transferred per kg. of fetus was less than for manganese. Size, weight and charge of the particles being transferred (Hansard, 1965) may have contributed to the differences in transfer rates.

Manganese and copper were found in all tissues in the maternal and fetal bodies indicating, in agreement with Cotzias (1962), Adelstein and Vallee (1962), that these minerals may function on a cellular basis and that manganese concentration is relatively consistent across species. However, the same is not true for copper.

Further study is suggested to determine specific cellular functions and maternal-fetal utilization of these two minerals.

TABLE XXXII. TRANSFER OF RADIO-MANGANESE AND RADIO-COPPER PER Kg. FETUS

| Species | Hours After Dose Administration | | | | | | |
|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 48 | 96 | 168 |
| | $^{54}Mn$[a] | $^{64}Cu$[a] | $^{54}Mn$ | $^{64}Cu$ | $^{54}Mn$ | $^{54}Mn$ | $^{54}Mn$ |
| Swine | 0.5 | 0.3 | 1.1 | 0.4 | 1.8 | 2.4 | 2.9 |
| Sheep | 0.4 | 0.1 | 0.6 | 0.4 | 0.6 | 0.6 | 0.6 |
| Rats | 180.0 | --- | 204.0 | --- | 204.0 | --- | --- |

[a]Expressed as percent dose per kg. fetus.

# CHAPTER V

## SUMMARY AND CONCLUSION

Fifteen yearling gilts (73 fetuses) and 15 yearling ewes (13 fetuses) were used to study absorption, tissue-organ distribution and movement of radio-manganese and radio-copper across placental membranes. Objectives were: (1) to obtain an understanding of the basic metabolic pathways, (2) to investigate placental transfer, and (3) to determine maternal-fetal relationships.

Results of this study indicated that liver serves as the main metabolic pool for both copper and manganese. Stage of gestation apparently does not influence manganese or copper retention, tissue-organ distribution or placental transfer of radio-manganese. However, a higher transfer rate for radio-copper was observed during the second trimester of pregnancy in swine and sheep.

The manganese time study clearly illustrated a distinct species difference in the amount and rate at which radio-manganese moved across the placental membranes. At 168 hr. post-dose administration, an 8,000-gm. swine litter contained 22.8 percent of the intravenously injected $^{54}Mn$; while a 5,000-gm. lamb fetus contained 3.0 percent. Fetal weight and need within species were the main determining factors in total percent transfer. Percentages of radio-copper transferred 24 hr. post-dosing in swine and sheep were 4.2 and 2.2, respectively, indicating that (if this trend continued) fetal weight and need, rather than species, would determine the total percent transferred.

A species difference was noted also with respect to retention of manganese, ewes retaining 7.9 percent and gilts 28.5 percent of the average daily intake. However, this difference was not observed for copper, ewes and gilts retaining 24.3 and 26.8 percent, respectively.

Results indicated that only small amounts of manganese and copper are needed by sheep and swine; however, small but consistent daily retentions and wide distribution throughout the maternal and fetal bodies supported the hypothesis that both these minerals have important functions in animal nutrition.

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## LITERATURE CITED


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# VITA

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