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Placental Transfer and Maternal-Fetal Utilization of Manganese by Third Trimester Pregnant Heifers

Boyce P. Wanamaker

University of Tennessee - Knoxville

Recommended Citation

To the Graduate Council:

I am submitting herewith a thesis written by Boyce P. Wanamaker entitled "Placental Transfer and Maternal-Fetal Utilization of Manganese by Third Trimester Pregnant Heifers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Sam L. Hansard, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
September 10, 1971

To the Graduate Council:

I am submitting herewith a thesis written by Boyce P. Wanamaker entitled "Placental Transfer and Maternal-Fetal Utilization of Manganese by Third Trimester Pregnant Heifers." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

[Signature]
Major Professor

We have read this thesis and recommend its acceptance:

[Signatures]

Accepted for the Council:

[Signature]
Vice Chancellor for Graduate Studies and Research
PLACENTAL TRANSFER AND MATERNAL–FETAL UTILIZATION OF MANGANESE
BY THIRD-TRIMESTER PREGNANT HEIFERS

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Boyce P. Wanamaker
December 1971
ACKNOWLEDGMENTS

The author wishes to express his appreciation and thanks to the following persons who contributed to the completion of his graduate study.

To Dr. S. L. Hansard, for serving as major professor, and for his continued encouragement and guidance in the collection of these data.

To Dr. K. M. Barth, Dr. M. C. Bell, and Dr. C. C. Chamberlain for serving as the graduate committee and for their understanding and assistance.

To Mr. H. M. Crowder, Mrs. Nancy Nuchols, Mr. F. C. Madsen, Mr. M. C. Sanwal, Mr. S. P. Bertsch, Mrs. Sandra Eddlemon, and Dr. G. M. Merriman for their technical assistance.

To his parents, Mr. and Mrs. Preston Wanamaker, for their encouragement and assistance during his graduate study.
ABSTRACT

Although manganese has been demonstrated to be required for normal life processes, little information is available on the placental transfer and maternal-fetal utilization of this mineral in the bovine. Nine pregnant and three non-pregnant heifers were used in this study. They were dosed with a tracer level of radio-manganese for blood balance and subsequent placental transfer and maternal-fetal manganese utilization studies. Results of this study suggested total blood radio-manganese levels to drop from 14% of the administered dose at 3 hours to 1.8% after 144 hours. Blood clearance studies indicated that equilibrium between red cells and plasma was reached 38 hours post-dosing, and further data suggested that approximately 50% of the plasma manganese was protein bound. Eighteen percent of an intravenous $^{54}$Mn dose was excreted by pregnant heifers 144 hours after dosing. Bile appeared to be a major excretory pathway for manganese, and urine a minor excretory pathway. Liver served as the main metabolic pool for manganese in both the dam and fetus. Data suggested that heifers retained a calculated 1.16 mg manganese per day and deposited 82 μg (7.0% of that retained) in the total products of conception. Third-trimester bovine fetuses (272 days) contained 19.1 mg stable manganese; placenta, 2.10 mg and placental fluids 1.20 mg, for a total of 22.4 mg manganese in the total products of conception. Pregnant heifers deposited in the total products of conception a calculated 82 μg manganese per day, suggesting that 82 μg manganese per day was needed to support the products of conception in the pregnant heifer.
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CHAPTER I

INTRODUCTION

Maternal-fetal nutritional relationships have interested investigators for hundreds of years. Maternal control of fetal environment and anatomical inaccessibility have prevented direct studies of fetal-placental and fetal-maternal physiology (Hansard, 1970). Recent advances in methodology and instrumentation, however, have allowed new approaches to the complex physiological mechanisms of pregnancy.

Although food energy is often the critical nutrient involved (Hansard and Berry, 1969), the micronutrients critical to normal tissue differentiation and growth become more important as trends toward animal confinement increase.

Many studies have been conducted to determine nutritional requirements of manganese, however, specific requirements for the gestating bovine have not been established. Also, there is a lack of information on the regulatory mechanisms of mineral absorption, placental transfer, metabolism and utilization. It has been established that manganese is widely distributed in nature and has varied functions in the animal body. Its consideration is, then, of practical importance to the livestock producer and the animal nutritionist.

The introduction of radiotracer techniques into the fields of nutrition and physiology greatly facilitated the study of mineral interrelationships and maternal-fetal nutrition. This has become, in fact, the primary way of studying placental transfer and metabolic
utilization of mineral elements. Hansard (1965) has discussed the use of indicators and radioactive labeled substances for predicting membrane permeability and transport rates, fetal accretion and estimations of deposition and pregnancy anabolism.

This experiment was conducted to determine the placental transfer and maternal-fetal utilization of manganese and its radioisotope in yearling gravid third-trimester heifers.
CHAPTER II

LITERATURE REVIEW

I. MANGANESE

**History.** Long before Scheele (1742-86) first isolated manganese, the Romans recognized its "female" magnetic properties. At that time the term magnesia was applied interchangeably to the ores of magnesium and manganese.

Cotzias (1958) demonstrated that manganese was essential to life, and many functions have been assigned to it. As analytical methods have improved, however, the amounts of manganese that can be detected have become smaller and smaller.

**Methods of analyses.** It has been recognized that the analysis of the manganese content of a substance is difficult. The problem lies in the ease of contamination with external manganese and loss of manganese from the sample. Cotzias (1962) has outlined the various procedures for manganese analyses.

**Functions in metabolism.** Oxidative phosphorylation is thought to require manganese ions. The work of Cotzias and Maynard (1955) who found a rapid accumulation of injected radio-manganese in the mitochondria, supports this view.

It has been demonstrated (Mohammed and Greenberg, 1945) that arginase preparations are markedly stimulated by divalent metals. Manganese activation of other enzymes has been demonstrated:
cystine desulfhydrase (Binkley, 1943); thiaminase (Reddy, Giril and Das, 1948); deoxyribonuclease (McCarty, 1946); and enolase (Malstrom, Vanngard and Larson, 1958).

Manganese has played a major role in the metabolism of fatty acids in the intact organism. This proposal was supported by the work of Amdur, Norris and Heuser (1946) and Curran (1954, 1955). Manganese has been found to act as a cofactor in cholesterol synthesis. Tchen (1958) has shown manganese at physiological concentrations to be an efficient activator of purified mevalonic kinase. Cotzias (1962) has reported manganese to be important in mitochondrial function, and it may function as a respiratory cofactor (Maynard and Cotzias, 1955).

Everson (1970) presented evidence to suggest that manganese was involved in normal reproduction and normal bone and cartilage formation. Erway, Hurley and Fraser (1970) reported manganese to be involved in otolith formation in mice, which appeared to be the result of improper mucopolysaccharide synthesis. Thomas (1970) has reported that manganese may be incorporated into the heme molecule, in vivo and in vitro. Parker et al. (1955) have reported that since a manganese deficiency may cause abnormal bone development without seriously altering the chemical composition of the bone, this element may function in the process involved in the orientation and rearrangement of the bone mineral crystals. Chamberlain and Burroughs (1962) suggested manganese to play a role in cellulose digestion, in vitro. Cotzias and Bertinchamps (1960) suggested that manganese was transported by a specific plasma protein, transmaganin, in the living organism.
Nutritional requirements. Cotzias (1962) has stated that manganese is considered an essential nutrient because:

1. It is always present in feedstuffs.
2. Its concentration in mammalian 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 portion of their deficiency.
6. When given as a dietary supplement, it prevents symptoms of deficiency from appearing.

In spite of this knowledge, the precise dietary requirements for manganese can only be surmised. Nutritional experiments have been carried out using inorganic manganese; since manganese in food is probably chelated, much of the work using inorganic forms might not be totally valid.

It is well accepted that heavy dietary loads with compounds of one element are capable of interfering with utilization of other elements. This has been demonstrated by the creation of perosis in birds in the presence of ample manganese with diets high in calcium or phosphorus (Thomas, 1970).

The view that birds require more manganese than mammals (Underwood, 1966) has been based on the required concentration of inorganic manganese per unit weight of diet and not on the actual amount of metal consumed (Cotzias, 1962).

In mammals, supplements of inorganic manganese to deficient diets prevent deficiency. Smith and Ellis (1947) have suggested that 300 mg per animal per day was adequate manganese for normal growth in the rabbit. The same level was not adequate for the rat.
Maynard and Loosli (1969) reported 0.4 mg/kg diet to be sufficient for the baby pig. Plumlee et al. (1956), however, found a deficiency when weanling pigs received 3.5 mg/kg diets. Grummer et al. (1950) observed impaired reproduction in sows fed a ration containing 12 mg/kg manganese. The manganese requirement for beef cattle has been set by the National Research Council at 10 mg/kg diet. Maynard and Loosli (1969) reported that the same level was probably sufficient for sheep. Rojas, Dyer and Cassatt (1965) stated that the gestating beef cow requires about 200 ppm manganese. A satisfactory level of manganese for dairy cattle was set at 20 mg/kg diet (Bentley and Phillips, 1951).

Generally, manganese deficiency has been prevented in poultry by addition of 50 mg/kg diet.

Man's daily requirements are unknown. The occidental diet contains roughly 4 mg per day, an amount apparently adequate (Sandstead, 1967).

**Deficiency symptoms.** Underwood (1966) has reported that the manifestations of manganese deficiency are impaired growth, skeletal abnormalities, disturbed reproductive function and ataxia in the newborn. Expression of the signs vary with the extent and duration of the deficiency and with the age or growth stage of the animal.

Plumlee et al. (1956) found irregular estrus, resorption of the fetus, birth of small, ataxic and weak pigs, poor udder development and an absence of milk when swine were fed rations low in manganese throughout growth, gestation and lactation.

Rojas et al. (1965) reported deformed calves born to beef cows
on rations containing 15.8 and 16.9 ppm manganese. The calves exhibited enlarged joints, stiffness, twisted legs and a general physical weakness.

Bentley and Phillips (1951) found that dairy heifers raised on low manganese rations were slower to exhibit estrus and slower to conceive.

Erway et al. (1970) demonstrated that otoliths were reduced in size or were absent in manganese deficient mice. The absence of otoliths was caused by a disorder in synthesis of the organic matrix, which appears to be composed of acid mucopolysaccharides.

Everson (1970) fed guinea pigs a diet containing 2 to 3 mg/kg manganese. A high percentage of the young from mothers fed the deficient diet throughout the gestation period were ataxic at birth and continued to have abnormal head movement, and some incoordination during growth and on into adult life. The incidence of stillbirths was also unusually high. There was a shortening of the long bones, enlargement and malformation of the joints, a shortening and doming of the skull, deformities of the ribs or missing ribs and anterior-posterior flattening of the chest. It was also indicated that there was a reduction in all acid-mucopolysaccharides, suggesting manganese to be involved in the normal development of cartilage.

Skinner, Peterson and Steenbock (1931) and Smith, Medlicott and Ellis (1944) found that manganese concentration in the rat and rabbit reached a peak at birth and again at weaning. They also indicated that adding manganese to the diet of pregnant rats affected manganese levels in the progeny, but that adding manganese to the lactation diet of deficient mothers or suckling young on normal mothers did not correct
a deficiency in the young. It can be assumed, then, that there is sufficient placental transfer of manganese to supply body stores at birth, but that a deficiency cannot be corrected through milk.

Rudra (1941) reported a vicarious appetite termed "manganese hunger" in the manganese-deficient rabbit.

Wilgus, Norris and Heuser (1937) were the first workers to link perosis with a manganese deficiency in chicks. Perosis was characterized by shortening and thickening of the bones accompanied by slipping of the epiphysis and of the Achilles tendon. Maynard and Loosli (1969) reported that choline, in addition to manganese, was necessary to prevent perosis in the chick.

Concentration in feedstuffs. Of the cereal grains used in animal feeds, corn was low (6 ppm), barley (18 ppm) and oats (43 ppm) were intermediate and wheat (55 ppm) was highest in manganese content (National Research Council, 1970).

Sandstead (1967) reported the removal of outer layers of grains in milling to result in loss of much of the manganese.

Cotzias (1962) found soybean meal to contain 30 ppm manganese and protein supplements of animal origin to contain low manganese content. Bentley and Phillips (1951) indicated forages in the United States to contain 50 to 150 ppm manganese.

Concentration in animal tissue. Liver has been considered to be a site of manganese storage, but bone may serve this function as well since it is the site of highest concentration in the animal body
(Cotzias, 1962). The pancreas and kidney have been reported to contain high levels as well (Maynard and Cotzias, 1955).

Absorption and excretion. The exact location of manganese absorption in the gastrointestinal tract is not known. However, Hobbs and Hansard (1952) have reported the amount of metal absorbed to be proportional to that presented for absorption.

Lassiter (1966) reported that manganese was poorly absorbed from the gut, and that net manganese absorption may be affected by dietary fat levels.

Britton and Cotzias (1966) proposed a variable excretion rather than a variable absorption to regulate the concentration of manganese in animal tissues. They further suggest the existence of an obligatory loss for the metal.

Manganese has been reported to be excreted primarily in the feces, with less than 1 percent of the intake in the urine (Gamble et al., 1971). This finding was substantiated by Watson et al. (1971). Cotzias (1962) suggested the gastrointestinal contents to be both the source and the sewer for manganese. This makes it difficult to determine between the ions being absorbed and those being excreted.

Excretion of manganese via the bile was investigated by Bertinchamps and Cotzias (1958). They reported that concentration in the bile paralleled that of the blood by a ratio of 10:1. Papavasiliou, Miller and Cotzias (1966) suggested that bile formation constitutes the main regulatory route of manganese under ordinary conditions, but under conditions of manganese overloading, auxiliary gastrointestinal routes participate.
Cotzias (1962) observed the excretion of manganese to be influenced by food intake, drugs and body load of stable manganese.

Lassiter, Morton and Miller (1970) reported that calcium plays an important role in manganese metabolism other than by affecting absorption, and suggested that dietary calcium can have a great effect upon the retention of absorbed manganese.

**Effects of excess dietary manganese.** Thomas (1970) reported that levels of 50 to over 4,000 ppm manganese in the diet of rats, dogs, swine and ruminants have caused reduced hemoglobin, growth depression, abnormal bones and other symptoms. In cows fed 5,000 ppm manganese the rumen bacterial population changed, and less propionic acid was produced (Cunningham, Wise and Barrick, 1966).

**Interrelationships with other nutrients.** Thomas (1970) has reviewed the interrelationships with manganese, and has reported that high levels of manganese shift copper deposits within the body. He further suggested interactions with organic molecules such as choline, inositol, thiamine, aureomycin, phenothiazine and hydralazine. In some conditions, increased dietary manganese has increased body thiamine and vice versa (Thomas, 1970). High thiamine levels that cause low reproductive rates can be counteracted by increasing manganese intake. Manganese may interfere with carotene conversion or increased vitamin utilization (Robinson et al., 1960).

Manganese interacts with calcium and phosphorous within the gastrointestinal tract (Thomas, 1970). Gallup, Nelson, and Darlow (1952) reported that all levels of increased manganese intake caused
increased phosphorus excretion in the feces with steer calves. Watson et al. (1971) indicated that a high-manganese diet resulted in decreased absorption of iron and phosphorus in lambs.

Hansard et al. (1960) reported that high dietary manganese depressed iron absorption in the rat. They further reported that excess dietary manganese added to a low phosphorus diet drastically reduced iron utilization by the red blood cells, and progressively decreased tissue iron concentration in the liver, spleen, kidney and femur. Diez-Ewald, Weintraub and Crosby (1968) indicated that iron deficient rats absorbed more manganese than control rats; iron loaded rats absorbed less manganese than the controls.

Gubler et al. (1954) reported that the administration of large amounts of manganese to rats was associated with an increase in plasma copper and a decrease in kidney copper. These investigators proposed that manganese may form a complex with copper which makes it unavailable, or in some manner blocks the action of copper containing enzymes.

Cotzias (1958) indicated that manganese deficiency in chicks can be aggravated by feeding high phosphorus or high calcium diets. He further reported that large amounts of dietary calcium or iron fed chicks may cause perosis, even in the presence of adequate manganese.

Maynard and Loosli (1969) observed the high-calcium and high-phosphorus rations normally fed poultry to be a contributing cause of perosis. They also reported that choline and other B vitamins may be involved.

Placental transfer. The presence of manganese in the newborn pig was reported by Newland and Davis (1961). Therefore, placental
transfer of this mineral was evidenced. Rojas, Dyer and Cassatt (1966) studied the effect of the stage of pregnancy upon manganese transfer in rats. They found that radio-manganese was transferred to the fetus with a gradual increase in uptake which reached a maximum at the fifteenth day of pregnancy. These investigators further reported a moderate uptake of radio-manganese by the mammary gland, and subsequent transfer to the milk. They further reported a high ovarian affinity for manganese which suggested a role for this element in estrual-ovarian activity.

Reynolds (1949) indicated that the quantity of most materials traversing the placenta increases with the progress of gestation. Rate of transfer of a physiologic substance paralleled relative growth rate of the fetus. The fewer the layers of tissues intervening between circulations, the greater the rate of transfer of a substance across a unit weight of placenta.

Terry, Terry and Davies (1960) suggested that the increased uptake of radiozinc observed in the rabbit fetus might be the result of the maturation of some fetal acceptor organ, or an increased efficiency of the placental transport mechanism.

Bothwell et al. (1958) reported the placental transport of iron to reflect both fetal demands and the state of iron metabolism in the mother.

Flexnor and Pohl (1941) observed that the blood flow through the pregnant uterus of the rabbit about doubles during the last half of pregnancy, which might affect rate and amount of placental transfer.

Mansard (1965) indicated that absorbed minerals pass freely from
the fetus at all stages of gestation, with the quantity and rate increasing with weight and age to parturition. This increase in transmission was suggested to be due to (1) variations in molecular size, (2) exchange rate, (3) concentration gradients, (4) anion-cation properties, and (5) hormone effect upon membrane permeability.

Newland and Davis (1961) fed rations of natural feedstuffs containing high (100 ppm) and low (6 ppm) levels of manganese to sows at various stages of gestation, and used radio-manganese to study fetal development and placental transfer. They found no difference in fetal weights from sows on the two diets, nor did it appear that the level of dietary manganese affected 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.

Gamble et al. (1971) found that fetal mass appeared to be a major factor affecting total manganese transfer in gilts. They also observed that 168 hours post-dosing with radio-manganese 25.7% of the retained $^{54}$Mn was transferred to the conception products, 87% of which was in the developing 110-day old fetuses. The total amount of elemental manganese transferred to a 5,000 gm sheep fetus (135 days of age) was 2.2 mg.
CHAPTER III

PROCEDURE WITH HEIFERS

Non-pregnant and third-trimester pregnant Hereford heifers (Table I) were utilized in this experiment to study the kinetics of manganese transport across the placental membranes, and the subsequent maternal-fetal manganese distribution and utilization. Placental transfer of manganese as a function of time after dose administration of radio-manganese was studied in six third-trimester gravid yearling heifers. Three non-pregnant heifers were used as controls.

The heifers were bred and the breeding data were recorded. Prior to the balance trial, pregnant and control heifers consumed pasture and corn silage of unknown composition. During the experimental period all heifers were placed into individual metabolism units equipped for quantitative separate collection of urine and feces (Hansard, 1951a). Daily feed intake of the heifers was measured for calculation of total manganese and radio-manganese balance. The heifers were fed a cottonseed meal, cracked corn and ground hay ration containing 13.0 ppm manganese, 70.0 ppm iron, 6.0 ppm copper, 4.0 mg/g calcium and 2.4 mg/g magnesium. Dosing was oral or intravenous with a single tracer level of carrier-free $^{54}$MnCl for blood-balance studies (Hansard, 1951b) and subsequent sacrifice for tissue distribution, and for placental manganese transfer at specified time intervals after dose administration. Total and radio-manganese balances at 37, 120, 144 and 168 hours were conducted. Excreta were quantitatively collected, weighed and mixed ashed for 24 hours at 600°C. Suitable aliquots of ashed samples were taken, made to
### TABLE I. SUMMARY OF HEREFORD HEIFERS USED, $^{54}\text{Mn}$ DOSING AND SACRIFICE SCHEDULE AND BALANCE RECORDS

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Wt. (kg)</th>
<th>$^{54}\text{Mn}$ Dose</th>
<th>Sacrifice HAD $^c$</th>
<th>Days Pregnant</th>
<th>Fetus Wt. (kg)</th>
<th>Sex</th>
<th>Dose MC $^d$</th>
<th>Total Mn (mg) $^e$</th>
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<tr>
<td>2319</td>
<td>232</td>
<td>OS</td>
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<td>IV</td>
<td>144</td>
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<td>IV</td>
<td>168</td>
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<td>168</td>
<td>275</td>
<td>26.8</td>
<td>M</td>
<td>560</td>
<td>283</td>
</tr>
</tbody>
</table>

$^a$ Third-trimester pregnant and non-pregnant yearling Hereford heifers.

$^b$ Method of $^{54}\text{Mn}$ dose administration.

$^c$ Hours after dose administration that heifers were killed.

$^d$ Microcurie dose of carrier-free $^{54}\text{MnCl}$.

$^e$ Milligrams for the period indicated in note $c$. 

volume with 2 to 5 ml 6N-HCl, transferred to graduated tubes, and diluted to appropriate volume with deionized water for radiochemical and total manganese analyses. Periodic blood samples were taken using a needle attached to a heparinized syringe. Heifer total blood volume was calculated as 6.5% total animal weight (Hansard et al., 1953). Radioactivity was measured in a Nuclear Chicago 1085 automatic gamma well-counter. The blood samples were centrifuged and the plasma removed for manganese analyses. From these data plasma and RBC $^{54}$Mn clearances were calculated.

Total-plasma whole-blood manganese measurements were made from pooled samples after drying in an oven at 100°C for 12 hours, and ashing for 24 hours at 600°C. All samples were taken into solution with 2 to 3 ml 6N-HCl and diluted with deionized water to a minimum volume. Aliquots were taken for manganese determinations with a Perkin Elmer 303 atomic absorption spectrophotometer by conventional procedures (Anonymous, 1964).

At sacrifice, the following maternal-fetal tissues, organs and fluids were taken: whole blood, plasma, bile, brain, pituitary, thyroid, heart, aorta, kidney, liver, spleen, adrenal, gastrocnemius muscle, skin, mandible, sternum, rib shaft, rib end, femur shaft and femur end. Placentae were removed intact for weighing and sampling of fluids, membranes and fetuses. Since whole-body fetal analyses were not practical due to the size of the fetuses, fetal manganese and radio-manganese values were calculated on a 26.4% bone and 41.2% flesh basis (Hedrick, 1968). Samples were placed in tared flat-bottom counting tubes for weighing and radio-manganese measurements.
Additional samples were taken in tared crucibles for subsequent ashing and chemical analyses. Samples were ashed at 24 hours at 600°C. The ash was taken into solution using 2 to 3 ml 6N-HCl, transferred to graduated tubes and made to volume with deionized water for mineral analyses, using a 303 Perkin Elmer spectrophotometer. Manganese concentrations (mg) were calculated by the following formula:

\[
\frac{(\text{dilution}) \times (\text{sample absorption}) \times (\text{concentration of standard})}{(\text{fresh weight}) \times (\text{standard absorption})}
\]

For convenience of data presentation and for comparative purposes, all maternal tissue and non-pregnant heifer tissue values were corrected to 227 kg bodyweight and balance data were employed to correct radio-manganese values to that absorbed and retained by the dam (Hansard, 1970).

Since this study was concerned with a basic understanding of placental transfer and maternal-fetal utilization of manganese, standard deviation was the only statistical analysis used.

Because tissue and organ distribution levels were consistently low in the 168 hour pregnant heifer, these values were discarded.
CHAPTER IV

RESULTS AND DISCUSSION

Circulating manganese in the bovine. $^{54}\text{Mn}$ characteristic curve indicating the disappearance of injected radio-manganese from the blood of third-trimester heifers is shown in Figure 1. A very sharp decline in the curve was observed immediately after dose administration indicating that radio-manganese was being removed from the circulating blood. Blood concentration levels dropped from 14% of the total dose at 3 hours after dosing to 1.8% of the total dose at 144 hours after dose administration. Clearances indicated that red cell and plasma levels of $^{54}\text{Mn}$ reached equilibrium 38 hours post-dosing. Other data indicated the blood partition of radio-manganese to be approximately 50% in plasma and 50% in red blood cells. Stable manganese levels of 0.10 ppm were observed in samples of whole blood, and plasma contained 0.07 ppm manganese.

Absorption and excretion. The accumulative fecal excretion of $^{54}\text{Mn}$ as a function of time after intravenous dose administration is shown in Figure 2. Approximately 18% of an intravenous dose had been excreted 144 hours after dosing. Manganese was excreted almost exclusively in the feces with less than 1% of the total excretion in the urine. Bile, which contained levels of radio-manganese 10 times greater than the blood, appeared to be a major excretory pathway.

Balance data showed that four out of seven heifers were in positive manganese balance. The remaining three animals were in
Figure 1. Percent $^{54}$Mn in the total circulating bovine blood as a function of time after intravenous dose administration.
Figure 2. Accumulative fecal excretion of $^{54}$Mn by the bovine as a function of time after intravenous dosing.
negative manganese balance. This was probably due to some failure to adjust to the experimental ration and scouring in the test heifers.

**Tissue distribution of manganese in the bovine.** The percent of radio-manganese per gram \(\times 10^{-4}\), manganese and specific activity in maternal tissues and organs of heifers as functions of time after dosing are shown in Table II. Radio-manganese concentration was highest in liver, kidney and placenta at 37 hours post-dosing. Tissue levels subsequently declined steadily thereafter. Tissue radio-manganese levels in non-pregnant heifers were similar to those of pregnant heifers.

The endocrine glands (adrenal, pancreas and thyroid) accumulated radio-manganese rapidly and maintained high concentrations through 144 hours after dosing. Of the endocrine glands, the pituitary behaved exceptionally, showing a steady and gradual increase in radio-manganese concentration to 144 hours. Brain apparently concentrated radio-manganese similarly to the pituitary; suggesting the blood-brain barrier to be permeable to manganese (Widdowson and Dickerson, 1964).

High levels of total manganese were found in rib, pituitary, femur, pancreas and liver, in that order. Muscle, bone marrow and plasma contained low manganese levels.

**Total organ manganese accretion.** Data showed that in cows, 144 hours post-dosing, the liver contained 79% of the radio-manganese contained in the major organs (Table III). Liver reached a peak in total radio-manganese 37 hours post-dosing, when it contained 81.49% of the total retained dose. At 144 hours it had dropped to 45% of the
<table>
<thead>
<tr>
<th>Tissue</th>
<th>μg/gm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3</th>
<th>37</th>
<th>120</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood</td>
<td>0.10 ± 0.02</td>
<td>7.01   70.1</td>
<td>1.04  10.4</td>
<td>0.85  8.5</td>
<td>0.91  9.1</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.07 ± 0.03</td>
<td>36.14  516.28</td>
<td>13.86  198.0</td>
<td>0.28  4.0</td>
<td>0.10  1.43</td>
</tr>
<tr>
<td>Bile</td>
<td>0.25 ± 0.08</td>
<td>65.46  261.84</td>
<td>11.41  45.64</td>
<td>7.90  31.6</td>
<td>10.01 40.04</td>
</tr>
<tr>
<td>Pituitary</td>
<td>1.96 ± 0.44</td>
<td>4.26   2.17</td>
<td>6.22  3.17</td>
<td>8.35  4.26</td>
<td>12.46 6.36</td>
</tr>
<tr>
<td>Brain</td>
<td>0.33 ± 0.09</td>
<td>0.24   0.73</td>
<td>1.11  3.36</td>
<td>1.51  4.58</td>
<td>2.24  6.80</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.27 ± 0.05</td>
<td>123.73 458.26</td>
<td>50.80 188.15</td>
<td>23.19 85.89</td>
<td>52.36 193.92</td>
</tr>
<tr>
<td>Heart</td>
<td>0.34 ± 0.10</td>
<td>20.32  59.76</td>
<td>8.88  26.12</td>
<td>11.34 33.35</td>
<td>53.17 156.38</td>
</tr>
<tr>
<td>Liver</td>
<td>1.43 ± 0.11</td>
<td>235.86 164.94</td>
<td>250.61 175.25</td>
<td>160.95 112.55</td>
<td>138.52 96.87</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.18</td>
<td>125.68 184.82</td>
<td>135.63 199.45</td>
<td>84.83 124.75</td>
<td>50.14 73.74</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.23 ± 0.47</td>
<td>111.28 90.47</td>
<td>94.73 77.02</td>
<td>93.75 76.22</td>
<td>159.74 129.87</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.50 ± 0.16</td>
<td>218.16 145.44</td>
<td>217.30 144.87</td>
<td>207.91 138.61</td>
<td>316.07 210.71</td>
</tr>
<tr>
<td>Tissue</td>
<td>ug/gm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>S.A.c</td>
<td>37</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>---</td>
<td>-------------</td>
<td>-------</td>
<td>----</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.33 ± 0.08</td>
<td></td>
<td>55.77</td>
<td>169.0</td>
<td>54.90</td>
</tr>
<tr>
<td>Ovaries</td>
<td>0.58 ± 0.19</td>
<td></td>
<td>11.09</td>
<td>19.12</td>
<td>9.83</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.17 ± 0.06</td>
<td></td>
<td>2.63</td>
<td>15.47</td>
<td>1.05</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.19 ± 0.08</td>
<td></td>
<td>---</td>
<td>---</td>
<td>1.01</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>0.08 ± 0.03</td>
<td></td>
<td>0.78</td>
<td>9.77</td>
<td>---</td>
</tr>
<tr>
<td>Sternum</td>
<td>1.50 ± 0.37</td>
<td></td>
<td>8.95</td>
<td>5.97</td>
<td>5.62</td>
</tr>
<tr>
<td>Mandible</td>
<td>1.60 ± 0.23</td>
<td></td>
<td>0.83</td>
<td>0.52</td>
<td>1.38</td>
</tr>
<tr>
<td>Rib Shaft</td>
<td>2.57 ± 1.02</td>
<td></td>
<td>1.16</td>
<td>0.45</td>
<td>7.56</td>
</tr>
<tr>
<td>Rib Epiphysis</td>
<td>2.10 ± 0.67</td>
<td></td>
<td>8.52</td>
<td>4.06</td>
<td>7.22</td>
</tr>
<tr>
<td>Femur Shaft</td>
<td>1.87 ± 0.46</td>
<td></td>
<td>0.35</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>Femur Epiphysis</td>
<td>1.70 ± 0.48</td>
<td></td>
<td>---</td>
<td>---</td>
<td>0.77</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>0.40 ± 0.07</td>
<td></td>
<td>65.26</td>
<td>163.15</td>
<td>25.79</td>
</tr>
</tbody>
</table>
TABLE II (continued)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>µg/gm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hours After Dose Administration</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 %&lt;sup&gt;b&lt;/sup&gt; S.A.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37 %  S.A.</td>
<td>120 %  S.A.</td>
<td>144 %  S.A.</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>0.22 ± 0.07</td>
<td>2.60 11.82</td>
<td>4.67 21.23</td>
<td>3.41 15.5</td>
<td>1.51 6.86</td>
<td></td>
</tr>
<tr>
<td>Placental Fluids</td>
<td>0.14 ± 0.09</td>
<td>0.11 0.78</td>
<td>0.01 0.07</td>
<td>0.01 0.07</td>
<td>0.01 0.05</td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.66 ± ----</td>
<td>0.04 0.06</td>
<td>0.37 0.56</td>
<td>1.07 1.62</td>
<td>1.96 2.97</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Stable manganese--mean ± standard deviation.

<sup>b</sup>Percent dose $^{54}\text{Mn/gm} \times 10^{-4}$, corrected to 227 kg maternal weight.

<sup>c</sup>Specific Activity Calculated: $\frac{\% {^{54}\text{Mn/gm}} \times 10^{-4}}{\mu g \text{ Mn/gm}}$, fresh basis.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total %&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>85.22</td>
<td>100.00</td>
<td>89.43</td>
<td>100.00</td>
<td>59.06</td>
<td>100.00</td>
<td>56.97</td>
<td>100.00</td>
</tr>
<tr>
<td>Liver</td>
<td>76.00</td>
<td>89.18</td>
<td>81.49</td>
<td>91.12</td>
<td>52.34</td>
<td>88.62</td>
<td>45.00</td>
<td>78.99</td>
</tr>
<tr>
<td>Kidney (1)</td>
<td>4.40</td>
<td>5.17</td>
<td>4.74</td>
<td>5.30</td>
<td>2.97</td>
<td>5.03</td>
<td>1.75</td>
<td>3.07</td>
</tr>
<tr>
<td>Heart</td>
<td>2.81</td>
<td>3.29</td>
<td>1.23</td>
<td>1.38</td>
<td>1.57</td>
<td>2.66</td>
<td>7.36</td>
<td>12.92</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.92</td>
<td>2.25</td>
<td>1.89</td>
<td>2.11</td>
<td>2.11</td>
<td>3.57</td>
<td>2.73</td>
<td>4.79</td>
</tr>
<tr>
<td>Adrenal (1)</td>
<td>0.09</td>
<td>0.11</td>
<td>0.08</td>
<td>0.09</td>
<td>0.07</td>
<td>0.12</td>
<td>0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.0005</td>
<td>0.0006</td>
<td>0.0007</td>
<td>0.0008</td>
<td>0.0009</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent of the dose in selected total organs.

<sup>b</sup>Total percent $^{54}$Mn dose contained in each organ, corrected to 227 kg cow.
retained dose. The remaining major organs (kidney, heart and spleen) contained 10.72% of the retained dose at 3 hours post-dosing and 21.01% of the retained dose at 144 hours post-dosing.

**Placental transfer of manganese.** Radio-manganese, specific activity and total manganese values in the placental complex of third-trimester cows are summarized in Table IV. Radio-manganese levels reached a maximum in the placenta 37 hours post-dosing and subsequently declined through 144 hours. The placenta contained 92.10% (Table V) of the radio-manganese in the total placental complex at 3 hours after dosing. At 144 hours after dosing the placenta contained only 20.04% of the radio-manganese in the total complex. Placental fluid radio-manganese levels declined steadily from 0.09% of the retained dose at 3 hours post-dosing to 0.007% of the dose at 144 hours. At 3 hours post-dosing 3.40% of the total radio-manganese in the placental complex was contained in the placental fluids. At 144 hours only 0.10% of the radio-manganese in the complex was found in the fluids. The fetus showed a steady uptake of radio-manganese from 3 hours to 144 hours after dosing. The fetus contained 4.52% of the radio-manganese in the total complex at 3 hours post-dosing and 79.86% at 144 hours.

These relative amounts of retained radio-manganese in the placental complex of third-trimester cattle are demonstrated graphically in Figure 3. Soon after intravenous dosing of pregnant heifers with radio-manganese the placenta contained the majority of the retained dose in the placental complex. However, 144 hours subsequent to dosing, the fetus contained the largest percentage of the radio-manganese
### TABLE IV. SUMMARY OF MANGANESE, TOTAL RADIO-MANGANESE, AND SPECIFIC ACTIVITY IN THE THIRD-TRIMESTER BOVINE PLACENTAL COMPLEX

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Manganese mg</th>
<th>3</th>
<th>% Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S.A.&lt;sup&gt;b&lt;/sup&gt;</th>
<th>37</th>
<th>% Dose</th>
<th>S.A.</th>
<th>120</th>
<th>% Dose</th>
<th>S.A.</th>
<th>144</th>
<th>% Dose</th>
<th>S.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Complex</td>
<td>22.40</td>
<td>2.65</td>
<td>1.16</td>
<td></td>
<td>5.46</td>
<td>2.39</td>
<td></td>
<td>6.29</td>
<td>2.75</td>
<td></td>
<td>7.09</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>2.10</td>
<td>2.44</td>
<td>11.82</td>
<td></td>
<td>4.38</td>
<td>21.23</td>
<td></td>
<td>3.19</td>
<td>15.50</td>
<td></td>
<td>1.42</td>
<td>6.86</td>
<td></td>
</tr>
<tr>
<td>Placental Fluids</td>
<td>1.20</td>
<td>0.09</td>
<td>0.78</td>
<td></td>
<td>0.008</td>
<td>0.07</td>
<td></td>
<td>0.008</td>
<td>0.07</td>
<td></td>
<td>0.007</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Fetus (28.8 kg)</td>
<td>19.10</td>
<td>0.12</td>
<td>0.06</td>
<td></td>
<td>1.07</td>
<td>0.56</td>
<td></td>
<td>3.09</td>
<td>1.62</td>
<td></td>
<td>5.66</td>
<td>2.97</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent retained $^{54}$Mn dose, corrected to 227 kg cow.

<sup>b</sup>Specific Activity Calculated: $\frac{\% \; ^{54} \text{Mn/gm} \times 10^{-4}}{\mu \text{g Mn/gm}}$, fresh basis.
TABLE V. PARTITION OF RETAINED RADIO-MANGANESE IN THE PLACENTAL COMPLEX OF THIRD-TRIMESTER HEIFERS

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>37</td>
<td>120</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Complex</td>
<td>2.65</td>
<td>100.00</td>
<td>5.46</td>
<td>100.00</td>
<td>6.29</td>
<td>100.00</td>
<td>7.09</td>
<td>100.00</td>
</tr>
<tr>
<td>Placenta</td>
<td>2.44</td>
<td>92.08</td>
<td>4.38</td>
<td>80.25</td>
<td>3.19</td>
<td>50.73</td>
<td>1.42</td>
<td>20.04</td>
</tr>
<tr>
<td>Placental Fluids</td>
<td>0.09</td>
<td>3.40</td>
<td>0.008</td>
<td>0.15</td>
<td>0.008</td>
<td>0.13</td>
<td>0.007</td>
<td>0.10</td>
</tr>
<tr>
<td>Fetus (28.8 kg)</td>
<td>0.12</td>
<td>4.52</td>
<td>1.07</td>
<td>19.60</td>
<td>3.09</td>
<td>49.14</td>
<td>5.66</td>
<td>79.86</td>
</tr>
</tbody>
</table>

\(^a\text{Percent retained }^{54}\text{Mn dose, corrected to 227 kg cow.}\)

\(^b\text{Percent }^{54}\text{Mn in total placenta, fluids and fetus.}\)
Figure 3. Partition of retained radio-manganese in the placental complex of third-trimester heifers.
in the products of conception. This trend indicated a significant placental transfer of manganese. Data indicated placental fluids to play only a minor role in the maternal-fetal transfer of manganese.

Radio-manganese accumulated in the placenta at the rate of 0.12% of the total retained dose per hour until 37 hours after dosing. Subsequently, it disappeared from the placenta at the rate 0.03% of total retained dose per hour through 144 hours post-dosing. Radio-manganese levels in the placental fluids peaked 3 hours after dosing, and subsequently disappeared at the rate of 0.0006% of the total retained dose per hour. Fetal accretion of radio-manganese was 0.04% of the total retained dose per hour until 3 hours after dosing, 0.03% of the dose per hour until 37 hours post-dosing, 0.02% of the dose per hour through 120 hours and 0.10% of the dose per hour from 120 to 144 hours. Radio-manganese accretion by the third-trimester total placental complex occurred at the rate of 0.05% of the total retained dose per hour through 144 hours after dosing.

Specific activity values indicated the placenta to have the highest manganese turnover rate of the constituents of the placental complex. The total placental complex of third-trimester cattle contained 22.4 mg manganese, 1.20 mg were found in the placental fluids, 2.10 mg were contained in the placenta and 19.10 mg (85%) were found in the fetus.

**Fetal manganese tissue distribution.** Radio-manganese, specific activity and total manganese values of third-trimester cattle, fetal tissues and organs are summarized in Table VI. All fetal tissue and
TABLE VI. SUMMARY OF MANGANESE, RADIO-MANGANESE, AND SPECIFIC ACTIVITY VALUES OF THIRD-TRIMESTER BOVINE FETAL ORGANS AND TISSUES AS A FUNCTION OF TIME AFTER INTRAVENOUS DOSE ADMINISTRATION

<table>
<thead>
<tr>
<th>Tissue</th>
<th>μg/gm$^a$</th>
<th>Hours After Dose Administration</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>37</td>
<td>120</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S.A.</td>
<td>S.A.</td>
<td>S.A.</td>
<td>S.A.</td>
</tr>
<tr>
<td>Pituitary</td>
<td>4.75 ± 2.26</td>
<td>----</td>
<td>5.07</td>
<td>1.49</td>
<td>5.52</td>
<td>1.62</td>
</tr>
<tr>
<td>Brain</td>
<td>0.52 ± 0.19</td>
<td>0.02</td>
<td>0.05</td>
<td>0.09</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.96 ± 0.49</td>
<td>0.02</td>
<td>6.43</td>
<td>3.28</td>
<td>7.10</td>
<td>3.62</td>
</tr>
<tr>
<td>Heart</td>
<td>0.38 ± 0.21</td>
<td>0.04</td>
<td>0.21</td>
<td>0.55</td>
<td>2.40</td>
<td>6.31</td>
</tr>
<tr>
<td>Liver</td>
<td>2.92 ± 1.41</td>
<td>0.13</td>
<td>10.02</td>
<td>3.43</td>
<td>30.17</td>
<td>10.33</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.33 ± 0.12</td>
<td>0.04</td>
<td>0.62</td>
<td>0.67</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.70 ± 0.86</td>
<td>0.09</td>
<td>1.02</td>
<td>0.60</td>
<td>6.46</td>
<td>3.80</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.26 ± 0.41</td>
<td>0.04</td>
<td>0.27</td>
<td>0.12</td>
<td>9.43</td>
<td>4.17</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.37 ± 0.10</td>
<td>0.01</td>
<td>1.00</td>
<td>2.70</td>
<td>1.22</td>
<td>3.29</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.18 ± 0.04</td>
<td>0.02</td>
<td>0.21</td>
<td>1.16</td>
<td>0.52</td>
<td>2.88</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.30 ± 0.14</td>
<td>0.88</td>
<td>0.72</td>
<td>2.40</td>
<td>0.65</td>
<td>2.16</td>
</tr>
<tr>
<td>Sternum</td>
<td>1.30 ± 0.61</td>
<td>0.12</td>
<td>0.23</td>
<td>0.18</td>
<td>1.57</td>
<td>1.21</td>
</tr>
<tr>
<td>Tissue</td>
<td>μg/gm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>S.A.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37</td>
<td>S.A.</td>
<td>120</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>---------</td>
<td>------------------</td>
<td>---------</td>
<td>------</td>
<td>----------</td>
</tr>
<tr>
<td>Mandible</td>
<td>1.90 ± 0.71</td>
<td>0.18</td>
<td>0.10</td>
<td>0.39</td>
<td>0.20</td>
<td>----</td>
</tr>
<tr>
<td>Rib Shaft</td>
<td>2.22 ± 0.95</td>
<td>0.11</td>
<td>0.05</td>
<td>0.32</td>
<td>0.14</td>
<td>0.57</td>
</tr>
<tr>
<td>Rib Epiphysis</td>
<td>3.10 ± 0.86</td>
<td>0.24</td>
<td>0.08</td>
<td>0.47</td>
<td>0.15</td>
<td>2.56</td>
</tr>
<tr>
<td>Femur Shaft</td>
<td>2.22 ± 0.86</td>
<td>0.10</td>
<td>0.04</td>
<td>0.25</td>
<td>0.11</td>
<td>0.32</td>
</tr>
<tr>
<td>Femur Epiphysis</td>
<td>0.56 ± 0.28</td>
<td>0.02</td>
<td>0.04</td>
<td>0.13</td>
<td>0.23</td>
<td>1.67</td>
</tr>
</tbody>
</table>

<sup>a</sup>Stable manganese--mean ± standard deviation.

<sup>b</sup>Percent dose $^{54}\text{Mn/gm} \times 10^{-4}$.

<sup>c</sup>Specific Activity Calculated: $\% \frac{^{54}\text{Mn/gm} \times 10^{-4}}{\mu g \text{Mn/gm}}$, fresh basis.
organs showed a steady increase in radio-manganese concentration through 144 hours post-dosing.

Fetal liver contained high levels of radio-manganese at all sampling times after dose administration. Fetal endocrine glands (adrenal, pancreas, pituitary and thyroid) accumulated radio-manganese rapidly between 3 and 37 hours after dosing. Levels in the endocrine glands continued to increase through 144 hours. Fetal brain accumulated radio-manganese steadily but never contained as much as 1.0 x 10^{-4} percent dose per gram of fresh tissue. Fetal bone also demonstrated a gradual buildup of radio-manganese concentration. Generally, epiphyseal bone contained higher levels than bone shaft. Fetal muscle, like maternal muscle, contained low levels of radio-manganese.

Specific activity values were highest in fetal liver and, in general, were higher in soft tissue than in bone. These data indicated soft tissue to have a greater manganese turnover rate than bone. Fetal liver, thyroid and pituitary contained high concentrations of total manganese. Fetal pituitary contained roughly twice as much total manganese as did maternal pituitary. The large standard deviation on the fetal pituitary concentration suggested some error in this value, however. In general, fetal bone contained a higher concentration of total manganese than did soft tissue.

**Partition of manganese in fetal organs.** Radio-manganese levels in third-trimester fetal organs as a function of time after dose administration is shown in Table VII. Fetal liver contained 5.83% of the radio-manganese in major fetal organs 3 hours after dose
<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total %&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
<th>% Dose</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus, total</td>
<td>0.12 100.00</td>
<td>1.07 100.00</td>
<td>3.09 100.00</td>
<td>5.66 100.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.007 5.83</td>
<td>0.58 54.20</td>
<td>1.77 57.28</td>
<td>3.03 53.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.0009 0.75</td>
<td>0.004 0.37</td>
<td>0.05 1.62</td>
<td>0.03 0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney (2)</td>
<td>0.0004 0.33</td>
<td>0.006 0.56</td>
<td>0.006 0.19</td>
<td>0.06 1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal (2)</td>
<td>0.00004 0.03</td>
<td>0.0006 0.06</td>
<td>0.004 0.13</td>
<td>0.012 0.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.00006 0.05</td>
<td>0.006 0.56</td>
<td>0.007 0.23</td>
<td>0.04 0.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td>---- --</td>
<td>0.0002 0.02</td>
<td>0.0003 0.01</td>
<td>0.0004 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent retained $^{54}$Mn dose in total fetal organs.

<sup>b</sup>Percent of the total fetal dose (100%) in total fetal organs.
administration. After 144 hours the liver contained 53.53% of that radio-manganese deposited in the major fetal organs. Fetal heart, kidneys, adrenals, spleen and pituitary contained only 0.001% of the radio-manganese in the major fetal organs at 3 hours post-dosing. These organs subsequently contained 2.46% of the radio-manganese in the major fetal organs at 144 hours after dose administration.
Although manganese has been demonstrated to be required for normal life processes, little information is available on the placental transfer and maternal-fetal utilization of this mineral in the bovine. Nine pregnant and three non-pregnant heifers were used in this study. They were dosed with a tracer level of radio-manganese for blood balance and subsequent placental transfer and maternal-fetal manganese utilization studies. Results of this study suggested total blood radio-manganese levels to drop from 14% of the administered dose at 3 hours to 1.8% after 144 hours. Blood clearance studies indicated that equilibrium between red cells and plasma was reached 38 hours post-dosing, and further data suggested that approximately 50% of the plasma manganese was protein bound. Eighteen percent of an intravenous $^{54}$Mn dose was excreted by pregnant heifers 144 hours after dosing. Bile appeared to be a major excretory pathway for manganese, and urine a minor excretory pathway. Liver served as the main metabolic pool for manganese in both the dam and fetus. Data suggested that heifers retained a calculated 1.16 mg manganese per day and deposited 82 μg (7.0% of that retained) in the total products of conception. Third-trimester bovine fetuses (272 days) contained 19.1 mg stable manganese; placenta, 2.10 mg and placental fluids 1.20 mg, for a total of 22.4 mg manganese in the total products of conception. Pregnant heifers deposited in the total products of conception a calculated 82 μg manganese per
day, suggesting that 82 μg manganese per day was needed to support the products of conception in the pregnant heifer.
LITERATURE CITED


VITA

The author was born March 13, 1948 in McMinnville, Tennessee, the only child of Doris and Preston Wanamaker. He attended public school in Warren County, Tennessee, and in 1966, upon graduation from McMinnville City High School, he enrolled in the College of Agriculture, the University of Tennessee, Knoxville.

At the University of Tennessee, he was a member of Alpha Zeta, Gamma Sigma Delta, Phi Kappa Phi and the Block and Bridle Club. He was awarded the B.S. degree with a major in Animal Husbandry in June, 1970. In September, 1970, he entered the Graduate School, University of Tennessee and began work toward an M.S. degree.