MANGANESE NUTRITION OF THE PIG

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ABSTRACT

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By

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Four experiments involving 104 pigs were conducted to study different aspects of manganese (Mn) nutrition in swine.

In the first experiment, a basal diet (16.2 ppm Mn) and basal diet supplemented with 10 ppm of Mn from $MnSO_4 \cdot H_2O$, $MnCO_3$ or MnO were compared for Mn availability to the growing pig. Growth rates were equal on all diets. Mn availability as measured by Mn balance data and tissue Mn concentrations indicated that Mn from the supplemented diets was more available than that from the basal diet. Mn retention, as a percent of intake, was higher on Mn from the supplemented diets than the basal diet. Regardless of dietary Mn source, over 90% of excreted Mn was recovered in the feces. Within the supplemented diets, Mn was essentially equally available to the growing pig. Hemoglobin, hematocrit, serum Mn and serum alkaline phosphatase did not differ significantly due to dietary treatment.

In the second experiment, flux patterns across the wall of the gastrointestine of Mn from different sources was studied. Net absorption of Mn from the basal diet was evident in two sections of the gut, the stomach and the cecum, whereas Mn from the supplemented diets was apparently absorbed in the stomach, cranial small intestine and cecum.

The net cecal absorption of Mn from the basal diet was higher than that of Mn from the supplemented diets. Net Mn secretion in the caudal small intestine and the rectum was much higher on the supplemented than on the basal diet, but this trend was reversed in the colon. The pH values of the gut contents from different sections of the tract were not significantly different between dietary treatments.

In the third experiment, two ratios of Ca to P, two levels of Ca and P and two levels of Mn were studied using a factorial feeding trial. Mn supplementation significantly increased heart Mn levels and significantly depressed rib Ca and Mg values. Mn supplementation did not affect serum Ca, inorganic P, Mg and alkaline phosphatase levels. Dietary Mn levels had no significant effect on rib and metacarpal physical measurements, breaking strength and related parameters. A 2 to 1 Ca to P ratio significantly ($P<0.05$) depressed rib Mn content. The increased levels of Ca and P supplementation significantly ($P<0.01$) increased rib, pancreas and serum Mn levels but significantly ($P<0.01$) depressed metacarpal Mn concentration. There was a signifiicnt ($P<0.01$ to $P<0.05$) interaction between levels of Ca and P and ratios of Ca to P on the levels of serum, liver and pancreas Mn, and on metacarpal Mn values. High levels of Ca and P in both ratios had a depressing effect on metacarpal Mn concentration. Feeding Mn along with Ca and P, in a 2 to 1 ratio, increased liver Mn. Metacarpal Mg was depressed when Ca and P were given in a 1 to 2 ratio.

The interaction between Ca and P levels and Mn levels was significant ($P<0.05$) with respect to rib ash content, Ca and P, metacarpal Mn and serum inorganic P. With lower Ca and P levels, Mn supplementation increased metacarpal Mn and serum inorganic P but depressed rib ash, Ca and P concentration. The significant effects of Mn supplementation on

rib Mg, metacarpal internal vertical diameter and heart and serum Mn disappeared when Ca and P supplements were also fed. The 3-way interaction between level of Ca and P, ratio of Ca to P and level of Mn was significant ($P<0.05$) relative to rib and serum Mn levels, pancreas dry matter and metacarpal Mg content and elasticity. With high or low Ca and P levels in a 2 to 1 ratio, Mn supplementation increased rib and serum Mn and pancreas dry matter but depressed metacarpal elasticity. With low Ca and P levels in a 1 to 2 ratio, Mn supplementation increased serum and metacarpal Mn, but high Ca and P levels in the same ratio depressed rib and serum Mn, metacarpal Mg and pancreas dry matter and increased metacarpal elasticity.

Mn supplementation produced more nearly normal histologic structure of the epiphysis than the basal diet, but animals on high Mn levels had significantly ($P<0.05$) less compact bone in the diaphysis. However, the thickness of the epiphyseal cartilagenous plate was not affected. There was a significant ($P<0.05$) interaction between Ca to P ratio and Mn level on the histology of the epiphysis. The interaction of diet Mn with Ca and P levels was significant ($P<0.05$) in relation to the thickness of the epiphyseal cartilagenous plate. These changes were not typical of rickets but were changes in which there was failure of production of compact bone in the region of the diaphysis. However, the deleterious effects on weight gain, feed efficiency and histology of bone of a low dietary P level (0.35%) from soybean meal were much more pronounced than the effects of excessive dietary levels of Ca and P or of an inverse Ca to P ratio, regardless of dietary Mn supplementation.

In the fourth experiment, the Mn requirements of the baby pig born of sows fed a low Mn diet were determined using three dietary Mn concentrations. Growth, Mn balance data and serum Mn concentration were used

as measures of sufficiency. Average daily gain, serum Mn, Mn retention, fecal Mn excretion and urinary Mn excretion as percent of intake were significantly ($P<0.01$ or $P<0.05$) different between dietary treatments. The average daily gain and feed efficiency were highest on 2.67 ppm Mn in the diet. Mn intake was highly correlated with serum Mn and serum alkaline phosphatase activity but not with average daily gain and feed efficiency. Mn retention as percent of intake had very high negative correlations with feed efficiency and urinary Mn excretion as percent of intake. Fecal and urinary Mn excretion as percent of intake was significantly higher on the basal diet (0.46 ppm Mn). There was a negative Mn retention on this diet. Serum Mg levels substantially declined in pigs on the basal diet 28 days after the start of the experiment. Average daily gain was positively related to Mn retention as percent of intake and negatively related to fecal Mn excretion as percent of intake. Mn retention and fecal Mn excretion, both as percent of intake, were much more highly correlated with growth rate than absolute Mn intake, excretion and retention and the serum parameters examined.

Based on all criteria examined, the dietary Mn requirements of the baby pig on a semipurified diet are probably between 3 and 6 ppm.

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Dedicated to

My Beloved Parents

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INTRODUCTION

Nutritional studies with manganese (Mn) were stimulated by the iscovery that two poultry diseases, perosis and chondrodystrophy, were aused by inadequate intakes of Mn and could be prevented by Mn sup- lementation. Since then Mn has been assigned a growing number of iological functions vitally important to the organism. This element s now associated with normal activation of enzymes and co-enzymes ssociated with feed utilization. Manganese plays an important role n liver function, bile production, energy metabolism, reproduction and roper skeletal development. The occurrence of Mn in all tissues and he small range of Mn concentrations indicate a role of this element n general cell metabolism as opposed to metabolic processes character- stic of particular tissues.

Although Mn is an indispensable micro-element of considerable ractical importance in animal feeding, its metabolism in swine has ad limited research, with little attention to its availability, site f absorption and requirement at different stages of development. anganese interactions with other elements and vitamins, shown in other pecies, have not been studied in swine.

Manganese compounds have different chemical and physical properties hich are important in determining their availability to animals. Since he cost of Mn compounds varies widely, it is important not only to efine accurately the nutritive requirement of Mn for swine at

ferent stages of their development but also to define the less ensive but equally effective ingredients to use in the mineral mix.

The Mn flux pattern across the gastrointestinal mucosa in swine not been extensively studied. Such influx-efflux studies can vide information on the extent of absorption of the mineral and secretion, in various sections of the digestive tract.

Manganese interactions with Ca and P have been studied in some ail in poultry and laboratory animals, but most of those findings e not been examined in swine. Interactions of this element with er elements, such as copper and iron, and vitamins have not been died in swine. Verification of any Mn interactions in swine is ortant not only to the understanding of the utilization of Mn but o of other elements in the presence of low or high dietary levels Mn.

The National Research Council has suggested 20 ppm Mn as the uirement for a young growing pig. Manganese requirements that have reported in the literature vary widely. There is a definite need lefine accurately the Mn requirement of the baby pig. Modern manage- practices, increased knowledge of other nutrient needs and ever- ging breeding programs in the swine industry all call for reevalua- of Mn requirement data. The development of more sensitive and ific analytical methods also necessitates this reexamination.

The experiments to be reported in this dissertation were designed tudy the following aspects:

1. The availability of Mn from different Mn sources for the ing pig.

2. The flux pattern of Mn across the gastrointestinal tract in the pig.

3. The effect of high level supplementation of Ca and P and a Ca-P ratio less than 1.0 on Mn utilization.

4. The Mn requirement of the baby pig born of a Mn-deficient sow.

LITERATURE REVIEW

Manganese: An Essential Trace Element

1. Growth and Development

The first experiments with rats and mice attempting to demonate the essentiality of Mn using purified diets were suggestive but conclusive. In 1931 Kemmerer *et al.* found better growth rates of ale mice when 0.1 mg of Mn was added daily to a milk diet containing tional Fe and Cu. Since that time many workers have demonstrated essentiality of Mn for optimal growth in rats. Boyer *et al.* (1942) rted that young rats from Mn-deficient dams showed a markedly er growth rate and poorer feed utilization than similar rats on the diet supplemented with Mn. Randoin (1944) found similar subnormal th, followed by a rapid decline and death. Holtkamp *et al.* (1950) ed that a subcutaneous injection of colloidal Mn improved growth . Supplements of Mn improved growth rate and feed efficiency in ea pigs and rabbits (Everson, 1970; Ellis *et al.*, 1947). Rabbits a Mn-free diet showed signs of anorexia (Rudra, 1944a) and, when on this diet for a long time, the animals ceased to grow normally died. Postmortem examination revealed hemorrhages of the lungs, and intestinal capillaries.

Wachtel *et al.* (1943) reported that Mn deficiency in rats caused bone formation and Frost *et al.* (1959) observed delayed skeletal ation in rats on Mn-deficient diets. O'Dell (1961) described short

wed forelegs, extra sternebrae and fusion of the sternal and vertebral gments of Mn-deficient rats. Hurley *et al.* (1961a) confirmed the ortening of the long bones, both absolutely and relative to body ngth, and reported that the radii and tibia were greatly thickened distorted; longitudinal growth of the skull was reduced, and the th and height of the skull were slightly less in the deficient mals on an absolute basis, but in proportion to length of the skull y were greater. They also reported that ribs were either missing or ormed and the chest was anteriorly-posteriorly flattened. Abnormal e development due to Mn deficiency has been described in rabbits by th *et al.* (1944) and Ellis *et al.* (1947). Only the front legs were ed; the ulna and the humerus were significantly reduced in size, sity, Mn content and breaking strength. X-ray and microscopic dies showed changes distinctly different than those seen in rickets. narrowing of the zone of provisional calcification and the epiphyseal te, and a deficiency of spongy bone indicated a suppression of eogenesis (Smith *et al.*, 1944).

Concrete evidence that Mn is involved in bone formation was ained by Parker *et al.* (1955), who showed that a greater concentra- of injected radioactive Mn was found in the regions of greater formation, by Leach (1967), who showed that the changes in epiphyseal ilage of growing bone of Mn-deficient chick embryos were due to a ction in mucopolysaccharide production, and by Tsai and Everson 7), who reported a similar reduction in mucopolysaccharides due to duced hexose utilization for their synthesis in Mn-deficient guinea . The conclusion by Caskey *et al.* (1939) that abnormal bone develop- was probably the result of disturbance in Ca and P metabolism in absence of Mn was disproved by Parker *et al.* (1955), who showed that

he quantities of radioactive Ca and P deposited in bone were not ffected by Mn intake.

Shils and McCollum (1943) reported ataxia, incoordination and poor quilibrium in young rats from Mn-deficient dams. Hurley and Everson 1959) and Hurley *et al.* (1961b) found that the offspring of Mn-deprived others were slower to acquire reflexes and showed inadequate develop- ent of the bony labyrinth. Hurley *et al.* (1961b) found that the brain eights of Mn-deficient rats were absolutely smaller, but relative to he body weight, larger than the controls; the cerebrospinal fluid ressure did not differ significantly. Assays of tissues for a number enzymes, including acetylcholine esterase in the brain of Mn- ficient rats and guinea pigs did not reveal any biochemical faults an Reen and Pearson, 1955). Shrader and Everson (1967) and Hurley 969) concluded that abnormal development of the otoliths was sponsible for the congenital ataxia present in rats and guinea pigs en the maternal diets were Mn-deficient. Shrader and Everson (1967) ported abnormal curvatures of the semi-circular canals and misshapen pullae in Mn-deficient guinea pigs. However, hematoxylin and eosin ctions of the vestibular apparatus of ataxic rats failed to show ysical lesions (Hill and Holtkamp, 1954). Erway *et al.* (1970) showed at the incidence of ataxia increased in proportion to the severity d duration of the Mn deprivation of the mother.

There is a depressed growth rate in chickens on low dietary Mn allup and Norris, 1939a; Litricin, 1967). Improved growth rates due Mn supplementation of low Mn basal diets have been reported by tle *et al.* (1969), Belincenko (1968), and Van Reen and Pearson 955). Since Wilgus (1936, 1937) showed that addition of 25 ppm of to a basal diet containing 10 ppm of Mn completely prevented perosis,

ıer workers have confirmed this finding (Underwood, 1971). The
:urrence of perosis on low Mn diets has been reported in ducklings
ın Reen and Pearson, 1955) and in turkey poults (Vohra and Heil,
59). Wolbach and Hegsted (1953) found a suppression of the epiphyseal
:tilage developmental sequence, with immature cartilage cells, fol-
ved by retarded tunnelling and abnormal matrix in the zone of growth.
ıch (1968) reported a reduction in width of epiphyseal plate and
:aphysis. Creek *et al.* (1960) reported that bone deformities of the
:k joint in perosis were aggravated by weight applied to the leg.

Lyons and Insko (1937) reported that nutritional chondrodystrophy
caused by inadequate intakes of Mn, and Litricin and Andrejevic
66) found that in Mn deficiency the long bones of the legs and wings
e significantly smaller, had reduced diameter and length but did not
cken. Caskey and Norris (1940) did not find any significant effect
the sternum and metacarpus due to dietary Mn levels. Ataxia in the
spring of Mn-deficient chickens was first observed by Caskey and
ris (1940). Caskey *et al.* (1944) showed that ataxic chicks from
deficient parents grew normally on Mn-supplemented diets. They
cribed the ataxia as a tetanic spasm of the opisthotonic type.
tologically, the brain was normal, but chemical changes in lipid,
al phosphorus and phosphatase content were noted.

Manganese is involved in egg shell formation. Pullets fed rations
:aining low Mn with high levels of Ca and P produced eggs with
erior shell characteristics (Lyons, 1939; Longstaff and Hill, 1971).
owska and Parkhurst (1942) showed that feeding chickens Mn-deficient
:s significantly reduced the eggshell breaking strength, but Chubb
54) found no detectable difference in eggshell quality due to Mn
olementation. Recently Hill and Mathers (1968a), Mathers *et al.*

(1971) and Longstaff and Hill (1970) have shown that eggshell thickness was depressed by a low Mn diet when given before laying but not when given from the point of lay. They also showed that low Mn diets depressed significantly the acid mucopolysaccharide content of the shell matrix.

The nutritional significance of Mn in ruminant feeding has not been clearly verified. Bentley and Phillips (1951a) showed that heifers fed diets containing 7 to 10 ppm Mn were slower to mature, and more of their calves were born with weak legs and pasterns at first calving. Grashuis *et al.* (1953) has reported leg deformities with overknuckling and poor growth in calves of cows grazing Mn depleted pastures. Rejas *et al.* (1965) reported reduced breaking strength and length of the humerus of deformed calves from Mn-deficient cows. Hartmans (1970) did not find any Mn deficiency symptoms in grazing animals in Holland. Lanke and Groppel (1970) reported that there were no growth differences in female goats fed a ration containing 20 ppm Mn in the first year and ppm in the second year as compared to the controls consuming a similar ration supplemented up to 100 ppm of Mn. Lassiter and Morton (1968) have reported that Mn-deficient sheep developed poor bones with low ash concentration in the femur and reduced Ca, P and Mn content in the ash, implying an effect of Mn on bone ossification.

Manganese deficiency has been shown to cause poor growth and lameness in growing swine. Miller *et al.* (1940) reported the occurrence of lameness in pigs at 27 kg when they were fed high-corn diets containing 14 ppm Mn. The condition was characterized by a slight halting gait, progressing into enlarged hocks and crooked legs, becoming painful for the pigs to rise to their feet. Bone analysis of the Mn-deficient pigs showed normal mineral deposition. Higher Mn supplementation did

not cure the stiffness but did prevent it. This work was confirmed by Keith *et al.* (1942), but in addition they showed that Mn-deficient pigs were non-ataxic and had good appetites. Sandstedt and Carlquist (1951) showed similar bone deformities, characterized by swollen streaks on the posterior external contours of the hocks in pigs fed Mn-deficient diets. Johnson (1943) obtained satisfactory growth rate and no early lameness on purified diets containing less than 0.5 ppm of Mn. However, after long periods of time on this diet some signs of lameness appeared. Leibholz *et al.* (1962) found that 0.4 ppm Mn in the diet of baby pigs was sufficient to support maximum growth. Johnson (1944) reported satisfactory growth rates from weaning to market weight on low Mn diets, and Mn supplementation was only slightly beneficial when the ash content of the ration was raised above 10 percent. Giessler and Kirchgessner (1959) found that Mn supplementation did not improve weight gains, feed intake or efficiency of conversion of Swabian-Hall pigs fattened from 20 to 96 kg on a diet of barley with 250 g of a standard German protein concentrate daily. But Williams and Noland (1949) found that Mn supplementation, along with other trace elements, improved both the growth rate and feed efficiency in swine.

Grummer *et al.* (1950) fed pigs a corn-soy basal diet containing 12 ppm Mn supplemented with 40, 80, and 160 ppm Mn. Pigs consuming diets supplemented with 40 ppm gained significantly faster than those on the basal diet but performance of the pigs was not improved with higher levels of Mn. There was a slight depression of bone ash of the pigs on the basal diets. Plumlee *et al.* (1956) showed no significant difference in growth rate and feed efficiency between groups of Duroc boars fed on diets ranging from 0.5 to 40 ppm Mn. Pigs on the low Mn diets showed a tendency towards sickle-hocks; and X-ray photographs of the legs of

-deficient gilts showed that growth of the radii was prevented by
osure of the distal epiphyseal plate. Plumlee *et al.* (1956), feeding
its from Mn-deficient sows on Mn-deficient diets, observed signs of
deficiency manifested by pain and weakness of the legs at 27 kg
veweight, and shortened and thickened front legs becoming bowed at
kg. The deficient pigs were short in the body and excessively fat.
ner *et al.* (1956) reported similar defects in young pigs on purified
ets of low Mn content. They showed generalized rarefaction of leg
nes, but the lameness and rarefaction disappeared after 335 days on
perimental diets, whereas the other signs of Mn deficiency persisted.

2. Reproduction

Kemmerer *et al.* (1931) reported that mice reared on cows' milk
plemented with Fe and Cu failed to ovulate normally, but mice on the
e diet with 0.01 mg of Mn added daily exhibited normal estrous cycles.
nt and McCollum (1931) fed young rats on Mn-free diets; the females
ibited normal estrous cycles, but all their litters died, apparently
to deficient lactation. The males displayed testicular abnormali-
s. Boyer *et al.* (1942) reported irregular or no estrous cycles in
ale rats raised on low Mn diets, and there was a marked delay in the
ning of the vaginal orifice; the males had testicular degeneration
complete sterility owing to lack of spermatozoa production when fed
imilar diet. Shils and McCollum (1943) reported that when Mn-
icient females were mated to normal males, only 1.5 percent of the
ng survived to weaning as compared to 56 percent of the supplemented
up. Male rats on low Mn diets from weaning did not show differences
sperm mortality and testicular weight. There were increased still-
ths and depressed survival rates when male and female Mn-deficient

lbino rats were mated (Barnes *et al.*, 1941). Hurley *et al.* (1958) onfirmed these effects and reported that they were aggravated in ubsequent generations. Smith *et al.* (1944) and Ellis *et al.* (1947) lso reported testicular degeneration and lack of libido in male abbits on low Mn rations, and reduction in size of ovaries and uterii n females. In guinea pigs, the omission of Mn from the maternal diet esulted in a decrease in litter size and an increase in percentage of oung born prematurely or ataxic or dead (Everson *et al.*, 1959).

Gallup and Norris (1939b) showed that Mn deficiency in the diet f chickens resulted in decreased egg production, fertility and hatcha- ility. Others have reported improved reproductive performance when asal diets were supplemented with Mn (Underwood, 1971; Atkinson *et l.*, 1967). Christiansen *et al.* (1940) reported that Mn and flavin ere the critical factors involved in the subnormal hatchability associ- ced with soybeans. Hoogendorn (1940) found that with birds kept ndoors, the addition of Mn to the diet improved egg production and atchability. Gutowska and Parkhurst (1942) and Hill and Mathers 1968b) did not find any improvement in egg production, fertility and atchability due to Mn supplementation. Chubb (1954) found that pullets ed on low Mn diets produced twice as many infertile eggs and dead-in- ell embryos as those on 50 ppm Mn diets. An increased incidence of ead retractions in newly hatched chicks and chondrodystrophic embryos s associated with lines of birds producing eggs of low Mn content olton, 1957).

Bentley and Phillips (1951a) showed that Mn-supplemented Holstein lves came into heat earlier than those on low Mn diets, suggesting a imulating effect of Mn on sexual maturity; the number of services per nception and number of calves born and calves born dead were unchanged

by dietary treatments, but the calves born of Mn-supplemented cows in the second generation were noticeably heavier at birth. Experimental and field data show that Mn is necessary for fecundity in cattle (Rojas *et al.*, 1965; Bentley *et al.*, 1951). Bentley *et al.* (1951) found a significantly lower Mn content in the ovaries of repeat-breeders, but there was no direct relationship between the content of Mn in feed, tissue and organs, and infertility. Werner and Anke (1960) showed a relationship between the Mn supply and number of services per conception. Dyer (1960, 1961) and Dyer *et al.* (1964) reported a positive relationship between a low Mn intake by gestating cows and the incidence of neonatal deformities in their calves. Pastures with 15 ppm Mn or below resulted in greater incidence of infertility and abortion. Bonomi (1966) and Wilson (1966) reported that high dietary levels of CaO and P_2O_5 seemed to cause Mn deficiency, which precipitated functional infertility in cattle. Manganese supplementation has been shown to improve the fertility of cattle in Europe (Grashuis *et al.*, 1953; Wilson, 1965, 1966; Krolak, 1968). Anke and Groppel (1970) found that goats on low Mn diets came into estrus late, the symptoms of estrus were weak, and more inseminations per conception were needed. The low Mn group produced more male kids but also had more abortions. Similar observations were reported in guinea pigs by Everson *et al.* (1959).

Manganese involvement in reproductive processes of cattle was further proven by Anke and Groppel (1970), who showed a higher concentration of the element in the ovaries after an injection of radioactive Mn, and in Archibald and Lindquist (1943), who showed that there was a substantial transfer of Mn into the milk by the ovine mammary gland.

Johnson (1940) reported that bred sows fed a semi-purified diet containing 0.3 ppm Mn produced apparently normal pigs; however, the pigs

contained one tenth as much Mn as those fed 6.0 ppm Mn. Sows fed diets containing 6.0 ppm Mn raised their litters satisfactorily. Johnson (1944) showed satisfactory reproduction through two generations on a ration of 8.6 ppm Mn and 1.78 percent ash. Grummer *et al.* (1950) reported that sows on diets containing 12 ppm Mn tended to be less fertile, hard to settle and gave birth to smaller and abnormal litters. Supplementation of the basal diets with 40, 80, or 160 ppm Mn for sows during reproduction and lactation resulted in a significant difference in performance of their litters. Gligor *et al.* (1966) reported no significant effect on weight of sows at farrowing or weaning or 60 days later, or on number and weight of piglets. Speer *et al.* (1952) showed that pigs from sows supplemented with 70 to 90 ppm Mn during gestation and lactation made highest gains during the growing and fattening periods. Plumlee *et al.* (1956) fed female swine either low or high Mn diets throughout growth, gestation and lactation, and reported that the growth rate and feed efficiency of their litters were satisfactory at both levels. In another experiment, Plumlee *et al.* (1956) reported that when female pigs were fed on either low or high Mn diets, the differences in number of pigs farrowed or viability or birth weights of litters could not be attributed to Mn levels given. But when gilts were depleted of Mn by feeding Mn-free purified diets, the reproductive organs were histologically normal, and they ovulated normally but showed irregular estrus and sometimes would not accept the boar. When mated, they farrowed weak ataxic litters, unable to suck, and most of them died even when transferred to Mn-supplemented diets. The litters from supplemented gilts were normal, vigorous and healthy. Boars from gilts getting either low or high Mn levels in their diets, all reared on 3.3 ppm Mn ration, performed equally well and showed normal

ermatogenesis (Plumlee *et al.*, 1956). Neher *et al.* (1956) reported at sows on a low Mn diet farrowed abnormal pigs with reduced birth- ights. The above findings were not sustained by the results of ibholz *et al.* (1962) and Newland and Davis (1961), who found, dependently, that sows on low Mn diets farrowed or produced normal tuses without apparent reduction in birthweights. The involvement Mn in swine reproduction is further shown by the findings that there a rapid, unlimited transfer of the element to the fetus, colostrum d milk (Plumlee *et al.*, 1956; Newland and Davis, 1961; Leibholz *et* ., 1962).

Biological Roles of Mn

1. Enzyme Activation

Lehninger (1970) lists many enzymes involved in intermediary tabolism that require Mn ions for activation. Mn is an integral part many enzyme systems of the body. Some of the specific enzymes for ch Mn is known to be essential as an activator are acetyl CoA boxylase (Wells and Remy, 1965), cytochrome oxidase (Vorob'eva, 0), enolase (Wacker *et al.*, 1964; Babin *et al.*, 1964), fructose 1,6 hosphatase (Pontromoli *et al.*, 1969), heparinase (Dietrich *et al.*, 2) and succinic dehydrogenase (Babin *et al.*, 1964). Other divalent ions, especially Mg, with similar properties, can replace Mn in the ivation of many enzyme systems.

Rubenstein *et al.* (1962) showed that high levels of Mn induced ere hypoglycemia due to increased enzyme activation, and Johnson *et* (1959) reported that preincubation with Mn ions increased consider- the proteolytic activity of rat and chick pancreatic homogenates. terjee *et al.* (1960) found that Mn ions restored the conversion of

;luconate to ascorbic acid by goat liver microsomal enzymes. Skinner
nd McHargue (1944) found that Mn supplementation of rats increased
o-carboxylase activity. Reineke and Turner (1945) showed that enzymes
nvolved in the iodination of tyrosine to diiodotyrosin and subsequent
xidation to thyroxine were best catalyzed by Mn, especially colloidal
nO_2. Van Reen and Pearson (1955), studying a number of enzymes in
n-deficient and Mn-supplemented ducks, showed that Mn had no effect
n the activity of liver diphospho-pyridine nucleotidase, cytochrome
xidase, catalase and isocitrate dehydrogenase. Leach (1967) found that
he enzymes, polymerase and galactotransferase, involved in the chon-
roitin sulfate synthetic system are activated by Mn.

In vitro studies indicated that Mn ions activated phosphatase, but
ddition of Mn ions to an enzyme preparation from the bones and blood
f a perotic chick did not raise the activity to nearly the same level
s that found for a chick without perosis (Weise *et al.*, 1939). Wachtel
t al. (1943) found that Mn deficiency caused a significant increase in
lood serum phosphatase but not bone phosphatase. But Ellis *et al.*
1947), Leibholz *et al.* (1962) and Rojas *et al.* (1965) showed a depres-
ion of bone alkaline phosphatase activity on low Mn diets. Combs *et*
l. (1942) found an intimate relationship between the phosphatase
ctivity of the bones and Mn deficiency. Lowering of the phosphatase
ctivity retarded bone development. Phosphatase activity was greatly
educed by withdrawing Mn. Lassiter *et al.* (1970) reported that sub-
rmal bone alkaline phosphatase activity does not invariably occur with
deficiency. Hurley and Everson (1959) showed that both Mn-deficient
ts and controls showed the same pattern of enzyme activity and there
re no significant differences between them. Nielsen and Madsen (1942)
owed that blood phosphatase significantly increased in perotic

rkeys, but Van Reen and Pearson (1959) found that the enzyme activity livers of Mn-deficient ducks was only one-half that of the livers of pplemented birds. Lassiter *et al.* (1970) found that serum alkaline osphatase activity of lambs fed 1 ppm Mn was significantly below that controls receiving 29 ppm Mn, but kidney alkaline phosphatase tivity was increased. Leibholz *et al.* (1962) showed that the alkaline osphatase activities of the kidney, liver and serum of baby pigs given 4 ppm Mn in the diet were not affected.

Boyer *et al.* (1942), Rehner and Stelte (1970) and Rehner and Cremer 970) reported the arginase activity of liver preparations from Mn-ficient animals was greatly increased by Mn additions. Mn supplementa-on increased urea in blood, urine and saliva of cattle (Zerebeov *et* ., 1970; Rozybakiev, 1966). Others have shown a depression in ginase activity of rats and guinea pigs on Mn-deficient diets (Ellis *al.*, 1947; Everson, 1970). But Leibholz *et al.* (1962) found that Mn eatments did not significantly affect the liver and kidney arginase tivity in baby pigs.

2. Energy Metabolism

Lehninger (1970) lists a number of key enzymes involved in the ycolytic pathway, gluconeogenesis and beta-oxidation requiring Mn as for activation. Early investigations by Ray and Deysach (1942) wed that subcutaneous injection of Mn into guinea pigs in small es raised oxygen consumption, but higher doses up to 100 mg per kg bodyweight progressively depressed oxygen consumption. Wachtel *et* (1943) found no Mn effect on basal metabolic rate (BMR) in rats. Bentley *et al.* (1951b) found that phosphorylation of chicken liver genates was increased 41 percent by Mn above that on a choline-

ficient, Mn-deficient ration. Hurley *et al.* (1970) showed a depres-
on in oxidative phosphorylation in Mn-deficient mice. Buccellato
953) found *in vitro* that a compound formed between pyridoxine and
lloidal Mn had an active role in carbohydrate metabolism. Scrutton
al. (1966) found that pyruvate carboxylase is a Mn metalloprotein,
d Mildvan *et al.* (1966) showed that Mn functions in the transcarboxyla-
on part of the pyruvate carboxylase reaction.

Manganese involvement in glucose utilization was shown by Everson
d Shrader (1968) and Shrader and Everson (1968). Newborn guinea pigs,
verely deficient in Mn, exhibited a marked hypoplasia of all cellular
mponents of the pancreas, with fewer and less intensely granulated
ta cells than the controls. When glucose was administered orally or
travenously to young adults which were congenitally Mn-deficient,
ey showed glucose responses resembling the diabetic subject, whereas
ntrol animals always returned promptly to normal glucose levels. Mn
plementation of deficient animals completely reversed the reduced
cose utilization in guinea pigs and cattle (Everson and Shrader,
8; Zerebeov *et al.*, 1970). The administration of this element to
betic subjects has a hypoglycemic effect (Mehrolera *et al.*, 1964;
enstein, 1962); and both pancreatectomy and diabetes have been
related with decreased Mn levels in blood and tissues (Konseko,
5).

Manganese supplementation was shown to reduce liver and bone fat
Mn-deficient rats (Amdur *et al.*, 1946). Plumlee *et al.* (1956) showed
Mn-deficient gilts were excessively fat by 25 to 40 kg liveweight.
an (1954) showed that Mn stimulates the hepatic synthesis of
esterol and fatty acids in rats. Mn ions are necessary for the
version of mevalonic acid to squalene by mevalonic kinase (Amdur

al., 1957); and the phosphorylated derivative of mevalonic acid, essary for this reaction, requires Mn for its synthesis. Barron 66) showed that Mn was a necessary co-factor for mitochondrial fatty d synthesis together with NAD and citrate; and others have reported t Mn inhibits lipoamide dehydrogenase (Lehninger, 1970).

3. Hemoglobin Formation

Manganese has been shown to either increase or decrease or e no effect on hemoglobin values in a variety of animal species. htel *et al.* (1943) and Smith *et al.* (1944) found that hemoglobin els were not significantly affected by lack of Mn. But Skinner and argue (1946a), using dry or milk diets, showed that Fe, Cu and Mn e higher hemoglobin values than Fe and Cu added alone.

High levels of Mn (100 to 3000 ppm) had a small but significant ression on hemoglobin levels of calves (Cunningham *et al.*, 1966). oglobin regeneration was greatly retarded and serum iron depressed anemic and normal lambs, rabbits and pigs by feeding high levels of The levels causing reduction of hemoglobin ranged from 50 to 5000 (Robinson *et al.*, 1960; Matrone *et al.*, 1959; Hartman *et al.*, 5). With baby pigs, Matrone *et al.* (1959) showed that the regenera- n of hemoglobin, when Fe intake was low, was depressed by excess Mn akes but the depression was overcome by extra Fe. When the anemic pigs, given 25 ppm Fe and 5 ppm Cu in the diet, were supplemented n 125, 250 or 2000 ppm Mn, all the levels of Mn depressed hemoglobin nation and the drop was sharp and significant. Moinuddin and Lee 0) found a decline in hemoglobin concentration, a reduction in red d cell count and an increase in white blood cell count due to feed- high Mn levels. In 1960, Sullivan noted similar changes in rats

ven a manganese edetate supplement. The changes were greater in
ung rats.

4. Ascorbic Acid Synthesis

Rudra (1939) found that rat and guinea pig livers were able to
nthesize ascorbic acid when incubated with mannose or galactose in
e presence of Mn which acts as a co-enzyme in the conversion. Later
showed that intraperitoneal injection of 20 mg of mannose in the
esence of 0.04 percent Mn to guinea pigs gave a small increase of
corbic acid in body tissues and protected against scurvy (Rudra,
44b). He concluded that Mn was essential for the synthesis of vitamin
in animals and that failure to synthesize it is due to insufficiency
the metal at the site of ascorbic acid synthesis.

Injections of mannose plus Mn given to scorbutic guinea pigs did
t stimulate ascorbic acid synthesis from mannose *in vivo* (Skinner and
Hargue, 1946b). The Mn involvement in ascorbic acid synthesis is
nplicated by vitamin E (Caputto *et al.*, 1958). Using enzyme prepara-
ons from vitamin E-deficient rats, 70 to 90 percent less ascorbic
id was produced than from a preparation taken from animals given
fficient vitamin E, and addition of Mn to *in vitro* systems increased
ascorbic acid produced by vitamin E-deficient preparation 315
rcent.

5. Genetics, Disease and Immunity

A difference in Mn requirement to prevent perosis among various
eds of birds has been documented (Golding *et al.*, 1940; Pilla,
8). Caskey *et al.* (1944) reported that offspring of ataxic female
male chickens grew normally on a diet supplemented with Mn, sug-
ting the ataxia was not complicated by the inheritance of a simple

ecessive character influencing Mn retention. Hurley (1969) showed that taxia caused by maternal Mn deficiency is indistinguishable from that aused by certain genes in mutant mice. A supplement of 1000 ppm Mn o pregnant females on a diet that normally produced 68 percent ataxic oung completely prevented the condition and, when the normal offspring ere mated and fed a normal diet, the normal offspring subsequently roduced 68 percent ataxic young. Thus, the high level of Mn prevented xpression of the genetic abnormality without influencing genetic onstitution.

Hoogendoorn (1940) reported that adding 12 mg of manganese sulfate o 100 kg of poultry meal afforded additional resistance to disease. arot and Durand (1944) found that the Mn content of malignant and enign tumors was much lower than that of healthy tissues. Sandstedt *t al.* (1951) showed that daily supplementation with 0.5 g of manganese ulfate gave rapid recovery from acetonemia in cattle, and preventive reatment over the years greatly reduced the incidence of the disease. mofal (1961) found that the level of Mn in the diet was a decisive ctor in the occurrence of goiter. Kamchatrov (1959) and Hakimova *et* . (1969) showed that excess dietary Mn affects thyroid metabolism. ellis (1970) showed a depression of the protein-bound iodine fraction. rlier Kamchatnov (1953) had noted a relationship between Mn content feed items and the distribution of enzootic and non-enzootic goiter gions. Angelico *et al.* (1965) and Antanova (1968) have reported that supplementation significantly delayed the death of rats and increased e survival rate after nitrogen mustard poisoning and coliform bac- rial infection.

Weinberg (1964) discovered that certain bacteria had specific uirements for Mn, in excess of that needed for growth, in order to

roduce antibiotic, bacteriophage, and protective antigens. Antanova 1968) and Antanova *et al.* (1968) reported that agglutinin response and agocytic activity were greater with higher Mn intakes in rabbits when munized with coliform and typhoid bacteria. Muraleedharan and Pande 1968) found that with Mn-deficient diets, infection with *Prosthogonimus atus* seemed to hasten death.

Mn Metabolism

1. Absorption

Little is known about the mechanism of Mn absorption from the strointestinal tract. Scott *et al.* (1958) showed that a Mn compound pable of dissolving and being converted to manganese chloride in the id medium of the gastrointestinal tract can be absorbed.

The uptake of Mn by the intestinal mucosa is very rapid (Miller *et* ., 1972), and the element is bound to the serum beta-globulin fraction all species studied (Panic and Ekman, 1967). While Saltman *et al.* 956) thought that simple diffusion constituted the driving force for e transport of Mn, Rothstein *et al.* (1958) and Weed and Rothstein 958) presented evidence for active transport for Mn. Gutowska *et al.* 941) showed that the amount of Mn absorbed from a solution of manganese ifate was proportional to its concentration; an average of a third of amount injected in the intestines was absorbed in two hours. There no significant sex difference in absorption of Mn in chickens. y reports have indicated that only 3 to 4 percent of an oral dose of is absorbed (Britton and Cotzias, 1966; Watson *et al.*, 1973). But ble *et al.* (1971) and Brown and McCracken (1965) have reported sub- ntial Mn absorption. Pregnant sows absorbed up to 28 percent of ested Mn (Gamble *et al.*, 1971).

Manganese absorption is affected by various factors present in diet. Poll *et al.* (1967) and Hill and Holtkamp (1954) reported t Mn was more readily absorbed at a lower than at a higher dietary concentration. Cotzias and Greenough (1958) and Zajcev (1959) found t Mn absorption was not affected by its valency state in the com- nds used. Many workers have reported that high dietary levels of and P aggravated Mn deficiency due to reduction in Mn absorption derwood, 1971). Intestinal absorption of Mn was increased in rats e iron deficient (Pollack *et al.*, 1965). Saltman *et al.* (1956) nd that Mn competitively inhibited Fe uptake and release by liver ces, which indicated that both Fe and Mn have a common pathway. o and Edwards (1968) reported that Mn absorption was enhanced by thylenetriamine pentaacetic acid (DTPA) and decreased by ethylene- mine tetraacetate (EDTA). Leibholz *et al.* (1962) found that pigs casein diets containing 0.4 and 40.4 ppm Mn grew at a more rapid e on less food per pound of gain than did pigs fed soybean protein ions containing 11.8 and 51.8 ppm Mn. Davis *et al.* (1962) found a tor in soybeans which tends to bind Mn and makes Mn unavailable. Settle *et al.* (1969) found no appreciable binding of Mn in feather l diets. Gilbert (1957) reported that thiamine, given in excess, cipitated a Mn deficiency, but Holtkamp *et al.* (1950) found no dence of antagonism between dietary Mn and thiamine.

2. Excretion

Everson and Daniels (1934) observed that total Mn urinary etion is virtually constant, irrespective of age, and that fecal xcretion varies directly with age and therefore with total dietary ntake. Many workers have reported that most of Mn administered

orally or intraperitoneally quickly appeared in bile and was excreted in the feces, and very little was excreted in the urine (Kent and McCance, 1941; Mahoney and Small, 1968; Starodubova, 1968). The predominance of the fecal route for Mn excretion has been verified in simple stomached animals (Britton and Cotzias, 1966; Miller, 1973), in sheep (Watson *et al.*, 1973), in cattle (Miller *et al.*, 1973), and in man (North *et al.*, 1960; Cotzias and Greenbough, 1958).

Under normal conditions, the bile flow is the principal regulatory mechanism of Mn excretion, and the concentration of Mn in bile can be increased tenfold or more by the animal (Underwood, 1971). The other routes of Mn excretion include pancreatic juice (Papavasiliou *et al.*, 1966; Burnett *et al.*, 1952) and secretions of the duodenum, jejunum and, to a smaller extent, the terminal ileum (Bertinchamps *et al.*, 1966). Excretion via the kidney is negligible normally or during jaundice or after a marked oral dose. The administration of chelating agents such as EDTA produces a marked rise in urinary excretion of Mn (Maynard and Fink, 1956).

Lassiter *et al.* (1970) reported that the body pool is small and the body does not ordinarily accumulate Mn. Total body excretion is continuous and very nearly equal to intake, and much of the Mn in the body pool is replenished daily. Animals placed on a low Mn diet continue to excrete Mn, suggesting an obligatory loss of Mn (Zajcev, 1959; Starodubova, 1968). The excretion of Mn administered parenterally was much lower on a 1.0 percent Ca diet than on a 0.6 percent Ca diet, but raising P levels had no comparable effect on the excretion or retention of Mn (Lassiter *et al.*, 1970). Underwood (1971) noted that Ca influences Mn metabolism by affecting its absorption. The normal excretion of Mn from the body is prevented by rectal and biliary ligation (Papavasiliou

al., 1966), and there is no appreciable interdependence of these
tes of excretion (Bertinchamps *et al.*, 1966).

3. Retention

Everson and Daniels (1934) reported that Mn retention varies
ersely with age of children and therefore intake. North *et al.*
60) showed that college women retained about a third of the absorbed
and pullets retained about the same amounts (Brown and McCracken,
5). Gamble *et al.* (1971) showed that 7 days after the administra-
n of a radioactive dose of Mn, pregnant sows retained 26 percent of
oral and 78 percent of an intravenous dose. A highly significant
relation between intake and retention has been found (Mathers and
l, 1967; Murty, 1957), but Hughes *et al.* (1966) reported that only
mall change in tissue Mn level can be effected by a large change in
tary Mn intake. There is a linear relationship between Mn turnover
level of dietary Mn intake, and the half-life of body Mn is
eased with increasing Mn intake (Britton and Cotzias, 1966). The
rbed Mn is found principally in the liver and bone. At lower Mn
kes the liver retains more Mn than intestinal tissue, but at high
kes the latter retains more (Underwood, 1971). Passive transendo-
ial transport occurs immediately after intravenous administration
n, and about 70 percent of the blood Mn leaves the circulation each
te and is mainly taken up by the liver (Cotzias, 1958; Thomas,
). Borg and Cotzias (1958) showed that most of the endogenous Mn
ts in highly labile intracellular combinations.

Tissue Mn concentrations generally are remarkably constant even
gh consumption levels vary greatly (Underwood, 1971). These might
egulated through variable excretion rates (Britton and Cotzias,

6), and absorption differences (Howes and Dyer, 1971; Miller, 1973). siter *et al.* (1970) showed that 0.9 percent dietary P caused signifi- tly higher Mn retention of orally administered Mn than did 0.4 per- t P, but there were no comparable effects on intraperitoneally inistered Mn. Hughes and Cotzias (1961) found that administration exogenous glucocorticoid hormone markedly affected the tissue distri- ion of radioactive Mn, but adrenalectomy did not affect retention ghes *et al.*, 1966). Hill (1967) found that vitamin D reduced Mn nover, and Suso and Edwards (1968) reported that EDTA reduced the ention of intravenously injected Mn, and greatly increased Mn trans- (Sahagian *et al.*, 1967). Gamble *et al.* (1971) reported that gnancy in swine had no significant effect upon maternal tissue ention, organ distribution or turnover rate of Mn. Fournier *et al.* 72) reported increased Mn retention in all organs as a result of ose ingestion.

Manganese Requirement

1. Factors Affecting Mn Requirement

There is a very wide margin of safety between the minimum and c levels of Mn for all species. The minimum dietary requirements n depend upon the species, the criteria of adequacy employed, the ical form in which the element is ingested, and the nature of the of the diet (Underwood, 1971).

Manganese requirements differ between and within species. Chickens ire more Mn than any other species (Thomas, 1970). A level of 13 Mn in the diet resulted in signs of perosis in White Leghorns and Hampshires but not in Rhode Island Reds (Pilla, 1958). Chondro- rophy was confined to Barred Rock chicks only and did not affect

hite Leghorns, and the response to Mn supplementation was greatest in ew Hampshires and least in Leghorns (Golding *et al.*, 1940). Gutowska t *al.* (1941) and Mathers and Hill (1967) reported no significant sex ifference in Mn utilization in chickens. But Barnes *et al.* (1941) eported that female chicks were more sensitive to Mn deficiency in he mother's diet than male chicks. And Pilla (1958) found that the esponse to Mn was greater in males than females. There is evidence howing that Mn levels necessary for growth are less for reproduction Rojas *et al.*, 1965; Grummer *et al.*, 1950; Bentley *et al.*, 1951a).

In attempting to define dietary requirements of Mn, the composi- ion of the diet is crucial. There are interactions that occur in the ood or in the gastrointestinal lumen. Manganese interacts with Ca and within the digestive tract. In 1939, Caskey and Norris observed that was made unavailable by high levels of Ca and P in the diets of ickens. High dietary levels of Ca and P have been shown to decrease e growth rate and increase the incidence of perosis in chickens on ets having Mn levels ordinarily adequate to prevent perosis, and ditional Mn prevented the development of perosis (Underwood, 1971). lgus and Patton (1939) explained the perosis-producing action of lcium phosphate as being due, at least in large part, to the removal Mn from solution in the intestinal tract by adsorption or chemical mbination. High levels of ferrous citrate also increased the inci- nce of perosis, presumably through similar intraluminal action (Wilgus d Patton, 1939). Addition of ferric oxide to a Mn and Cu mixture emed to offset its beneficial effect on hatchability (Hoodengoorn, 40). In intracellular fractions, the concentration of Mn and Fe have en shown to have a reciprocal relationship (Thiers and Vallee, 1957). ere is some evidence of dietary Mg-Mn and Zn-Mn interactions

akemore *et al.*, 1937; Cotzias, 1960; Sahagian *et al.*, 1966). Diez-
ld *et al.* (1968) and Kolomijceva and Veznesenskaja (1968) have
orted interrelationships between Fe, Cu and Mn metabolism.

In some other circumstances, high levels of dietary thiamine intake
rease body Mn storage (Hill and Mathers, 1954), but Sandberg *et al.*
39) found that the state of thiamine deficiency caused great increases
Mn retention. Anderson and Parker (1955) found no thiamine level
ect on liver and heart Mn content. Holtkamp *et al.* (1950) found no
dence of antagonism between thiamine and Mn. Perla and Sandberg
39) reported that high thiamine levels causing low reproduction rates
be counteracted by increasing Mn intakes. Riboflavin, at high levels,
shown to complicate perosis in ducklings (Turton, 1953).

The protein source of the diet may affect Mn metabolism. The
lability of Mn to chicks was better when either fishmeal or dried
milk was the protein source as compared to soybean meal (Morimoto
al., 1959; Kealy and Sullivan, 1966). Davis *et al.* (1962) showed
soybean protein contains a component which combines with Mn leading
conditioned deficiency. Settle *et al.* (1969) found no such binding
In when feathermeal was fed as the source of protein.

The Mn compound fed may determine Mn availability and requirements.
emer *et al.* (1940) showed that precipitated $MnCO_3$ protected against
sis at a lower level than the naturally occurring carbonates which
ed useless even when used in large amounts due to low solubility.
ible and Bandemer (1942) reported that chemical forms of Mn com-
ds as diverse as carbonates, oxides, sulfates and chlorides were
lly valuable as sources of Mn in poultry rations. Anke *et al.*
7) and Watson *et al.* (1970) have shown that the chloride and sulfate
better utilized than the oxides in cattle. Lyons (1939) and

eshi *et al.* (1963) found that Mn supplied in rice bran appeared to s well utilized as the inorganic sulfate. The method of administra- of Mn is important in determining its effectiveness. Caskey and is (1939) showed that small amounts of Mn injected intraperitoneally more effective than ingested materials against perosis.

A number of feed additives have been shown to either depress or nce Mn availability to the animal. The inclusion of chlortetra- ine in low Mn diets for chickens reduced the incidence of perosis he pullets but not in their progeny (Pepper *et al.*, 1952, 1953). on (1955) showed that giving estradiol increased plasma and liver Hart (1953, 1954) reported that giving Vevoron, an antithyroid aration containing methylthiouracil for fattening, significantly essed the Mn content of the liver. Hydrazine administration can e symptoms in animals similar to those of Mn deficiency (Thomas,).

There are other factors which may influence Mn requirements. stiansen *et al.* (1939) concluded that sunlight exerts a sparing on on the hens' Mn requirements. Urban (1959) showed that hepa- omy aggravated Mn deficiency.

2. Mn Requirement of Swine

There are conflicting data in the literature on the exact Mn irements of pigs for proper growth, skeletal development and repro- ion. Keith *et al.* (1942), using a high corn ration fortified with rals and containing 11 to 14 ppm Mn, found that the growth of pigs not impaired but skeletal development was poor, and a supplemental l of 50 ppm Mn prevented skeletal deformities but did not cure them. er *et al.* (1940) had a similar response with 60 ppm supplementation.

ohnson (1940) reported that 6 ppm was sufficient for successful repro-
uction of sows; and satisfactory growth was obtained on 0.3 ppm Mn,
ut reproduction was unsatisfactory and, at this level, tissue Mn
ontent was significantly depressed. Johnson (1944) showed that pigs
rew well from weanling to market weight on rations containing 7 to 10
pm Mn. Grummer *et al.* (1950) reported that a diet containing 12 ppm
n was adequate for skeletal development and growth but not adequate
or optimum reproductive performance. When the same diet was supple-
ented with 40, 80 and 160 ppm Mn, the highest average daily gains were
btained on the 40 ppm level. Higher supplementation had no added
enefit. Plumlee *et al.* (1956) reported that dietary Mn concentrations
anging from 0.5 to 34 ppm Mn did not show significant differences in
ig performance. Speer *et al.* (1952) showed that pigs from sows
upplemented with 70 to 90 ppm Mn performed best during growing and
ttening as compared to those from unsupplemented groups. Leibholz
al. (1962) defined the baby pig Mn requirement at 0.4 ppm Mn for
ximal growth rate. Leibholz *et al.* (1962) and Newland *et al.* (1961)
und independently that sows farrowed or produced normal fetuses when
d 89 to 117 ppm Mn or 6 to 100 ppm Mn in their diets, respectively.
e Mn requirements for growth of pigs are extremely low, well below
e levels ordinarily found in practical swine diets, although the
tional Research Council (NRC, 1973) recommended 20 ppm Mn in the diet.

3. Mn Requirement of Other Species

The Mn requirements of many species have been estimated by
ny investigators and reviewed by Thomas (1970) and Underwood (1971).
rds have higher Mn requirements than mammals. The best evidence
dicates that to prevent deformities in the fetus, cows should receive

diet containing 20 ppm Mn. In calves 8.6 mg per day were not suffi-
ient but 36 mg per day were optimum for growth. Poultry requirements
ave been put at 40 to 70 ppm Mn in the diet. Children require at
east 1.2 mg per day; and older humans 3 to 5 mg. Requirements for the
at have been set at 0.5 to 1.0 mg per day, and the low amount of 1 ppm
n the diet produces fetal abnormalities. Rabbits require about 1 mg
er day, and sheep 50 to 60 ppm on a feed dry matter basis.

Mn Toxicity

Mn is one of the least toxic of the trace elements to mammals and
irds. Richards (1930) fed pigs 3.5 g of manganese citrate daily for
ine months without any adverse effects. Mussehl and Ackerson (1939)
owed that turkeys can tolerate 385 ppm Mn in their diets and Insko
al. (1938) reported that hens tolerated 600 to 1000 ppm Mn. However,
hers have reported adverse effects due to high level feeding of Mn.
mimura (1938) found that rabbits fed 0.5 to 6 g per kg bodyweight
ily were stunted and their bone development impeded. Similar effects
ve been shown in rats and cattle (Chornock *et al.*, 1942; Cunningham
al., 1966). Wessinger *et al.* (1943) found that injections of solu-
ons containing 180 to 975 ppm Mn as $MnCl_2$ into white rats caused
amel hypoplasia, interrupting amelogenesis in the apical quarter of
e zone of matrix formation. Heller and Penquite (1937), feeding a
tion containing 4800 ppm Mn, showed the element to be highly toxic
young chickens.

High level feeding of Mn interferes with Fe, Cu and P metabolism
nderwood, 1971). Urinary Cu excretion is depressed and Cu retention
the tissues is increased, causing a microcytic, hypochromic anemia in
ts fed very high Mn levels (Gubler *et al.*, 1954). Earlier, Edgar

942) supplemented sheep rations with 25 mg of Mn as the sulfate and
mg of Cu as the sulfate daily and showed large but non-significant
creases in liver Cu levels over those of the controls receiving copper
lfate alone. Experimentally, dietary Mn levels causing a suppression
hemoglobin formation are 1000 to 2000 ppm for anemic lambs (Hartman
al., 1955), 5000 ppm for normal lambs (Robinson *et al.*, 1960), 1250
n for mature rabbits (Matrone *et al.*, 1959), and 2000 ppm for baby
gs (Matrone *et al.*, 1959). Hartman *et al.* (1955) and Cunningham *et*
. (1966) reported that high levels of dietary Mn resulted in decreased
ncentrations of Fe in liver, kidney and spleen of ruminants, and
pressed hemoglobin formation.

Blakemore *et al.* (1937) found that the Mn content of pastures in
stricts where lactation tetany was prevalent was 700 ppm on a dry
tter basis as compared to 50 ppm of pastures on which tetany had never
en recorded. The feeding of Mn to rabbits, sheep and cows in amounts
pplied in the pastures on which tetany occurred brought about transi-
ry falls in levels of Mg in blood. Robinson *et al.* (1960) and
nningham *et al.* (1966) found that cattle fed high Mn levels produced
ss rumen propionic acid. Keith *et al.* (1942) showed that growing
gs fed on diets containing 2000 ppm Mn grew poorly, lost weight and
petite. They vomited and had nausea, diarrhea and dermatitis.
ummer *et al.* (1950) showed that pigs do not tolerate high levels of
etary Mn since 500 ppm in the diet reduced growth rate, feed
ficiency and depressed appetite of growing and finishing swine.
Leibholz *et al.* (1962) showed no toxicity signs in baby pigs fed
00 ppm Mn; however, there was evidence of reduced growth rate at
00 ppm. This work showed a high tolerance by the baby pig and a
nsiderable margin of safety between levels of Mn likely to be

ngested in the diet and detrimental levels. In man the contamination
f air and water by large amounts of Mn causes locula manganica, dis-
urbance of the extrapyramidal tract and atrophy and disappearance of
erve cells of the globus pallidus (Cotzias, 1958; Belani *et al.*, 1967).
otzias (1968) and Mena *et al.* (1968) showed a decreased turnover of
n in patients suffering from chronic Mn poisoning.

EXPERIMENTAL PROCEDURES

Introduction

Four experiments involving 104 pigs were conducted to study Mn tabolism in swine. These were:

Experiment 1. The relative availability of Mn from a 16% corn-soy sal diet and the basal diet supplemented with 10 ppm of Mn from $SO_4 \cdot H_2O$, $MnCO_3$ or MnO for the growing pig.

Experiment 2. Study of the gastrointestinal flux pattern of Mn om different Mn sources using chromic oxide (Cr_2O_3) as an indicator.

Experiment 3. The effect of high level Ca and P supplementation d an inverse Ca-P ratio on Mn utilization by the growing pig.

Experiment 4. The Mn requirement of the baby pig from sows fed a w Mn diet.

Yorkshire, Hampshire and Yorkshire-Hampshire crossbred pigs from e Michigan State University herd were used. All trials were conducted the university swine farm facilities.

Experiments

1. Experiment 1

Before the diets for this experiment were made, three Mn com- unds (manganous sulfate, manganous carbonate and manganous oxide) were bjected to a 0.4% HCl availability study.

A liter of 0.4% HCl was heated in a 1500 ml beaker to a constant mperature (37°C) in a gyrotory water bath shaker, Model G76.[1] One am of each compound, ground to pass through a 100 mesh screen, was ded; the temperature and agitation were maintained for one hour. The sulting solution was filtered at once through a dry Whatman #42 filter per, discarding approximately the first 50 ml. The filtrate was oroughly shaken, sub-sampled into three 20 ml aliquots, and Mn was termined by atomic absorption spectroscopy.

Twelve crossbred weanling pigs, 9 males and 3 females, from two tters, were allotted to the four diets shown in Table 1, equalizing r sex, litter and weight. The supplemented diets were made using the me compounds used in the above study. The basal diet was supplemented th 10 ppm of Mn from $MnSO_4 \cdot H_2O$, $MnCO_3$ or MnO to make diets 2, 3, and respectively.

The pigs weighed about 8 to 10 kg initially. They were housed in dividual stainless steel metabolism cages for a 7-day adjustment riod followed by two balance trials. The first was a replicated tin square design in which all animals were fed all the diets during ur collection periods. The second was a split-plot design in which ree repeated collections were made on the animals maintained on the ne diets. The pigs were removed from the cages three times daily and dividually fed an amount of food and water which could be consumed thin a 5- to 10-minute period. The feed was mixed with water to make slurry for quick consumption. Following feeding, the pigs' mouths re wiped clean to avoid contamination of excreta. The pigs were then turned to the cages.

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[1] New Brunswick Scientific, New Brunswick, N.J.

BLE 1. DIETS USED IN EXPERIMENT 1

gredient	Diet			
	Basal	+ $MnSO_4 \cdot H_2O$	+ $MnCO_3$	+ MnO
rn, shelled, ground	79.2	78.2	78.2	78.2
ybean meal, dehulled solvent (49% CP)	17.9	17.9	17.9	17.9
lt	0.5	0.5	0.5	0.5
mestone, grd (38% Ca)	0.9	0.9	0.9	0.9
calcium phosphate	1.0	1.0	1.0	1.0
M premix[1]	0.5	0.5	0.5	0.5
$SO_4 \cdot H_2O$ premix		1.0		
CO_3 premix			1.0	
O premix				1.0
	100	100	100	100
vel of CP (calculated), %	16	16	16	16
vel of Mn (analyzed), ppm	16.2	26.0	24.8	27.0

[1]See Appendix A, Table A-1.

Fecal and urine collections were made over a 3-day period, with -day intervals in between each collection. Feces were separated from ine by means of a fine screen placed over the urine collection funnel. e pigs consumed near *ad libitum* quantities of feed daily, and constant ily feed intakes were maintained throughout the balance periods. consumed feed was collected after each feeding during the 3-day llection period to get an accurate measure of feed intake during the lance trial.

Feces were oven-dried for 24 hours, equilibrated to room temperature r 12 hours, weighed, ground and stored in sealed plastic containers. fused feed was air-dried, weighed and discarded. Urine was collected polyethylene containers and acidified with 6N HCl. Following the llection period, the urine volume was recorded and 100 ml aliquots re stored in acid-washed polyethylene bottles at 4°C.

The animals were bled weekly from the anterior vena cava before e start of each collection period for the determination of serum kaline phosphatase and serum Mn concentration. All the animals were lled after the second balance trial. The following tissues were llected, weighed and stored in a freezer in polyethylene bags: liver, dneys, spleen, testes, heart, left femur, 8th left rib, pancreas and n muscle from the left leg. Hair samples were collected from the imals at the beginning and at the end of the experiment from the loin gion of each animal.

. Experiment 2

The gastrointestinal flux pattern of Mn from different Mn sources. romic oxide was added (at 0.3%) to each of the diets used in Experiment Pigs were fed these diets for a period of 4 days before they were

Feces voided were weighed and grab samples of the feces were
for further analyses.

gs were killed one and one-half to three hours postfeeding. The
ary tract was quickly exposed and sectioned into the stomach,
small intestine, caudal small intestine, cecum, colon and
The sections were ligated to prevent movement of the contents.
were removed quantitatively from each section, weighed, and the
rmined on a small portion. The remainder was thoroughly mixed
ided into 20 g portions. These were placed in polyethylene bags
zen for later analysis.

e indicator method for determining digestibility was used to
e absorption and secretion of Mn along the gastrointestinal
The following equation was used:

$$\text{tibility} = 100 - \left(\frac{\%\text{ indicator in feed}}{\%\text{ indicator in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in feed}}\right) 100$$

on and Harris, 1969). In order to measure the net absorption
retion in the different sections of the alimentary tract as the
moved posteriorly, two consecutive sections of the tract were
relation to the above equation. The digesta in a given section
sidered to be the feed for the next posterior section. The
in the second section would be equivalent to the feces for the
tion using the ratio technique equation. Thus net absorption or
on was calculated in the following alimentary sections as
ed:

Stomach - using feed as feed and stomach digesta as feces.

Cranial small intestine - using stomach digesta as feed and
small intestine digesta as feces.

3. Caudal small intestine - using cranial small intestine digesta
eed and caudal small intestine digesta as feces.

4. Cecum - using caudal small intestine digesta as feed and cecal
sta as feces.

5. Colon - using cecal digesta as feed and digesta in the colon
eces.

6. Rectum - using colon digesta as feed and the feces voided as
s.

With this method, a positive value indicated net absorption from
section and a negative value, net secretion into that section of
gastrointestinal tract.

Experiment 3

The effect of high levels of Ca and P supplementation and an inverse ratio on Mn utilization by the growing pig.

A feeding trial using a 2^3 factorial design was conducted. Eighty
ling pigs weighing approximately 8 kg were randomly allotted to
eatments shown in Table 2, equalizing for sex, litter and weight.
ng the trial, the animals were housed in confinement on slotted
rete floors. Feed and water were provided *ad libitum*. The pigs
weighed every 2 weeks and 2 animals per lot, selected at random,
bled every 4 weeks to monitor changes in blood constituents.
l samples were taken from the anterior vena cava.

After 10 weeks, the experiment was terminated when the animals
ied an average of 60 kg. Four animals from each lot, including the
mals that were regularly bled, were slaughtered at the university
Laboratory and tissue samples collected for chemical, physical
istopathological analyses. The tissues collected included bones,

LE 2. DIETS USED IN FEEDING TRIAL (EXPERIMENT 3)

	1	2	3	4	5	6	7	8
% (calculated)	.7	.7	.35	.35	.7	.7	1.4	1.4
% (calculated)	1.4	1.4	.7	.7	.35	.35	.7	.7
ppm (analyzed)	15.3	56.4	15.9	55.7	15.2	56.1	16.0	55.8
	753	743	789	779	791	781	761	751
ean meal de-lled solvent, 9% CP)	188	188	183	183	181	181	186	186
	5	5	5	5	5	5	5	5
estone					17	17	23	23
lcium phosphate	26	26	12	12			19	19
premix[1]	5	5	5	5	5	5	5	5
remix [2]		10		10		10		10
biotic[3]	1	1	1	1	1	1	1	1
sodium phosphate	22	22	5	5				
	1000	1000	1000	1000	1000	1000	1000	1000

[1]MSU vitamin - trace mineral premix without manganese. See ndix A, Table A-1.

[2]Containing 4000 ppm Mn from $MnSO_4 \cdot H_2O$ reagent grade.

[3]Containing 22 g of chlortetracycline per kg.

rt, pancreas, liver and the left kidney. The bones taken included first left rib and the lateral and medial metacarpals from the t leg. The medial metacarpals were split longitudinally, fixed in fered 10% formalin, sectioned and stained with hematoxylin and eosin histopathologic examination. Two sections were taken from each e, one through the epiphyseal cartilagenous plate from the proximal of the bone and one section through the diaphysis. Sections of the physis and diaphysis were coded as follows: 1, normal, if they had y little cartilage and osteoid; 2, very slight change, cartilage and eoid persisting in the bony spicules distal to the epiphyseal tilagenous plate or distal from the periosteum of the diaphysis; 3, slight change, 4, moderate change, or 5, severe change, depending the degree of persistence of cartilage in bony spicules.

Experiment 4

The Mn requirement of the baby pig from sows fed a low Mn diet.

nt one-month pregnant first litter gilts were fed a low Mn diet wn in Table 3 throughout the remainder of the gestation period. er farrowing, 20 baby pigs were taken from the sows at 5 days of age placed in stainless steel rearing cages equipped with stainless el feeders and water troughs. The room temperature was maintained 30°C for the period of the trial. The pigs were weaned to the basal purified diet (no. 1) shown in Table 4. Pigs were taught to consume feed by placing small amounts in the animal's mouth. Some pigs lily adapted to the dry feed, but many were affected with diarrhea, me weak, refused to eat and eventually died.

After adapting to the diets, 12 healthy pigs were randomly allotted he three dietary treatments shown in Table 4. The pigs were fed

3. LOW MANGANESE GESTATION DIET (EXPERIMENT 4)

Ingredient	Amount, kg[2]
Corn	1915
Limestone	20
Dicalcium phosphate	30
Salt	10
VTM premix[1]	10
Vit. E premix	5
Lysine (50%)	10
	2000

[1]See Appendix A, Table A-1 supplied all the trace minerals : Mn.

[2]Level of manganese 11.2 ppm as analyzed.

LE 4. PURIFIED DIETS USED IN EXPERIMENT 4

m	Diets 1	2	3
ein	30	30	30
elose	55	52	46
lulose	3	3	3
d	5	5	5
amin premix[1]	1	1	1
premix[2]		3	9
eral premix[3]	6	6	6
	100	100	100
concentration, ppm analyzed)	0.46	2.67	6.34

[1]See Appendix A, Table A-3.

[2]Manganese premix containing 100 ppm Mn from $MnSO_4 \cdot H_2O$, J. T. er reagent grade.

[3]See Appendix A, Table A-1.

libitum and had free access to water, which was changed 3 times a . All feed was weighed daily and individual feed consumption was orded. Pigs were individually weighed every 4 days throughout the day trial. One pig (234-4F) on the basal diet died suddenly and the se of death was determined to be heart failure. The pigs were bled the 9th, 21st, and 28th day of the feeding trial.

After the feeding trial, the pigs were used for a Mn balance dy. Another pig (241-11) on diet 3 was eliminated from the study to an infection in the left rear leg. The pigs were housed, treated, fed as described in Experiment 1. The collection and treatment of feces, urine and refused feed was similar to the procedures described Experiment 1.

Analyses

1. Hematological Determinations

a. Hemoglobin. In Experiments 1 and 3, hemoglobin was ermined by the cyanmethemoglobin method of Crosby *et al.* (1954). oleman Junior II spectrophotometer was used to determine optical sity at 540 nm.

b. Hematocrit. Hematocrit was determined by the micro method Govern *et al.*, 1955) in Experiments 1 and 3. Blood samples were trifuged for 5 minutes at 10,000 rpm in an International "Hemacrit" trifuge.

2. Physical Determinations

a. Bone. In Experiment 1, the left femur and the 8th left rib e removed and cleaned of all connective tissue and periosteum and

d in air-tight polyethylene bags. The ribs were used for specific
ty determinations and the femurs for breaking strength and related
eters.

In Experiment 3, the 5th metacarpal was removed from the left foot,
ed of connective tissue and stored in a cold room in air-tight
thylene bags. The first left rib was obtained and treated as the
arpal. Bone strength and density tests were made on both the
and the metacarpals.

Specific gravity was determined according to the following formula:

$$\text{Specific gravity} = \frac{\text{weight in air}}{\text{weight in air - weight in water}}$$

In Experiments 1 and 3, the strength of the metacarpals, femurs,
ibs was determined using an Instron Testing Instrument, Model-TT
equipped with an FM-compression load cell having 100 kg full scale.
ross-head speed and chart speed were 0.2 cm/minute and 2 cm/minute,
ctively. Metal fulcra were used to support the metacarpals and
and the distance between fulcra was maintained constant at 3 and
respectively. The femurs varied in size and therefore the dis-
between supporting fulcra varied from bone to bone. The formulas
alculating the various strength characteristics were those
ibed by Miller *et al.* (1962) and are as follows:

Maximal bending moment	$M = Wl/4$
Moment of inertia	$I = (Bd^3 - bd^3)/64$
Maximal stress	$S = MD/2I$
Elasticity	$E = Wl^3/48Iy$

[1]Instron Engineering Corporation, Canton, Mass.

W = maximal load

l = distance between fulcra

B,b = outer and inner horizontal diameter

D,d = outer and inner vertical diameter

y = deflection at center of bone when load W is applied

3. Chemical Determinations

a. Blood. Upon withdrawal, the blood was placed in acid-ed centrifuge tubes, allowed to clot and centrifuged at 2000 g for inutes. The cell-free serum was then harvested and placed in acid-ed vials. Small amounts were stored in the cold room (4°C) for rmination of alkaline phosphatase activity, and the remaining serum ies were frozen for subsequent mineral analysis.

(1) Serum alkaline phosphatase. Determination of serum line phosphatase activity was made according to the procedure ribed in Sigma Technical Bulletin No. 194 (1963). The Sigma 104 hatase substrate was used in the enzyme activity determination. kman Model DU spectrophotometer was used for optical density rminations in Experiments 1, 3 and 4.

(2) Serum calcium and phosphorus. To one milliliter of from Experiment 3 was added 4 ml of 12.5% TCA. Serum proteins ipitated by the 12.5% TCA were centrifuged out at 2000 g for 15 es, and the resulting protein-free supernatant was diluted 1:1 with tium mixture A[1] to suppress phosphate interference. Calcium was

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[1] 61.0 g strontium chloride ($SrCl_2 \cdot 6H_2O$) + 10.0 g sodium chloride).

determined at 422.6 nm by atomic absorption spectrophotometry using Jarrell-Ash[1] Model 82-516 spectrophotometer equipped with a Hetco l consumption burner and an air-hydrogen flame, as described by ey *et al.* (1967).

For phosphorus determinations the Gomorri (1942) method was used. optical density was determined on a Coleman Junior II spectrophoter at 700 nm after a 45-minute incubation period.

(3) Serum magnesium. Cell-free serum samples from Experis 3 and 4 were diluted 1:100 with strontium mixture B[2] to avoid phate interference. Magnesium was determined with a Jarrell-Ash ic absorption spectrophotometer at 285.2 nm in Experiment 3, and xperiment 4 an Instrumentation Laboratories, Inc.,[3] Model 453 ic absorption spectrophotometer was used.

(4) Serum manganese-neutron activation

(a) Principle. Mn in serum from Experiments 1 and s determined by modification of the neutron activation procedure of *et al.* (1968). The principle involved exposure of a small amount erum to a thermal neutron flux in a nuclear reactor and the follownuclear reaction occurred:

$$^{55}Mn(n, \gamma)\rightarrow ^{56}Mn$$

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[1] Jarrell-Ash Co., Waltham, Mass.

[2] 30.5 g strontium chloride ($SrCl_2 \cdot 6H_2O$) + 5.0 g sodium chloride l).

[3] Instrumentation Laboratories, Inc., Lexington, Mass.

The induced ^{56}Mn has a 2.56 hour half-life and emits gamma rays of
5 Mev. The activity of ^{56}Mn can be measured by using gamma-ray
ctrometry, comparing the areas of the 0.85 Mev gamma-ray photopeaks
h those of the induced standards (Anong-Nilubol *et al.*, 1968).

(b) Sample preparation. One milliliter of cell-free
um was pipetted into an acid-washed 250 ml Phillips beaker and 5 ml
concentrated HNO_3 acid were added. The contents were heated gently
boiling and evaporated to dryness. Three milliliters of 1N HNO_3
e added and heated again gently to boiling. After cooling, the
ume was made up to 5 ml. A like method was used to prepare the
ndards and nitric acid blanks.

(c) Irradiation. Four milliliters were pipetted into
yethylene vials,[1] 1.5 cm in diameter. The vials were thoroughly
aned (Jacobson *et al.*, 1961)[2] before loading. The vials were heat-
led, loaded into and activated by the MSU Triga Mark 1 reactor.[3]
samples were subjected to a thermal neutron flux of 2 X 10^{12} n/cm^2/
for a period of 15 minutes. The samples were removed from the
ctor and the radioactivity of the polyethylene vials and samples was
the mrem/hour range and presented no special handling problem.

(d) Radiochemical separation. Radiochemical separa-
n was performed according to the method of Hahn *et al.* (1968). The
adiated samples were transferred to separatory funnels. The vials

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[1] 1.5 cm diameter - Olympia Plastics, Los Angeles, Calif.

[2] See Appendix A, Table A-4.

[3] Gulf General Atomic, Inc., San Diego, Calif.

were rinsed with 8 ml of deionized, distilled water. Two drops of 0.1% brilliant yellow were added and the pH was adjusted to neutral using 5N NH_4OH. Then 2 ml of 5.7% sodium diethyldithiocarbamate and 5 ml of carbon tetrachloride were added. The contents were shaken for exactly 3 minutes and 4 ml of the organic layer were pipetted into clean vials for counting.

(e) Counting procedure. Counting was done about one hour after the samples had been removed from the reactor on a 5.2 multi-channel analyzer - computer series one-thirty[1] utilizing a 3" X 3" NaI well crystal.[2] The voltage was set at 1100 volts. The counts of the integrated areas under the peaks were corrected for both background, decay time and blank. Sample counts were compared to standard counts for quantitation.

(5) Serum manganese - flameless atomic absorption. Mn in the serum samples from Experiment 4 was determined by flameless atomic absorption spectrophotometry on the Instrumentation Laboratories, Inc., Model 355 accessory to the Model 453. Serum samples were diluted 20% w/v. The diluted serum was divided into three 25 µl aliquots. To all but one of these were added known amounts of manganese, 10 µl of either 5 or 10 ngm Mn/ml. The solutions were then placed on the tantalum ribbon and analyzed at 279.4 nm.[3] The samples were dried, pyrolyzed for 90 to 120 seconds and analyzed at a higher temperature than the one used during pyrolysis. The Mn in the serum was calculated using the method of additions (Slavin, 1968).

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[1]Tektronic, Inc., Portland, Ore.

[2]Packard Model, 1212 WSP serial 101-769.

[3]See Appendix A, Table A-5.

b. Bone. The cleaned ribs and metacarpals from Experiments nd 3 used in the strength and density studies were used in the mical analyses as well.

(1) Bone ash. The bones were cut in small pieces with a er hand saw, wrapped in cheesecloth and extracted 24 hours with olute ethanol and 24 hours with anhydrous diethyl ether in a Soxhlet ractor to remove water and fat. The dry, fat-free bone was ashed in uffle furnace at 600°C for 18 hours. The percent ash was calculated m the following formula:

$$\frac{\text{weight of ashed bone X 100}}{\text{weight of dry, fat-free bone}} = \% \text{ ash on a dry, fat-free basis}$$

(2) Bone magnesium, manganese, calcium and phosphorus. The ed bone was finely ground and approximately 300 mg of the powdered e ash were dissolved in 5 ml of 6N HCl. Two milliliter aliquots of resulting ash solution were diluted 1:1 with strontium mixture A and was determined on a Jarrell-Ash atomic absorption spectrophotometer at .4 nm. Bone Mn was expressed as ppm on a dry, fat-free basis.

The remainder of the ash solution was diluted 1:20 with deionized, tilled water for further mineral analysis. The resulting solutions e further diluted 1:100 with strontium mixture B and Ca and Mg were ermined by atomic absorption spectrophotometry as previously described blood serum. Phosphorus was determined by the colorimetric method viously described for serum inorganic P. Bone Ca, P and Mg were ressed as percent of the dry, fat-free bone.

c. Feed, feces and digesta

(1) Manganese. A wet ashing procedure was used. A 0.5 o 1.0 g sample was weighed into an acid-washed 250 ml Phillips beaker. ne milliliter of strontium mixture A was added and the contents igested in 60 ml of concentrated HNO_3 acid on a hot plate to near dry- ess and cooled. A second digestion with 7 ml of 72% perchloric acid as performed. The contents were protected from excessively rapid vaporation by a small water glass. They were heated to near dryness, ooled and samples diluted to volume with deionized, distilled water. tandards were prepared in a like manner. Mn content was determined y atomic absorption spectroscopy using a Jarrell-Ash Model 82-516 pectrophotometer for feed and fecal samples from Experiments 1 and 3. or digesta, feed and fecal samples from Experiments 2 and 4, Mn was etermined with the Instrumentation Laboratories, Inc., Model 453 atomic bsorption spectrophotometer.

(2) Chromium. Chromium was determined by the method of olin *et al.* (1952). A 100 to 500 mg sample of feed, feces, or digesta as weighed into a 50 ml Erlenmeyer flask and 5 ml of oxidizing reagent[1] ere added. The flask was then heated on a hot plate to digest the ixture until it was clear. The mixture was cooled and 2 ml of 72% erchloric acid added and reheated. The flask was cooled to room emperature and diluted to 50 ml using distilled, deionized water. The amples were then read at 470 nm using distilled, deionized water as a lank. The standard curve was prepared by oxidizing known amounts of he reference chromic oxide and diluting as described above.

[1] 10.0 g sodium molybdate + 150 ml distilled deionized water + 50 ml concentrated sulfuric acid and 200 ml 72% perchloric acid.

d. Urine. A twofold concentration and digestion procedure devised due to the low Mn content in urine. Twenty milliliters of ne were pipetted into a Phillips beaker, 1 ml of strontium mixture as added and the contents were digested with 50 ml of concentrated $_3$ acid to near dryness. The flasks were cooled and diluted to 10 ml h deionized, distilled water. Manganese was determined by atomic orption spectroscopy on the Jarrell-Ash unit for urine from Experiment nd on the Instrumentation Laboratories, Inc., Model 453 atomic orption spectrophotometer for urine from Experiment 4. The method specifications of determination were as described previously.

e. Tissues. The tissues included liver, kidney, pancreas, rt, spleen, testes and muscle from both Experiments 1 and 3. Mag- ium and P were determined on only the liver, kidney, pancreas and rt samples from Experiment 3, and Mn was determined on all the ples from both experiments.

(1) Tissue dry matter. Approximately 2 g samples were ghed into disposable aluminum dishes and dried in a vacuum oven for ours at 90°C. Dry matter was calculated as follows:

$$\text{Percent dry matter} = \frac{\text{tissue dry weight X 100}}{\text{tissue fresh weight}}$$

(2) Minerals. Tissue homogenates containing about 1 to of the tissues were pipetted into 250 ml Phillips beakers and a wet ing procedure was used as described previously for feed. The digesta e appropriately diluted and Mn, Ca and Mg were determined by atomic orption spectroscopy and P by the colorimetric method described viously.

f. Hair. Hair samples were soaked for 15 to 20 minutes in
eionized, distilled water, drained on filter paper and then placed
n 95% ethanol for 15 to 20 minutes to remove adhering contaminant
aterials. After removing from the ethanol, the samples were air-
ried and weighed into a 250 ml Phillips beaker and wet-ashed. Mn was
etermined by atomic absorption spectrometry as reported previously.

). Statistical Analysis

All data from Experiments 1, 2, 3, and 4 were subjected to analysis
of variance on a CDC[1] 3600 computer at the Michigan State University
Computer Laboratory Center. The same computer was used to calculate
simple correlations. Differences between means were determined by
Tukey's test for non-additivity.

[1]Control Data Corporation, Minneapolis, Minn.

RESULTS AND DISCUSSION

. Experiment 1: The relative availability of Mn from 16% corn-soy basal diet supplemented with 10 ppm of Mn from either $MnSO_4 \cdot H_2O$, $MnCO_3$ or MnO for the growing pig

1. *In vitro* Solubility of Manganese Compounds

The solubility of manganous sulfate monohydrate ($MnSO_4 \cdot H_2O$), anganous carbonate ($MnCO_3$) and manganous oxide (MnO) is shown in Table 5. The sulfate was soluble in water but the carbonate and oxide were practically insoluble. The sulfate and the carbonate were equally soluble in 0.4% HCl but the oxide was slightly less soluble. These findings are in agreement with those of Watson *et al.* (1971).

TABLE 5. CHEMICAL COMPOSITION AND SOLUBILITY OF DIFFERENT MANGANESE COMPOUNDS[1]

Compound	Mn %	Water			0.4% HCl		
		% Solubility	pH		% Solubility	pH	
			Initial	Final		Initial	Final
$nSO_4 \cdot H_2O$	32.6	96.3	7.0	4.15	100.0	1.03	1.06
nCO_3	44.3	0.91	7.0	7.70	100.0	1.03	1.04
nO	65.0	0.12	7.0	7.65	89.5	1.03	1.10

[1]Water-bath was maintained at 37°C.

The pH dropped sharply after the sulfate dissolved in water (Table , presumably due to dissociation of manganous sulfate and the formation

a predominantly acid medium. With the carbonate and oxide, the pH e slightly. The pH changes after these compounds were dissolved in % HCl were very small.

2. Mn Availability Studies Using a Split-Plot Design (where imals were fed their respective experimental diets for three consecu- ve Mn balance trials and three blood collections)

Weight gains, physical bone measures, and tissue Mn concentra- ons were not affected significantly by source of Mn (Tables 6 and 7). imals receiving supplemental $MnCO_3$ had significantly ($P<0.05$) heavier vers as a percent of bodyweight than those of animals receiving sup- lemental $MnSO_4 \cdot H_2O$. The kidneys, hearts and testes as a percent of dy weight of animals on the basal diet were slightly heavier than ose of animals on the other diets (Table 8).

The physical bone measures presented in Table 9 did not show any nsistent pattern of variation with respect to Mn sources. Mn from l sources was equally well utilized for bone formation. Weight gains d tissue Mn concentration (Table 10) showed that Mn from all the com- unds studied was equally utilized by the pig. This study also suggests at Mn requirements of young pigs for growth are not higher than 16.2 m, based on weight gains on all the diets. The low Mn levels of ssues from pigs on the basal diet may be a reflection of its low Mn ntent. In this study only liver, pancreas, spleen and testes Mn ncentrations showed a substantial response to Mn supplementation.

Hemoglobin, hematocrit, serum Mn concentration and serum alkaline sphatase activity did not differ significantly due to source of Mn; serum Mn, serum alkaline phosphatase and hematocrit did differ nificantly ($P<0.01$) within the different Mn sources due to time of

BLE 6. ANALYSIS OF VARIANCE FOR MN SOURCE EFFECTS ON BONE AND BLOOD PARAMETERS (SPLIT-PLOT DESIGN)

tegory	Mean	Min.	Max.	P-level of F-statistic
emur, left leg				
Weight, g (fresh basis)	72.6	63.9	85.5	0.40
Length, cm[1]	9.2	8.5	9.5	0.12
External diameter,[2] cm				
Horizontal (B)	1.42	1.31	1.5	0.65
Vertical (D)	1.35	1.25	1.5	0.93
Internal diameter, cm				
Horizontal (b)	1.02	0.81	1.31	0.89
Vertical (d)	0.98	0.80	1.20	0.79
Breaking strength, kg	165	140	187	0.67
Inertia, cm^4	1.15	1.03	1.26	0.07
Stress at the center, kg/cm^2	972	856	1089	0.06
Elasticity, 1000 kg/cm^2	27.6	21.6	34.2	0.33
ib, 8th left				
Weight, g (fresh basis)	4.40	3.20	5.49	0.36
Ash, % of dry, fat-free rib	58.6	57.9	59.8	0.19
Specific gravity (fresh basis)	1.25	1.20	1.31	0.70
lood parameters				
Serum Mn, mcg/100 ml	1.65	0.91	2.71	0.28
Hemoglobin, g/100 ml	10.8	9.0	12.2	0.68
Hematocrit, %	33.4	30.2	37.7	0.70
Serum alkaline phosphatase, Sigma units	7.12	5.0	11.4	0.72

[1]Measured from the mid-medial condyle to the fovea.

[2]The horizontal and vertical diameters were measured at mid-haft when the bone was positioned in such a way that the medial and ateral condyles were facing downwards.

BLE 7. ANALYSIS OF VARIANCE FOR MN SOURCE EFFECTS ON MN BALANCE, TISSUE MN CONCENTRATIONS, ORGAN WEIGHTS AND GROWTH DATA (SPLIT-PLOT DESIGN)

ategory	Mean	Min.	Max.	P-level of F-statistic
alance data				
Mn intake, mg/day	8.70	5.94	10.1	<0.0005
Mn excretion, mg/day				
Fecal	3.82	1.77	5.63	0.001
Urinary	.05	.04	.07	0.355
Mn retention, mg/day	4.84	2.52	6.15	<0.0005
Mn retention, % of intake	55.3	41.8	69.6	0.56
Mn excretion, % of intake				
Fecal	44.1	29.6	57.4	0.61
Urinary	.60	.39	.99	<0.0005
Tissue, Mn, ppm (dry matter basis)				
Liver	6.93	5.14	9.03	0.11
Kidney	6.60	5.55	7.87	0.12
Pancreas	4.92	3.93	6.02	0.24
Spleen	2.38	1.94	2.76	0.83
Bone	1.12	0.77	1.54	0.52
Muscle	1.84	1.50	2.22	0.22
Heart	2.51	2.33	2.77	0.80
Testes	3.88	3.22	4.39	0.82
Hair				
Initial value	1.00	0.80	1.33	0.59
Final value	1.17	0.96	1.44	0.22
Organs, % of body weight				
Liver	2.12	1.93	2.33	0.05
Kidney	.33	.29	.37	0.35
Pancreas	.14	.11	.20	0.49
Spleen	.20	.14	.26	0.08
Heart	.49	.44	.58	0.96
Testes	.16	.10	.25	0.36
rowth data				
Average daily gain, g	187	176	225	0.73

TABLE 8. THE EFFECT OF MN SOURCE ON THE PHYSICAL CHARACTERISTICS OF DIFFERENT ORGANS[1]

Item	Diet								
	Basal		+$MnSO_4 \cdot H_2O$		+$MnCO_3$		+MnO		
Mn conc., ppm	16.2		26.0		24.8		27.0		
No. of pigs	3		3		3		3		
	Abs.[3]	% BW[4]	Abs.	% BW	Abs.	% BW	Abs.	% BW	SE[2]
Liver	324	2.10	287	1.90	311	2.20[6]	315	2.10	.03
Kidney	51	0.34	48	0.32	47	0.33	49	0.33	.08
Pancreas	23	0.15	20	0.13	18	0.12	22	0.15	.01
Heart	80	0.53	74	0.49	70	0.48	69	0.46	.08
Spleen	32	0.21	23	0.15	30	0.21	32	0.22	.06
Testes[5]	34	0.22	18	0.12	20	0.13	20	0.14	.07

[1]Based on fresh basis.

[2]Statistical analyses made on percent bodyweight only.

[3]Absolute weight in grams.

[4]As percent of body weight.

[5]Data based on two males on diets 1, 3 and 4 and 3 males on diet 2.

[6]Significantly ($P<0.05$) greater than least value.

TABLE 9. THE EFFECT OF MN SOURCE ON THE PHYSICAL MEASUREMENTS OF THE FEMURS AND RIBS, BREAKING STRENGTH AND RELATED PARAMETERS OF THE FEMURS

Item	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
Mn conc., ppm	16.2	26.0	24.8	27.0
No. of pigs	3	3	3	3
Femur, left				
Weight, g (fresh basis)	73.2	77.0	68.3	71.7
Length, cm[1]	9.3	9.0	9.0	9.5
External diameter,[2] cm				
Horizontal (B)	1.46	1.43	1.37	1.45
Vertical (D)	1.37	1.35	1.32	1.36
Internal diameter, cm				
Horizontal (b)	1.05	1.03	1.03	0.96
Vertical (d)	1.05	0.94	0.94	1.00
Breaking moment, kg	171	166	166	157
Inertia, cm^4	1.12	1.17	1.21	1.09
Stress at center, kg/cm^2	1049	951	909	978
Elasticity, 1000 kg/cm^2	27.6	24.4	30.7	27.8
Rib, 8th left				
Weight, g (fresh basis)	4.9	4.6	3.8	4.5
Ash, % of dry fat-free rib	58.9	58.5	58.6	58.3
Specific gravity (fresh basis)	1.27	1.23	1.26	1.23

[1] Measured from the mid-medial condyle to the fovea.

[2] The horizontal and vertical diameters were measured at mid-shaft when the bone was positioned in such a way that the medial and lateral condyles were facing downwards.

TABLE 10. THE EFFECT OF MN SOURCE ON THE AVERAGE DAILY GAIN (ADG) AND TISSUE MN DISTRIBUTION

Item	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
Mn conc., ppm	16.2	26.0	24.8	27.0
No. of pigs	3	3	3	3
Avg. daily gain, g	200	186	180	182
Tissue Mn, ppm[1]				
Bone, 8th left rib	1.16	1.07	0.95	1.32
Liver	5.7	7.4	7.3	7.3
Kidney	6.7	6.7	6.1	6.9
Heart	2.5	2.6	2.5	2.5
Pancreas	4.5	5.1	4.8	5.3
Spleen	2.0	2.6	2.4	2.5
Testes[2]	3.4	4.1	4.1	4.0
Muscle	1.8	2.0	1.7	1.9
Hair	1.1	1.2	1.3	1.1

[1]Manganese expressed on dry matter basis except for hair and bone. Bone Mn was expressed on dry, fat-free basis and hair on ethanol-cleaned, air-dried basis.

[2]Based on 2 males on diets 1, 3 and 4 and 3 males on diet 2.

sampling (Table 11). Mn retention, fecal excretion and urinary excretion, all as percent of intake, showed significant ($P<0.01$) Mn source effects. Time of sampling, but not Mn source, had a significant ($P<0.05$) effect on urinary Mn excretion. Absolute Mn retention and urinary Mn excretion, as percent of intake, did not differ significantly source X time interaction. Only absolute urinary Mn excretion and urinary Mn excretion as a percent of intake showed a significant ($P<0.05$) source X time interaction.

The hematocrit value at the second sampling was significantly ($P<0.05$) greater than that at the third sampling for animals receiving supplemental $MnCO_3$ (Table 12). Serum alkaline phosphatase activity at the initial sampling of animals on the basal, basal supplemented with $MnSO_4 \cdot H_2O$ and basal supplemented with $MnCO_3$ were significantly ($P<0.05$) greater than at the third sampling. The hematocrit and serum alkaline phosphatase values dropped on the second and third sampling on all diets. Serum alkaline phosphatase levels were lowest on the basal diet. Serum Mn increased with time of sampling on the basal diet and basal diet supplemented with $MnSO_4 \cdot H_2O$. On diets supplemented with $MnCO_3$ and MnO, the levels rose on the second sampling and dropped substantially on the third.

The Mn balance data are summarized in Table 13. The low Mn intake and excretion on the basal diet were a reflection of the lower Mn content of the basal diet as compared to the supplemented diets. The absolute urinary excretion of Mn was the same on all diets. The absolute Mn retention was significantly ($P<0.01$) lower on the basal diet, essentially equal on diets supplemented with $MnSO_4 \cdot H_2O$ and $MnCO_3$, and slightly higher on the diet supplemented with MnO. When Mn retention was expressed as a percent of intake, the supplemented diets showed a

TABLE 11. THE EFFECT OF MN SOURCE AND TIME OF SAMPLING ON THE BLOOD AND BALANCE DATA[1] (SPLIT-PLOT DESIGN)

Item	Mn Source	Time of sampling	Source X time interaction
Serum alkaline phosphatase	0.72	<0.0005	0.41
Serum Mn	0.48	0.007	0.40
Hemoglobin	0.68	0.16	0.09
Hematocrit	0.70	0.001	0.59
Mn intake	<0.0005	0.009	0.28
Mn excretion, absolute			
Fecal	0.001	0.43	0.82
Urinary	0.36	0.028	0.03
Mn retention, absolute	<0.0005	0.38	0.87
Mn retention, % of intake	0.56	0.23	0.59
Mn excretion, % of intake			
Fecal	0.61	0.23	0.59
Urinary	<0.0005	0.13	0.03

[1]P-level of F-statistic.

TABLE 12. EFFECT OF MN SOURCE AND TIME OF SAMPLING ON SOME BLOOD PARAMETERS

Item	Diet											
	Basal			+$MnSO_4 \cdot H_2O$			+$MnCO_3$			+MnO		
Mn conc., ppm	16.2			26.0			24.8			27.0		
No. of pigs	3			3			3			3		
Samplings[1]	1	2	3	1	2	3	1	2	3	1	2	3
Hemoglobin, g/100 ml	10.9	10.4	10.2	11.1	10.1	11.2	11.0	10.7	10.6	10.9	11.3	10.9
Hematocrit, %	33.7	33.4	31.8	34.9	33.7	32.8	33.3	34.1[2]	31.4	34.1	33.8	32.9
Serum alkaline phosphatase, Sigma units	7.9[2]	6.0	5.3	9.3[2]	6.4	6.3	9.0[2]	6.8	6.3	8.1	7.3	7.2
Serum Mn, mcg/ 100 ml	1.1	1.2	1.3	1.5	1.6	1.7	2.0	2.1	1.5	2.0	2.9[2]	1.9

[1] Blood sampling weekly.

[2] Values under the same dietary source significantly ($P<0.05$) different than the least value.

TABLE 13. RETENTION AND ROUTES OF EXCRETION OF MN FROM BASAL DIET AND BASAL DIET SUPPLEMENTED WITH MANGANOUS SULFATE ($MnSO_4 \cdot H_2O$), MANGANOUS CARBONATE ($MnCO_3$) OR MANGANOUS OXIDE (MnO)

Item	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
Mn conc., ppm	16.2	26.0	24.8	27.0
No. of pigs	3	3	3	3
Mn intake, mg/day	6.0	9.6[3]	9.2[2]	10.0[4]
Mn excretion, mg/day				
Fecal	2.7	4.2[1]	3.8	4.2[1]
Urinary	0.1	0.1	0.1	0.1
Mn retention, mg/day	3.2	5.3[2]	5.3[2]	5.7[2]
Mn retention, % of intake	53.4	55.1	57.4	57.0
Mn excretion, % of intake				
Fecal	44.9	43.8	41.3	41.9
Urinary	1.7[4]	1.0	1.1	1.0

[1] Significantly ($P<0.05$) greater than least value.

[2] Significantly ($P<0.01$) greater than least value.

[3] Significantly ($P<0.01$) greater than least two values.

[4] Significantly ($P<0.01$) greater than least three values.

slight advantage over the basal diet. Mn from $MnSO_4 \cdot H_2O$ was retained to a lesser extent than that from the other two compounds. Fecal Mn excretion as a percent of intake was slightly higher on diets supplemented with $MnSO_4 \cdot H_2O$ than those supplemented with $MnCO_3$ and MnO. Urinary Mn excretion as a percent of intake was significantly ($P<0.01$) different between the basal and the supplemented diets, but not within the supplemented diets. Fecal and urinary Mn excretion, both as a percent of intake, on the basal diet were higher than that on the supplemented diets. Regardless of dietary Mn source, over 90% of the excreted Mn was found in the feces.

3. Mn Availability Studies Using a Replicated Latin Square Design (where all animals were subjected to all experimental diets in four collection periods)

The Mn source had no significant effect on the blood parameters (Table 14). There was a slightly lower serum Mn on the basal diet, presumably as a reflection of the lower Mn content of the diet. All sources were equally effective in maintaining the levels of hemoglobin, hematocrit and serum alkaline phosphatase (Table 15). Mn retention, fecal and urinary Mn excretion, all as percent of intake, were significantly ($P<0.01$) different between treatments (Table 16). Mn intake and absolute fecal Mn excretion and retention were significantly ($P<0.01$) different, but not absolute urinary Mn excretion.

The absolute fecal Mn excretion on the basal diet was significantly ($P<0.01$) lower than on the supplemented diets. Absolute fecal Mn excretion was slightly higher on diets supplemented with $MnSO_4 \cdot H_2O$ and MnO than those supplemented with $MnCO_3$. The absolute urinary Mn excretion was equal on all diets but, when expressed as a percent of intake, the

TABLE 14. EFFECTS OF MN SOURCE ON BLOOD AND MN BALANCE MEASURES (REPLICATED LATIN SQUARE DESIGN)

Item	Mean	Min.	Max.	P-level of F-statistic
Blood parameters				
Hemoglobin, g/100 ml	10.74	8.17	14.92	0.181
Hematocrit, %	33.95	31.00	44.80	0.528
Serum alkaline phosphatase, Sigma units	7.42	4.70	11.40	0.559
Serum Mn, mcg/100 ml	1.94	1.26	3.34	0.160
Balance data				
Mn intake, mg/day	7.08	4.65	9.41	<0.0005
Mn excretion, mg/day				
Fecal	3.63	2.54	4.98	<0.0005
Urinary	.030	.008	.067	0.264
Mn retention, mg/day	3.39	1.49	5.72	<0.0005
Mn retention, % of intake	47.37	30.00	67.02	<0.0005
Mn excretion, % of intake				
Fecal	52.14	32.82	69.11	<0.0005
Urinary	.14	.39	.88	<0.0005

TABLE 15. EFFECT OF MN FROM DIFFERENT SOURCES ON SOME BLOOD MEASUREMENTS

Item	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
Mn conc., ppm	16.2	26.0	24.8	27.0
No. of pigs	12	12	12	12
Hemoglobin, g/100 ml	11.1	10.4	10.9	10.6
Hematocrit, %	34.5	34.3	33.7	33.4
Serum alkaline phosphatase, Sigma units	7.56	7.39	7.67	7.06
Serum Mn, mcg/100 ml	1.70	2.15	1.76	2.25

TABLE 16. RETENTION AND ROUTES OF EXCRETION OF MN FROM BASAL DIET AND BASAL DIET SUPPLEMENTED WITH MANGANOUS SULFATE ($MnSO_4 \cdot H_2O$), MANGANOUS CARBONATE ($MnCO_3$) AND MANGANOUS OXIDE (MnO)

Item	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
Mn conc., ppm	16.2	26.0	24.8	27.0
No. of pigs	12	12	12	12
Mn intake, mg/day	5.17	8.31[4]	7.93[2]	8.63[6]
Mn excretion, mg/day				
Fecal	3.10	4.26[2]	3.91[2]	4.24[2]
Urinary	0.030	0.034	0.030	0.028
Mn retention, mg/day	2.04	4.02[2]	3.99[2]	4.36[2]
Mn retention, % of intake	38.8	48.0[1]	50.1[2]	50.3[2]
Mn excretion, % of intake				
Fecal	60.6[4/5]	51.6	49.5	49.4
Urinary	0.56[6]	0.39	0.36	0.31

[1]Significantly ($P<0.05$) greater than least value.

[2]Significantly ($P<0.01$) greater than least value.

[3]Significantly ($P<0.05$) greater than least two values.

[4]Significantly ($P<0.01$) greater than least two values.

[5]Significantly ($P<0.05$) greater than least three values.

[6]Significantly ($P<0.01$) greater than least three values.

pigs on the basal diet excreted significantly ($P<0.01$) more urinary Mn than the pigs on supplemented diets. Within the supplemented diets, urinary Mn excretion was not significantly different. Absolute Mn retention was significantly ($P<0.01$) higher on the supplemented diets than on the basal diet. Mn from the diet supplemented with MnO was retained in somewhat greater amounts than Mn from those diets supplemented with $MnCO_3$ and $MnSO_4 \cdot H_2O$, but these differences were not statistically significant. When expressed as a percent of intake, Mn retention on the basal diet was significantly ($P<0.05$ or $P<0.01$) lower than that on supplemented diets. Fecal Mn excretion, as a percent of intake, on the basal diet was significantly ($P<0.05$ or $P<0.01$) higher than that on the supplemented diets, but the differences between the supplemented diets were not significant. Irrespective of Mn source, the main route of Mn excretion was fecal, and urinary Mn excretion was very small and constant on all diets.

4. Discussion of the Results of Experiment 1

The low Mn retention on the basal diet and the high Mn excretion on the same diet agrees with the findings of Morimoto *et al.* (1959), who showed poor Mn availability to chicks when soy protein was used in the diet, and of Davis *et al.* (1962), who reported that soy protein contained a component which combined with Mn, making it unavailable. In this particular study, most of the Mn in the basal diet was supplied by soy protein. The dominance of the fecal route as a means of Mn excretion and the small but constant urinary Mn excretion found in this study have been reported by others in other species (Underwood, 1971; Thomas, 1970; Miller, 1973). The 40 to 50% Mn retention by the pig shown in this study parallels Mn retention in the human reported by North *et al.*

(1960). Brown and McCracken (1965) found that chickens retained 32% of the absorbed Mn.

The conclusion that Mn in $MnSO_4 \cdot H_2O$, $MnCO_3$, and MnO is equally available to a pig for growth agrees with the findings in other species. Schaible *et al.* (1938) and Gallup and Norris (1939) found that the sulfate, carbonate and oxide were equally effective in preventing perosis in chickens. Cotzias and Greenough (1958) and Zajvec (1959) have reported that the absorption of Mn was not affected by its valency state in the compounds used. But more recently others have reported that Mn oxides are not as available as the sulfate, carbonate and chloride (Anke *et al.*, 1967; Watson *et al.*, 1970, 1971).

Serum alkaline phosphatase and serum Mn levels were equal on all diets, which probably indicates that Mn from all sources was equally available to the growing pig or that the levels of Mn provided in all diets were high enough to sustain normal levels of serum alkaline phosphatase and serum Mn. Mn deficiency has been shown to reduce serum alkaline phosphatase and serum Mn levels in swine as well as other species (Plumlee *et al.*, 1956; Rojas *et al.*, 1965; Swaney and Kehar, 1958; Hawkins *et al.*, 1955; Ugnenko, 1972). The effect of time of sampling (age of the pig) on hematocrit and serum alkaline phosphatase values found in this study is supported by the findings of Miller *et al.* (1961), who showed a similar drop at a similar age of pigs. Long *et al.* (1965) also reported high serum alkaline phosphatase values in young pigs which gradually dropped with age.

In this study, liver, testes, and spleen Mn concentration increased with dietary Mn levels, which is in agreement with the reports of Leibholz *et al.* (1962), Johnson (1943, 1944), Grummer *et al.* (1950), and Underwood (1971). The response of spleen to Mn supplementation has

been shown in rats by Ugnenko (1972). Hair and bone, which were reported to respond to Mn supplementation by Leibholz *et al.* (1962), did not do so in this study. This may be due to the fact that Leibholz *et al.* (1962) used a 100-fold margin between the basal diet and the supplemented diet as compared to a twofold margin used in this study.

B. Experiment 2: Study of the gastrointestinal flux pattern of Mn from different Mn sources using chromic oxide (Cr_2O_3) as an indicator

The results of this study are summarized in Tables 17, 18 and 19 and Figures 2.1 through 2.4. The cranial small intestine was a major route of absorption for Mn from the supplemented diets but not for Mn from the basal diet. There was no net Mn absorption by the caudal small intestine. Hendricks (1967) showed that pigs fed a 16% soy protein diet supplemented wtih Mn from $MnSO_4 \cdot H_2O$ displayed net absorption in the stomach and cecum only. Since there is a large amount of Mn in the bile (Kent and McCance, 1941; Mahoney and Small, 1968; Starodubova, 1968), and bile is screted into the cranial small intestine, it is apparent that in the case of Mn from the supplemented diets, the cranial small intestine must be absorbing Mn faster than it is being secreted into this section of the gut. This finding makes the cranial small intestine a very important and efficient homeostatic mechanism for regulating Mn levels in the body. Britton and Cotzias (1966) concluded that it is the variable excretion rates rather than regulated absorption that seem to maintain constant tissue Mn levels. Howes and Dyer (1971) and Miller (1973) reported that absorption differences are the main homeostatic control mechanisms for body Mn levels.

Although digesta pH did not differ significantly between treatments (Table 18), there was a substantial treatment pH difference in the

TABLE 17. NET ABSORPTION AND SECRETION OF MN IN THE DIFFERENT SECTIONS OF THE GUT BY THE GROWING PIG FED DIFFERENT MN SOURCES[1]

	Diet							
	Basal		+$MnSO_4 \cdot H_2O$		+$MnCO_3$		+MnO	
Section of gut	Abs.[2]	Sec.[3]	Abs.	Sec.	Abs.	Sec.	Abs.	Sec.
Stomach	60		61		49		52	
Small intestine[4]								
Cranial		129	22		62		60	
Caudal		185		560[5]		879[6]		967[6]
Cecum	52		41		19		34	
Colon		39		33		24		12
Rectum		64		138		13		119

[1]Net flux as percent of Mn in feed or digesta in previous gut section.

[2]Net absorption.

[3]Net secretion.

[4]Divided roughly in two halves.

[5]Significantly ($P<0.01$) greater than least value.

[6]Significantly ($P<0.01$) greater than least two values.

TABLE 18. GUT CONTENT WEIGHTS AND pH[1]

Section of gut	Diet			
	Basal	+$MnSO_4 \cdot H_2O$	+$MnCO_3$	+MnO
pH values				
Stomach	2.75	3.36	3.19	3.07
Small intestine[2]				
Cranial	6.52	6.25	5.59	6.40
Caudal	6.61	6.13	6.77	6.70
Cecum	5.97	6.38	5.80	6.14
Colon	6.09	6.19	6.08	6.10
Rectum	6.45	6.42	6.39	6.39
Gut contents, g (wet basis)				
Stomach	459[5]	348	351	331
Small intestine				
Cranial	314	347	323	292
Caudal	361	323	330	358[3]
Cecum	442	498[4]	372	458[3]
Colon	74	91	163	125
Rectum	72	81	62	61

[1]Based on three animals per treatment.

[2]Divided roughly into two halves.

[3]Significantly ($P<0.05$) greater than least value.

[4]Significantly ($P<0.001$) greater than least value.

[5]Significantly ($P<0.05$) greater than least three values.

TABLE 19. MEAN, MINIMUM AND MAXIMUM VALUES OF pH, WET GUT CONTENTS AND NET ABSORPTION AND SECRETION IN DIFFERENT SECTIONS OF THE GUT

Item	Mean	Min.	Max.	P-level of F-statistic
pH				
Stomach	3.09	1.88	3.88	0.82
Small intestine[2]				
Cranial	6.21	5.02	7.20	0.29
Caudal	6.55	5.36	7.34	0.39
Cecum	6.07	5.38	6.83	0.50
Colon	6.12	5.72	6.38	0.92
Rectum	6.41	6.05	6.90	0.99
Gut contents, g				
Stomach	372	286	496	0.01
Small intestine				
Cranial	319	265	386	0.25
Caudal	343	278	405	0.75
Cecum	113	42	182	0.02
Colon	443	285	553	0.13
Rectum	69	50	93	0.21
Net absorption (+) or secretion (-)[1]				
Stomach	56	41	78	0.63
Small intestine				
Cranial	6	-224	78	0.01
Caudal	-648	-1176	2	0.02
Cecum	36	5	73	0.06
Colon	-21	-85	22	0.21
Rectum	-1002	-264	-1	0.24

[1]Net flux as percent of Mn in feed or digesta in previous gut section.

[2]Divided roughly into two halves.

Figure 2.1. Summary of net Mn flux from sections of the gastrointestinal tract of the growing pig fed the basal diet.

Section of the gut	PH	Digesta WT., GM.	Net Flux as Percent of Mn in Feed or Digesta in Previous Gut Section
Stomach	2.75	459.0	
Cranial S.I.	6.52	314.3	
Caudal S.I.	6.61	361.0	
Cecum	5.97	441.7	
Colon	6.09	73.7	
Rectum	6.45	72.3	

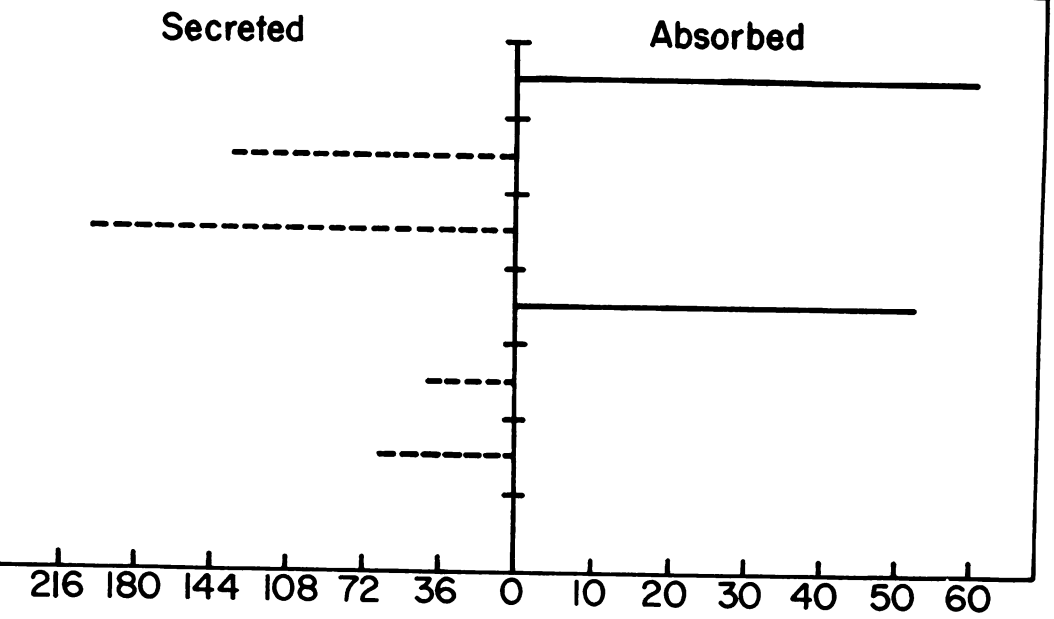

Figure 2.1.

Figure 2.2. Summary of net Mn flux from sections of the gastrointestinal tract of the growing pig fed the basal diet supplemented with $MnSO_4 \cdot H_2O$.

Section of the gut	PH	Digesta WT., GM.	Net Flux as Percent of Mn in Feed or Digesta in Previous Gut Section
Stomach	3.36	347.7	
Cranial S.I.	6.26	347.3	
Caudal S.I.	6.16	322.7	
Cecum	6.36	498.3	
Colon	6.19	90.7	
Rectum	6.47	80.7	

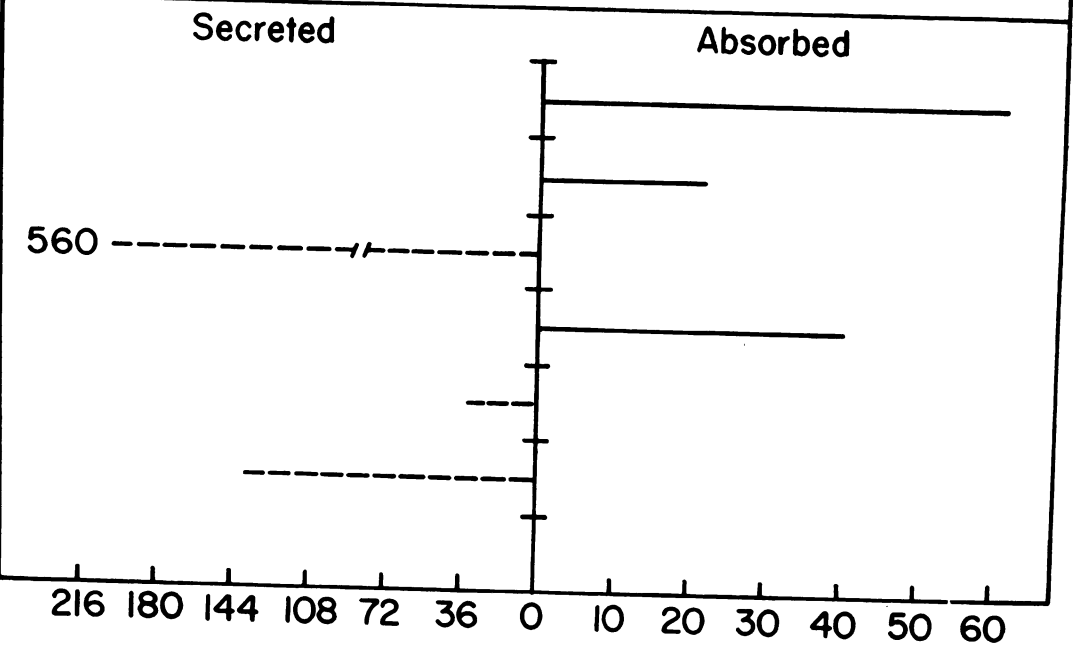

Figure 2.2.

Figure 2.3. Summary of net Mn flux from sections of the gastrointestinal tract of the growing pig fed the basal diet supplemented with $MnCO_3$.

Section of the gut	PH	Digesta WT., GM.	Net Flux as Percent of Mn in Feed or Digesta in Previous Gut Section
Stomach	3.19	351.0	
Cranial S.I.	5.59	322.7	
Caudal S.I.	6.77	330.0	
Cecum	5.80	371.7	
Colon	6.08	162.7	
Rectum	6.39	62.0	

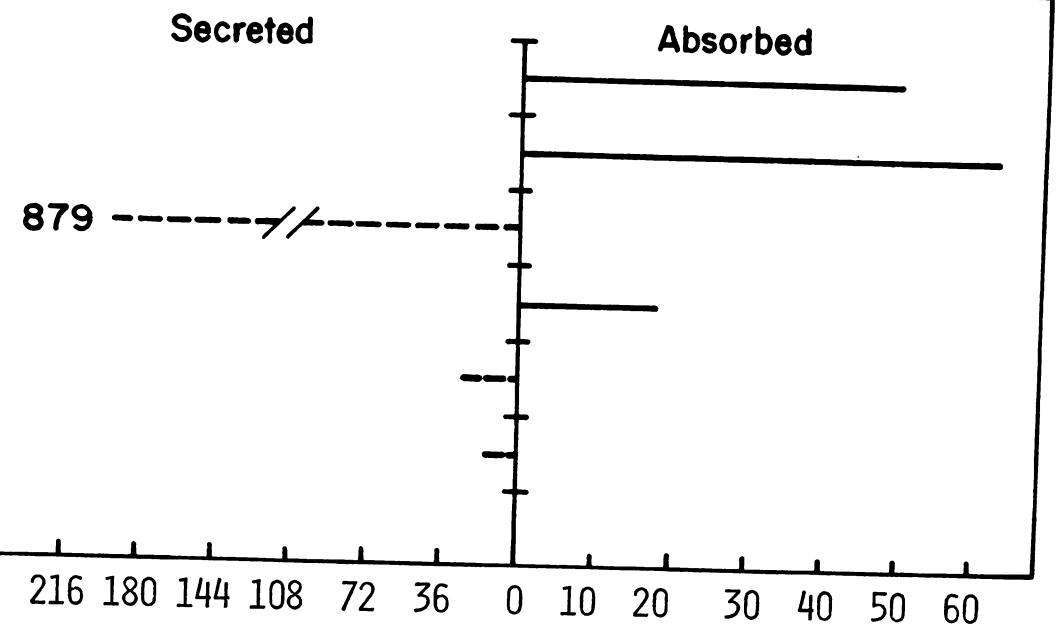

Figure 2.3.

Figure 2.4. Summary of net Mn flux from sections of the gastrointestinal tract of the growing pig fed the basal diet supplemented with MnO.

Section of the gut	PH	Digesta WT., GM.	Net Flux as Percent of Mn in Feed or Digesta in Previous Gut Section
Stomach	3.07	331.7	
Cranial S.I.	6.40	292.0	
Caudal S.I.	6.70	357.7	
Cecum	6.14	458.3	
Colon	6.10	124.7	
Rectum	6.39	61.3	

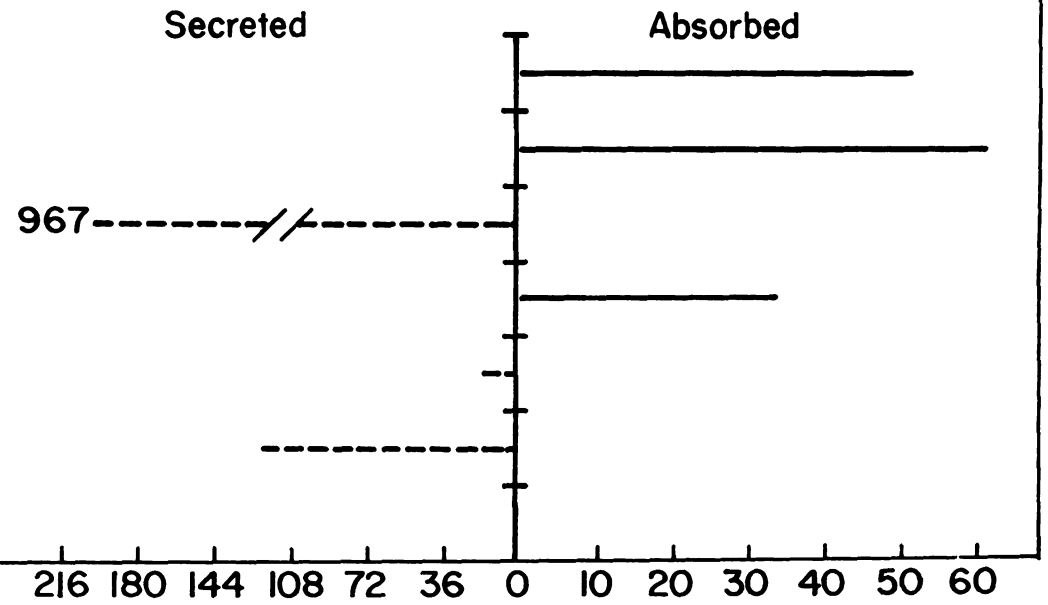

Figure 2.4.

cranial small intestine, being more acid in the case of pigs fed the supplemented diets than those fed the basal diet (6.08 vs 6.52). This might account for the differences in absorption rates. It could be postulated that the Mn absorption mechanisms might be more efficient at a lower pH in the cranial small intestine than at a higher pH. Manganese from the basal diet is presumably held in some form which makes it less available for absorption in this region of the gut. Davis *et al.* (1962) reported a factor in soybean which tends to tie up Mn and make it unavailable. Most of the Mn in the basal diet was supplied by soy protein.

In Experiment 1 it was shown that when soybean meal and corn were the sole sources of Mn, there was a relatively high fecal Mn excretion and a low Mn retention as a percent of intake, plus low tissue Mn concentrations. This indicated a lower net absorption of Mn from the basal diet, perhaps due in part to the fact that net absorption of Mn from the basal diet occurred only in the stomach and cecum.

The net absorption of Mn from the basal diet (feed sources) in the stomach was slightly higher than that of Mn from the supplemented diets, but in the cecum the net absorption of Mn from the basal diet was much higher than that of Mn from the supplemented diets. The gut fill in the stomach and cecum was significantly ($P<0.05$) different between treatments. The stomach contents of the animals on the basal diet were significantly ($P<0.05$) heavier than those of the animals on other diets. The wet weight of the cecal contents of animals on the diets supplemented with $MnSO_4 \cdot H_2O$ and MnO were significantly ($P<0.05$ or $P<0.01$) heavier than those of animals on the basal diet (Table 18). Within the supplemented diets, the net absorption of Mn from the diet supplemented with $MnSO_4 \cdot H_2O$ was highest and that from the diets

supplemented with $MnCO_3$ lowest in both the stomach and cecum. In the cranial small intestine, the net absorption of Mn from the diets supplemented with $MnCO_3$ was highest while that from the diets supplemented with $MnSO_4 \cdot H_2O$ was lowest. The net absorption of Mn from diets supplemented with MnO was intermediate in the stomach, cranial small intestine and cecum.

The net secretion of Mn was significantly ($P<0.01$) higher on the supplemented diets when compared to the basal diet in the caudal small intestine, slightly higher in the rectum but slightly lower in the colon. Within the supplemented diets, the net Mn secretion was significantly ($P<0.01$) higher on diets supplemented with MnO and $MnCO_3$ than on diets supplemented with $MnSO_4 \cdot H_2O$ in the caudal small intestine (Table 17). The net Mn secretion on diets supplemented with MnO or $MnCO_3$ was not significantly different. Net Mn secretion in the rectum was much greater on diets supplemented with $MnSO_4 \cdot H_2O$ and MnO than on the diets supplemented with $MnCO_3$. In the colon, the net Mn secretion was highest on diets supplemented with $MnSO_4 \cdot H_2O$ and lowest on diets supplemented with MnO. Hendricks (1967) reported net Mn secretion into the cranial small intestine, caudal small intestine, and colon when pigs were fed a 16% soy protein diet supplemented with Mn from $MnSO_4 \cdot H_2O$. He showed a high net Mn secretion in the colon, a low net Mn secretion in the cranial and caudal small intestine, and an absence of Mn secretion in the rectum.

C. Experiment 3: The effect of high level Ca and P supplementation and an inverse Ca-P ratio on Mn utilization by the growing pig

The effects of different Ca to P ratios, Ca and P levels and Mn supplementation on physical and chemical composition of pig tissues are summarized in Tables 20, 21, and 22. A 2 to 1 ratio of Ca to P significantly ($P<0.05$) depressed rib Mn content and slightly increased

heart, pancreas and serum Mn as compared to a 1 to 2 ratio. The increased levels of Ca and P supplementation significantly ($P<0.01$) increased rib and pancreas Mn concentration but also significantly ($P<0.01$) depressed Mn concentration of the metacarpal bone. The high levels of Ca and P slightly increased heart Mn and significantly ($P<0.05$) increased serum Mn concentration. This finding is at variance with those of Hawkins *et al.* (1955), who reported a suppression of serum Mn with high Ca and P intakes in cattle. Lassiter *et al.* (1970) reported that rats given a 0.9 percent P in the diet caused significantly higher retention of orally administered ^{54}Mn than did 0.4 percent P.

Mn supplementation increased heart Mn significantly ($P<0.01$) and slightly increased rib, pancreas and serum Mn, kidney Ca and serum inorganic P. High Mn levels in the diets significantly ($P<0.05$) depressed rib Ca and Mg and slightly depressed metacarpal Mn, P and Mg, serum Ca, and rib and kidney P. The slight increase of Mn in serum following increased Mn intakes is in harmony with other observations in swine (Plumlee *et al.*, 1956), and with some reports in cattle (Rojas *et al.*, 1965; Hawkins *et al.*, 1955), in rats (Ugnenko, 1972), and in poultry (Bolton, 1955). Other reports, however, are in contrast to these findings (Krieg, 1966; Bentley and Phillips, 1951a). In poultry, Nielsen and Madsen (1942) observed no appreciable difference in blood Ca concentration due to dietary Mn levels, and reported that acid soluble P of the blood and inorganic P of plasma did not differ significantly due to Mn supplementation.

Although Mn has been implicated in bone formation (Underwood, 1971), the results of this study showed no significant effect of Mn supplementation on rib and metacarpal physical measurements, breaking strength and related parameters (Tables 20 and 21). Dietary Mn levels did not

TABLE 20. EFFECT OF THE CA-P RATIO, CA AND P LEVELS AND MN SUPPLEMENTATION ON PHYSICAL AND CHEMICAL PARAMETERS OF THE FIRST, LEFT RIB

Item	Ca-P ratio		Ca-P level		Suppl. Mn, ppm		SE
	0.5	2.0	1X	2X	0	40	
Physical measurements							
Weight, g (fresh basis)	12.5[2]	10.9	9.4[1]	14.0	11.4	12.0	0.36
External diameter (B), cm	1.71	1.66	1.60[1]	1.77	1.70	1.67	0.04
Internal diameter (d), cm	.58[1]	.51	.57	.52	.56	.53	0.02
Specific gravity (fresh basis)	1.22	1.21	1.15[1]	1.27	1.22	1.21	0.01
Inertia, cm^4	.24	.22	.18[1]	.28	.24	.21	0.02
Breaking moment, kg	26.8	28.8	15.1[1]	40.5	28.4	27.2	1.04
Chemical measurements[3]							
Ash content, %	56.9	52.9	50.5	59.3	55.5	54.3	0.42
Calcium, %	23.0[1]	21.7	20.5[1]	24.2	22.6[1]	22.0	0.18
Phosphorus, %	10.9[1]	9.9	9.5[1]	11.2	10.5	10.3	0.09
Magnesium, %	.36[1]	.31	.30	.36	.35[1]	.32	0.01
Manganese, ppm	1.07[1]	.99	.95[1]	1.11	1.02	1.04	0.03

[1]Numbers on the same line under the same subheading, i.e., Ca-P ratio, Ca-P level, or Mn supplementation level, are significantly ($P<0.05$) different.

[2]$P<0.01$.

[3]Expressed on dry, fat-free bone.

TABLE 2(

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TABLE 20. EFFECT OF THE CA-P RATIO, CA AND P LEVELS AND MN SUPPLEMENTATION ON PHYSICAL AND CHEMICAL PARAMETERS OF THE FIRST, LEFT RIB

Item	Ca-P ratio		Ca-P level		Suppl. Mn, ppm		SE
	0.5	2.0	1X	2X	0	40	
Physical measurements							
Weight, g (fresh basis)	12.5[2/]	10.9	9.4[1/]	14.0	11.4	12.0	0.36
External diameter (B), cm	1.71	1.66	1.60[1/]	1.77	1.70	1.67	0.04
Internal diameter (d), cm	.58[1/]	.51	.57	.52	.56	.53	0.02
Specific gravity (fresh basis)	1.22	1.21	1.15[1/]	1.27	1.22	1.21	0.01
Inertia, cm^4	.24	.22	.18[1/]	.28	.24	.21	0.02
Breaking moment, kg	26.8	28.8	15.1[1/]	40.5	28.4	27.2	1.04
Chemical measurements[3]							
Ash content, %	56.9	52.9	50.5	59.3	55.5	54.3	0.42
Calcium, %	23.0[1/]	21.7	20.5[1/]	24.2	22.6[1/]	22.0	0.18
Phosphorus, %	10.9[1/]	9.9	9.5[1/]	11.2	10.5	10.3	0.09
Magnesium, %	.36[1/]	.31	.30	.36	.35[1/]	.32	0.01
Manganese, ppm	1.07[1/]	.99	.95[1/]	1.11	1.02	1.04	0.03

[1]Numbers on the same line under the same subheading, i.e., Ca-P ratio, Ca-P level, or Mn supplementation level, are significantly ($P<0.05$) different.

[2]$P<0.01$.

[3]Expressed on dry, fat-free bone.

TABLE 21. EFFECT OF THE CA-P RATIO, CA AND P LEVELS AND MN SUPPLEMENTATION ON PHYSICAL, CHEMICAL AND HISTOPATHOLOGICAL PARAMETERS OF THE 5TH METACARPAL

Item	Ca-P ratio		Ca-P level		Suppl. Mn, ppm		SE
	0.5	2.0	1X	2X	0	40	
Physical measurements							
Weight, g (fresh basis)	14.6	13.9	12.8[2]	15.8	14.4	14.1	0.39
External diameter (D), cm	1.38	1.38	1.33[2]	1.43	1.39	1.37	0.02
External diameter (B), cm	1.36	1.32	1.28[2]	1.40	1.35	1.34	0.02
Internal diameter (b), cm	.97	.96	.94	.99	.99[1]	.94	0.02
Specific gravity (fresh basis)	1.23	1.21	1.18[3]	1.25	1.22	1.21	0.01
Inertia, cm^4	1.04	1.12	.87[3]	1.30	1.08	1.09	0.09
Breaking moment, kg	42.6	43.0	29.2[2]	56.3	44.3	41.3	1.80
Stress, kg/cm^2	299	263	229[2]	333	292	270	20.01
Elasticity, 1000 kg/cm^2	13.7[2]	7.3	10.4	10.7	10.6	10.5	0.57
Chemical measurements[3]							
Ash content, %	58.1[2]	54.5	52.7[2]	59.9	56.8	55.8	0.57
Phosphorus, %	10.2[2]	9.4	9.1[3]	10.5	9.9	9.8	0.12
Manganese, ppm	.99[2]	1.04	1.09[2]	.94	.96[1]	1.07	0.03
Magnesium, %	.34[2]	.29	.29[2]	.34	.32	.30	0.01
Bone histology							
Epiphysis [4]	1.50	1.63	1.31[2]	1.81	1.75[1]	1.38	0.12
Thickness of compact bone of the diaphysis [5]	96.2[2]	51.8	45.8[2]	102.3	91.6[1]	56.4	10.59
Thickness of epiphyseal cartilagenous plate [5]	65.6	64.1	70.3[2]	59.4	62.5	67.2	2.33

[1] Numbers on the same line under the same subheading, i.e., Ca-P ratio, Ca-P level, or Mn supplementation level, are significantly ($P<0.05$) different.

[2] $P<0.01$.

[3] Expressed on dry, fat-free bone.

[4] 1 = normal; 2 = very slight change; 3 = slight change; 4 = moderate change; 5 = severe change.

[5] Percent of a 20X objective by 10X ocular field.

TABLE 22. EFFECT OF THE CA-P RATIO, CA AND P LEVELS AND MN SUPPLEMENTATION ON ORGAN AND BLOOD COMPOSITION

TABLE 22. EFFECT OF THE CA-P RATIO, CA AND P LEVELS AND MN SUPPLEMENTATION ON ORGAN AND BLOOD COMPOSITION

Item	Ca-P ratio		Ca-P level		Suppl. Mn, ppm		SE
	0.5	2.0	1X	2X	0	40	
Organ composition, dry matter basis							
Kidney calcium, ppm	258[2]	315	283	290	274	299	10.57
Kidney phosphorus, %	1.35[1]	1.40	1.38	1.37	1.39[2]	1.36	0.02
Heart manganese, ppm	2.80	2.86	2.76	2.90	2.43[2]	3.23	0.12
Pancreas manganese, ppm	6.03	6.11	5.70[2]	6.45	5.90	6.25	0.17
Blood parameters							
Hemoglobin, mg/100 ml							
2nd bleeding	11.2	11.1	11.7[1]	10.6	11.1	11.2	0.23
3rd bleeding	11.7	11.8	12.7[1]	10.9	11.7	11.8	0.31
Hematocrit, %							
2nd bleeding	34.5	33.6	35.5[1]	32.6	34.0	34.1	0.56
3rd bleeding	35.1	36.2	38.8[1]	32.5	35.5	35.9	0.91
Serum alkaline phosphatase, Sigma units							
2nd bleeding	5.0[1]	6.2	6.1	5.2	5.4	5.8	0.27
Serum Mn, mcg/100 ml							
1st bleeding	1.94	2.32	1.85[1]	2.40	1.96	2.25	0.09
3rd bleeding	8.9[1]	10.6	9.9	9.7	10.0	9.6	0.04
Serum Ca, mg/100 ml							
1st bleeding	9.9[2]	12.1	11.6[2]	10.4	11.1	10.9	0.01
3rd bleeding	9.7[2]	7.2	8.4	8.5	8.2	8.6	0.03
Serum inorganic P, mg/100 ml							

[1] Numbers on the same line under the same subheading, i.e., Ca-P ratio, Ca-P level, or Mn supplementation level, are significantly ($P<0.05$) different.

[2] $P<0.01$.

affect Ca and P metabolism in the bones, which is in accord with the findings of Parker *et al.* (1955) that Mn intake did not affect the quantities of radioactive Ca and P deposited in the bones of chicks. Ellis *et al.* (1947) reported no significant difference in breaking strength or Ca content of the humerus of rabbits due to dietary Mn levels, but this report is contrary to the reports of Smith *et al.* (1944), who showed a significant decrease in weight, density, length, breaking strength and ash content of the ulna and humerus of rabbits due to dietary Mn levels. Hemoglobin and hematocrit values were not affected by Mn supplementation. Sawney and Kehar (1958) reported similar findings in cattle. Similarly Mn supplementation did not significantly affect serum Ca, inorganic P, Mg, Mn and alkaline phosphatase. This work is in accord with the findings of Hawkins *et al.* (1955), Fain *et al.* (1952) and Sawney and Kehar (1958) in cattle, but Hawkins *et al.* (1955) and Fain *et al.* (1952) showed a depression of serum Mg due to Mn supplementation.

The slight increase in serum alkaline phosphatase activity due to Mn supplementation has also been reported by some (Lassiter *et al.*, 1970) but not others (Leibholz *et al.*, 1962). There was a time of sampling effect on serum Ca, inorganic P, Mn, Mg and alkaline phosphatase. In general, their levels increased within the first month except in the case of P, which declined drastically throughout the experimental period on diets 5 and 6 with low P content (0.35%) derived exclusively from corn and soybean meal. The serum Mg values tended to be higher on these same diets than on other diets.

A two-way interaction between ratio and level of Ca and P on Mn metabolism is shown in Tables 23 and 24. This interaction was significant ($P<0.01$ or $P<0.05$) relative to levels of serum, liver and pancreas

TABLE 23. EFFECT OF THE INTERACTION BETWEEN CA-P RATIO AND LEVEL OF CA AND P ON MN METABOLISM AND SOME OTHER BONE PARAMETERS[5]

Item	Ca-P Ratio 0.5 Ca-P level 1X	0.5 Ca-P level 2X	2.0 Ca/P level 1X	2.0 Ca/P level 2X	SE
Rib, lst left					
Weight, g (fresh basis)[2]	11.6	13.4	7.2	14.7	0.51
External diameter (D), cm[1]	.75	.72	.65	.76	0.03
Specific gravity[2] (fresh basis)	1.21	1.24	1.10	1.31	0.01
Inertia, cm^4 [2]	.22	.26	.13	.31	0.02
Breaking moment, k[2]	23.0	30.7	7.3	50.2	1.60
Ash content, %[2]	55.1	58.6	45.9	59.9	0.59
Calcium, %[2]	22.1	23.8	18.9	24.5	0.26
Phosphorus, %[2]	10.6	11.1	8.4	11.3	0.13
Magnesium, %[2]	.39	.34	.22	.39	0.01
Metacarpal, 5th					
Weight, g[2] (fresh basis)	14.8	14.4	10.7	17.2	0.56
External diameter (D), cm[2]	1.39	1.36	1.27	1.49	0.03
External diameter (B), cm[2]	1.36	1.37	1.20	1.44	0.03
Specific gravity[2] (fresh basis)	1.22	1.23	1.14	1.28	0.01
Inertia, cm^4 [2]	1.16	.93	.58	1.67	0.13
Breaking moment, kg[2]	41.7	43.4	16.7	69.3	2.55
Ash content, %[2]	57.1	59.1	48.2	60.7	0.81
Calcium, %[2]	21.0	20.8	18.2	21.2	0.71
Phosphorus, %[2]	10.1	10.4	8.1	10.7	0.16
Magnesium, %[2]	.36	.31	.22	.36	0.01
Manganese, ppm[2]	1.00	.98	1.18	.91	0.04
Elasticity, 1000 kg/cm^2 [2]	15.33	12.12	5.44	9.17	0.81
Bone histology					
Diaphysis 3/[1]	1.38	1.25	1.13	2.13	0.24
Epiphysis 3/[2]	1.63	1.00	1.38	2.25	0.17
Thickness of epiphyseal cartilagenous plate 4/[2]	65.63	75.00	65.63	64.06	3.29

[1] Significant ($P<0.05$).

[2] $P<0.01$.

[3] 1=normal; 2=very slight change; 3=slight change; 4=moderate change; 5=severe change.

[4] Percent of a 20X objective by 10X ocular field.

[5] Chemical constituents expressed on dry, fat-free bone.

TABLE 24. EFFECT OF THE INTERACTION BETWEEN CA-P RATIO AND LEVEL OF CA AND P ON ORGAN AND SERUM MN METABOLISM, AND SOME OTHER MEASURES

Item	Ca-P Ratio 0.5 Ca-P level 1X	0.5 Ca-P level 2X	2.0 Ca/P level 1X	2.0 Ca/P level 2X	SE
Organ chemical composition (dry matter basis)					
Liver Mn, ppm[1]	13.76	13.42	12.93	15.50	0.58
Kidney P, %[1]	1.39	1.31	1.37	1.43	0.03
Pancreas Mn, ppm[1]	5.41	6.66	5.99	6.23	0.23
Serum parameters					
Serum alkaline phosphatase (Sigma units)					
1st bleeding[1]	5.88	5.00	4.45	6.20	0.34
2nd bleeding[1]	5.08	6.83	7.98	6.15	0.39
3rd bleeding[1]	4.75	5.25	7.40	5.08	0.38
Serum Mn, mcg/100 ml[1]					
3rd bleeding	2.29	2.89	3.21	2.47	0.10
Serum inorganic P, mg/100 ml					
1st bleeding[2]	9.7	10.1	13.5	10.7	0.01
3rd bleeding[2]	10.4	9.0	6.4	8.0	0.04

[1] Significant ($P<0.05$).

[2] $P<0.01$.

Mn, and metacarpal Mn levels. High levels of Ca and P in a 1 to 2 ratio depressed pancreas Mn content appreciably and slightly depressed liver and metacarpal Mn content, but increased serum Mn. High levels of Ca and P supplementation in a 2 to 1 ratio increased liver and pancreas Mn but depressed the serum and metacarpal Mn concentration. Hawkins *et al.* (1955) reported that high intakes of Ca and P tended to suppress blood Mn.

The interaction between the ratio of Ca to P and Mn level on Mn and Mg metabolism is shown in Table 25. Feeding Mn along with Ca and P in a 1 to 2 ratio slightly depressed liver Mn but increased it when Ca and P were given in a 2 to 1 ratio. Although Mn supplementation alone did not significantly affect metacarpal Mg, feeding Ca and P in a 1 to 2 ratio did increase metacarpal Mg significantly ($P<0.05$). The interaction between Ca to P ratio and diet Mn level upon serum Mn concentration was not significant, contrary to Hawkins' *et al.* (1955) findings in cattle.

The interaction between diet Ca and P levels and Mn levels on some bone and serum parameters is shown in Table 26. This interaction was significant ($P<0.05$) relative to rib ash content, Ca and P, metacarpal Mn levels and serum inorganic P. With low Ca and P levels, Mn supplementation increased metacarpal Mn and serum inorganic P but depressed rib ash, Ca and P content. Mn supplementation, coupled with high Ca and P levels, did not affect metacarpal Mn, slightly increased rib ash, Ca and P, but depressed serum inorganic P. The interaction between diet Ca and P levels and Mn levels was not significant relative to Mg levels in the tissues. The significant effect of Mn supplementation on the metacarpal internal vertical diameter, rib Mg and heart Mn levels was suppressed when high Ca and P levels were also given in the diets.

TABLE 25. EFFECT OF THE INTERACTION BETWEEN CA-P RATIO AND MN LEVELS ON MN AND MG METABOLISM

Item	Ca-P ratio				SE
	0.5		2.0		
	Mn-level		Mn-level		
	0	40	0	40	
Metacarpal, 5th left					
Mg, % 1/	.36	.31	.29	.29	0.01
Liver					
Mn, ppm 1/	13.8	13.4	12.9	15.5	0.58
Bone histology					
Epiphysis 2/1/	1.88	1.63	1.13	1.63	0.17

[1]Significant ($P<0.05$).

[2]1 = normal; 2 = very slight change; 3 = slight change; 4 = moderate change; 5 = severe change.

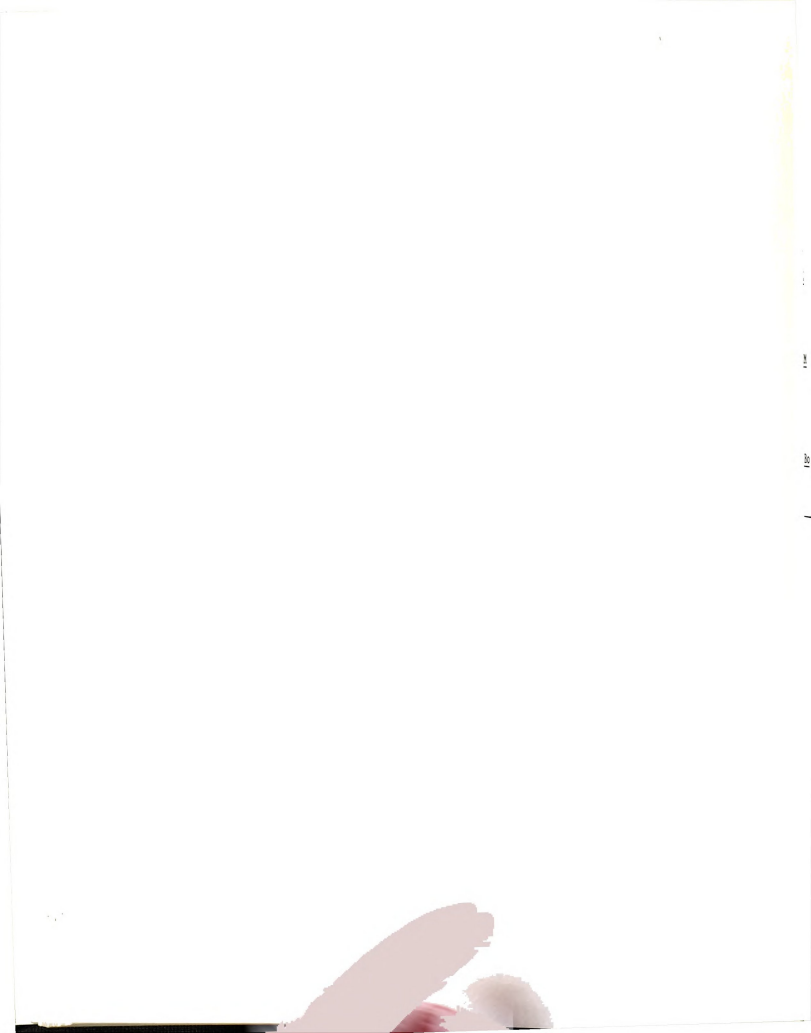


TABLE 26. EFFECT OF THE INTERACTION BETWEEN CA-P LEVELS AND MN LEVELS ON SOME PHYSICAL PARAMETERS

Item	Ca-P level				SE
	1X		2X		
	Mn-level		Mn-level		
	0	40	0	40	
Rib, 1st left					
Ash content, %[1]	51.81	49.13	59.14	59.42	0.59
Calcium, %[1]	21.08	19.86	24.17	24.18	0.26
Phosphorus, %[1]	9.77	9.31	11.15	11.22	0.13
Metacarpal, 5th left					
Manganese, ppm [1]	.98	1.19	.94	.94	0.04
Serum inorganic P, mg/100 ml[1]					
3rd bleeding	7.48	9.25	9.00	7.98	0.60
Bone histology					
Thickness of epiphyseal cartilagenous plate[2][1]	71.88	53.13	68.75	65.63	3.29

[1]Significant ($P<0.05$).

[2]Percent of a 20X objective by 10X ocular field.

Table 27 summarizes a three-way interaction between level of Ca and P, ratios of Ca to P and Mn levels on some measured parameters. The interaction was significant ($P<0.05$) with respect to rib and serum Mn content, pancreas dry matter, and metacarpal Mg content and elasticity. With low Ca and P levels in a 1 to 2 ratio, Mn supplementation increased serum and metacarpal Mn values, slightly increased pancreas dry matter and did not change metacarpal Mn and elasticity. High Ca and P levels in a 1 to 2 ratio with Mn supplementation depressed rib Mn, metacarpal Mg, pancreas dry matter and serum Mn, but increased metacarpal elasticity. With low Ca and P levels in a 2 to 1 ratio, Mn supplementation depressed rib Mn content, pancreas dry matter and serum Mn, and increased metacarpal elasticity but did not change metacarpal Mg. With high Ca and P levels in a 2 to 1 ratio, Mn supplementation increased rib and serum Mn levels and pancreas dry matter, and slightly increased metacarpal Mg but depressed metacarpal elasticity. The deleterious effects on weight gain and feed efficiency of a low dietary P level (0.35%), derived exclusively from corn and soybean meal, were much more pronounced than the effects of excessive dietary levels of Ca and P or of an inverse Ca to P ratio, regardless of dietary Mn concentration.

1. Histopathology[1]

a. Histopathologic examination. The histology of the epiphyseal cartilagenous plate was considered normal in all bones from all pigs regardless of treatment group. There was some persistence of cartilage

[1]Performed by Dr. K. K. Keahey, Department of Pathology, Michigan State University.

TABLE 27. EFFECT OF THE INTERACTION BETWEEN CA-P RATIO, CA AND P LEVEL AND MG LEVEL ON

TABLE 27. EFFECT OF THE INTERACTION BETWEEN CA-P RATIO, CA AND P LEVEL AND MN LEVEL ON SOME MEASURED PARAMETERS

Category									
Ca-P ratio	0.5				2.0				
Ca-P level	1X		2X		1X		2X		
Mn level	0	40	0	40	0	40	0	40	SE
Rib, 1st left									
Mn conc., ppm [1]	0.98	1.06	1.15	1.10	0.91	0.85	1.05	1.15	.05
Metacarpal, 5th left									
Mg content, % [2]	0.36	0.37	0.37	0.26	0.22	0.21	0.35	0.38	.02
Elasticity, 1000 kg/cm^2 [1]	15.4	15.3	11.1	13.2	5.0	5.9	11.0	7.4	1.15
Pancreas, dry matter % [1]	28.4	29.6	28.2	25.6	28.1	26.7	27.4	28.4	.97
Serum Mn, mcg/100 ml [1]									
1st bleeding	1.25	2.01	2.36	2.10	2.21	1.95	2.05	3.00	.24

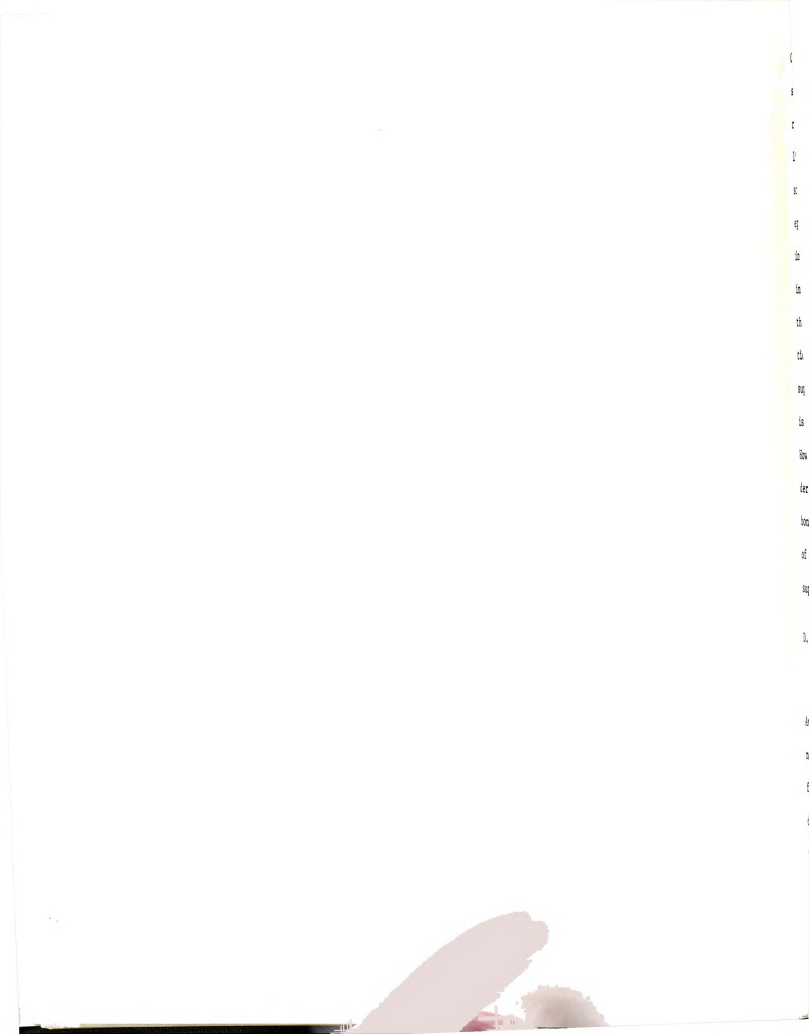
[1] Significant ($P<0.05$).

[2] $P<0.01$.

in the spicules. The most striking histologic change was in the diaphysis of the bone. Pigs that had a thickness of compact bone equivalent to 70% of the diameter of the microscope field[1] or greater had normal histologic structures. Those with less than 70% had incomplete ossification of the Haversian canal system. The center of the Haversian canal was occupied by a blood vessel which was surrounded by a significant zone of loose connective tissue and osteoid. The compact bone, therefore, appeared histologically like spongy bone instead of normal compact bone. Examination of a record kept at the time of trimming of tissues indicated that most of the soft bones had either a very thin layer of compact bone in the diaphysis or an absence of compact bone. It was concluded that the condition observed in the bones from pigs from some dietary regimes were not changes of rickets but were changes in which there was a failure of production of compact bone in the region of the diaphysis. This conclusion agrees with that of Smith *et al.* (1944), who, using X-ray pictures and microscopic studies, reported bone changes in Mn deficiency were distinctly different from those seen in rickets. Neher *et al.* (1956) also reported generalized bone rarefaction in Mn deficient pigs.

b. Statistical inferences. Manganese supplementation was associated with a significantly ($P<0.05$) higher histological normalcy score of the epiphysis as compared to no Mn supplementation. The histology of the diaphysis was not affected by diet Mn level. Animals on high Mn level diets had significantly ($P<0.05$) less compact bone in the diaphysis, but these levels did not significantly affect the thickness of the epiphyseal cartilagenous plate (Table 21). The ratio of

[1] 10X eyepiece and 20X objective.



Ca to P, level of Ca and P and the interaction between the two had a significant effect (Table 23) on the bone histologic structure as reported in many studies of Ca and P on bone metabolism (Underwood, 1971). The interaction between the Ca-P ratio and Mn levels was significant ($P<0.05$) relative to the histologic structure of the epiphysis (Table 25). With a 2 to 1 Ca to P ratio, Mn supplementation increased the incidence of change in bone histologic structure. Mn interaction with the Ca and P levels significantly ($P<0.05$) affected the thickness of epiphyseal cartilagenous plate (Table 26). Production of the normal histologic structure of the epiphyseal bone by Mn supplementation is in agreement with the established concept that Mn is involved in bone formation (Leach, 1967; Tsai and Everson, 1967). However, the deleterious effects of a low dietary P level (0.35%) derived exclusively from corn and soybean meal on the histology of bone were much more pronounced than the effects of high dietary levels of Ca and P or of an inverse Ca to P ratio, regardless of dietary Mn supplementation.

D. Experiment 4: The manganese requirement of the baby pig from sows fed a low manganese diet

Table 28 summarizes the results of the analysis of variance. Average daily gain was significantly ($P<0.01$) different between treatments but feed efficiency was not. Absolute Mn intake, and absolute fecal and urinary Mn excretion, were significantly ($P<0.01$ or $P<0.05$) different between treatments, but absolute Mn retention was not. Mn retention, fecal and urinary Mn excretion, all as percent of intake, were significantly ($P<0.01$ or $P<0.05$) different between dietary treatments. Of the serum parameters studied, serum Mg levels on the 28th day

TABLE 28. ANALYSIS OF VARIANCE OF EFFECT OF DIETARY MN LEVEL ON GROWTH, BALANCE AND BLOOD MEASURES

Item		Mean	Min.	Max.	P-level of F-statistic
Serum parameters					
Magnesium, mg/100 ml	9[1]	2.38	2.00	2.85	0.83
	21	2.57	1.87	3.05	0.49
	28	2.04	1.26	2.90	0.03
Alkaline phosphatase (SAP), Sigma units	9	5.8	3.4	8.4	0.04
	21	7.0	5.3	10.3	0.10
	28	6.2	3.0	10.7	0.11
Manganese, mcg/100 ml	9	1.20	.90	1.90	0.348
	21	0.94	.60	1.50	0.129
	28	0.90	.40	1.70	0.005
Growth data					
Average daily gain, g		253	206	299	0.02
Feed/gain		1.33	1.18	1.45	0.238
Balance data					
Mn intake, mg/day		1.82	0.18	4.06	<0.0005
Mn excretion, mg/day					
Fecal		1.40	0.24	3.45	0.006
Urinary		0.015	0.01	0.02	0.049
Mn retention, mg/day		.44	-.30	1.59	0.240
Mn retention, % of intake		3.6	-72	73	0.016
Mn excretion, % of intake					
Fecal		94	26	167	0.02
Urinary		2.1	0.3	5.6	<0.0005

[1]Days on experimental diets.

of the experiment, serum alkaline phosphatase (SAP) on the 9th day and serum Mn on the 28th day showed significant ($P<0.01$ or $P<0.05$) treatment effects.

There were no treatment effects on serum Mg levels 9 days after the start of the experiment, a slight rise on the 2.67 ppm Mn diet (diet 2) after 21 days, and a significant ($P<0.05$) drop on the basal diet by the end of the experiment (Table 29, Figure 4.1). Manganese supplementation appeared to sustain normal serum Mg levels. The SAP levels were high on the 6.34 ppm Mn diet (diet 3) and low on the basal diet (0.46 ppm Mn) and diet 2 throughout the duration of the experiment. On the 9th and 21st days, the animals on the basal diet had higher SAP levels than those on diet 2 and those values dropped slightly by the end of the experiment (Table 29, Figure 4.2). The serum Mn values were significantly ($P<0.01$) lower on the basal diet than on the diet containing 6.34 ppm Mn on the 28th day of the experiment. There was an appreciable and consistent drop of serum Mn values on the basal diet and diet 2 as the experiment progressed. In animals on the 6.34 ppm Mn diet, serum Mn values dropped slightly by the 21st day but returned to previous levels by the 28th day (Table 29, Figure 4.3).

Serum Mn response to dietary Mn levels has been reported in swine fed practical diets (Plumlee *et al.*, 1956; Newland and Davis, 1961), in ruminants (Hawkins *et al.*, 1955; Rojas *et al.*, 1965), and in chickens (Bolton, 1955). A rise in SAP levels in response to Mn supplementation has also been reported by some (Wachtel *et al.*, 1943; Lassiter *et al.*, 1970) but not by others (Leibholz *et al.*, 1962). The big drop of serum Mg and serum Mn levels by the 28th day on the basal diet may indicate that when animals are fed low Mn diets (0.46 ppm) for a long period their Mn stores are depleted and Mg substitutes in the functions that

TABLE 29. THE EFFECT OF DIETARY MN LEVELS ON SERUM AND GROWTH PARAMETERS

Item		Diets 1	2	3
Mn conc., ppm		0.46	2.67	6.34
No. of pigs		4	4	4
Serum Mg, mg/100 ml	9[3]	2.44	2.38	2.32
	21	2.38	2.72[1/]	2.62
	28	1.47	2.40[1/]	2.25
SAP,[4] Sigma units	9	5.6	4.6	7.3[1/]
	21	7.0	5.8	8.3
	28	4.5	5.5	8.6
Serum Mn, mcg/100 ml	9	0.98	1.33	1.31
	21	0.73	0.95	1.15
	28	0.41	0.88	1.28[2/]
Average daily gain, g		224	283[1/]	251
Feed/gain		1.37	1.27	1.35

[1] Significantly ($P<0.05$) greater than the least value on the same line.

[2] $P<0.01$.

[3] Days on experimental diets.

[4] Serum alkaline phosphatase.

usually involve Mn. Magnesium and Mn can replace each other in a number of enzymatic reactions since they have similar properties (Lehninger, 1950, 1970). Johnson (1943) found a lower body Mn content of pigs born of sows depleted of Mn. In this study, the correlations between serum Mg and serum Mn were positive and significant ($P<0.05$) (Table 32).

Average daily gain was significantly ($P<0.05$) greater on diet 2 than on diet 1 (Table 29, Figure 4.4). The average daily gain was also higher on diet 3 than on diet 1, but the difference was not statistically significant. Food efficiency was greater on diet 2 than on the other diets but those differences were not statistically significant. Many reports have shown increased growth rate with optimal Mn supplementation in the species studied (Underwood, 1971). The results of this study support those of Leibholz *et al.* (1962), who concluded that Mn requirements for baby pig growth were very low. For maximal growth, these data would indicate that 3.0 ppm Mn in the diet are adequate. This level is approximately equal to that reported by Plumlee *et al.* (1956).

Mn intake, fecal Mn excretion and Mn retention all reflected the dietary level of Mn (Table 30, Figure 4.5). The absolute Mn intakes of the three diets were significantly ($P<0.01$) different; the absolute fecal Mn excretion on diet 3 was significantly ($P<0.01$) greater than that on diets 1 and 2 but the difference between diets 1 and 2 was not statistically significant. Diets 2 and 3 resulted in significantly ($P<0.05$) greater absolute urinary Mn excretion than diet 1 but the absolute urinary Mn excretion on diets 2 and 3 was equal and did not reflect the differing dietary Mn levels. Other workers have also reported this small but virtually constant urinary Mn excretion in other species irrepsective of Mn ingested and the dominance of the feces as the principal excretion route of this element (Britton and Cotzias,

TABLE 30. RETENTION AND EXCRETION ROUTES OF MN FROM DIETS CONTAINING DIFFERENT LEVELS OF MN

Item	Diets 1	2	3
Mn conc., ppm	0.46	2.67	6.34
No. of pigs	4	4	4
Mn intake, mg/day	0.19	1.67[2]	3.63[2,4]
Mn excretion, mg/day			
Fecal	.30	1.05[2]	2.85[2,4]
Urinary	.01	.02[2]	.02[2]
Mn retention, mg/day	-.11	.60	.77
Mn retention, % of intake	-58	36[1]	22[1]
Mn excretion, % of intake			
Fecal	154[3]	63	78
Urinary	5.3[4]	1.2	0.6

[1] Significantly ($P<0.05$) greater than least value.

[2] $P<0.01$.

[3] Significantly ($P<0.05$) greater than least two values.

[4] $P<0.01$.

1966; Miller, 1973). Absolute Mn retention did not differ significantly between treatments. There was a negative absolute Mn retention on the low dietary Mn level (-0.11 mg/day). This finding agrees with the results of Zajcev (1959) and Starodubova (1968), who showed an obligatory loss of Mn when animals were placed on very low Mn diets. Mn retention, as a percent of intake, was significantly ($P<0.05$) lower on the basal diet than on diets 2 and 3. Mn retention as a percent of intake on diets 2 and 3 did not differ significantly. Hill and Holtkamp (1954) reported a greater net absorption at a low Mn concentration in the diet than at high concentrations, and Gutowska *et al.* (1941) showed that net Mn absorption was proportional to dietary Mn concentration. Fecal and urinary Mn excretion, as percent of intake, were significantly ($P<0.01$ or $P<0.05$) higher on the basal diet than on diets 2 and 3. The fecal and urinary Mn excretion, as percent of intake, on diets 2 and 3 were not significantly different.

Dietary Mn was significantly ($P<0.01$) related to levels of serum Mn on the 9th day, SAP on the 28th day and serum Mg on the 28th day of the experiment (Table 31). Mn intake was significantly ($P<0.01$) correlated to serum Mn on the 9th day, serum Mn on the 28th day, and SAP on the 28th day of the experiment. Krieg (1966) found no parallel between Mn in the diet and that in blood. There was a poor positive correlation between Mn intake and average daily gain, and feed efficiency. Average daily gain and feed efficiency were poorly correlated to all the serum parameters except SAP on the 9th day. Serum Mg on the 28th day was related to feed, urine and tissue Mn concentration. Absolute fecal Mn excretion had a significant ($P<0.01$) positive correlation with serum Mn on the 9th day and SAP on the 28th day of the experiment. Mn retention as percent of intake was significantly ($P<0.01$ or $P<0.05$)

TABLE 11. CORRELATIONS BETWEEN NN BALANCE MEASURES, SERUM PARAMETERS AND GROWTH

TABLE 31. CORRELATIONS BETWEEN MN BALANCE MEASURES, SERUM PARAMETERS AND GROWTH[4]

Item	Day[3]	Manganese level					Growth data		% of intake		
		Feed	Intake	FE	UE	RET	ADG	F/G	RET	FE	UE
SMn	9	.76[1]	.78[1]	.70[1]	.48	.55	.39	-.38	.71[1]	-.72[1]	-.88[1]
SMn	21	.49	.63[2]	.48	.56	.61[2]	.61[2]	-.39	.84[1]	-.84[1]	-.85[1]
SMn	28	.48	.81[1]	.61[2]	.41	.59[2]	.22	.09	.59[2]	-.59[2]	-.74[1]
SAP	9	.46	.39	.51	.15	.11	-.47	.76[1]	-.14	.15	-.08
SAP	21	.47	.36	.31	.28	.20	-.29	.58[2]	.05	-.05	-.09
SAP	28	.84[1]	.80[1]	.72[1]	.58[2]	.37	-.03	.20	.32	-.31	-.54
SMg	9	-.18	-.09	.00	.17	-.19	.01	.04	-.19	.19	.14
SMg	21	.59[2]	.11	.15	.62[2]	.50	.52	-.48	.40	-.40	-.22
SMg	28	.87[1]	.47	.12	.63[2]	.79[1]	.57	.30	.85[1]	-.85[1]	-.71[1]
ADG		.24	.31	.05	.57	.56	---	---	.65[2]	-.65[2]	-.61[2]
F/G		-.06	.15	.14	-.16	-.57	-.70[1]	---	-.61[2]	.61[2]	.39

[1]Significant (P 0.01).

[2]Significant (P 0.05).

[3]Days after start of the experiment.

[4]Abbreviations are as follows: SMn = serum manganese; SAP = serum alkaline phosphatase; SMg = serum magnesium; ADG = average daily gain; F/G = feed/gain; FE = absolute fecal Mn excretion; UE = absolute urinary Mn excretion; RET = absolute Mn retention.

positively related to serum Mn throughout the experiment, serum Mg on the 28th day and average daily gain. This may indicate that a high retention of Mn enhances growth rate. Fecal and urinary Mn excretion, both as a percent of intake, were significantly ($P<0.01$ or $P<0.05$) negatively correlated to serum Mn throughout the experiment, and serum Mg on the 28th day. Feed efficiency was significantly ($P<0.05$) negatively related to Mn retention as percent of intake. Mn retention and fecal Mn excretion, both as percent of intake, had high correlations with average daily gain and were better indicators of growth rate than absolute Mn intake, excretion or retention, or any of the serum parameters studied. Up to the 21st day of the experiment, none of the serum parameters were significantly correlated with each other. On the 28th day, serum Mn had significant ($P<0.05$) positive correlations with serum Mg and SAP (Table 32).

Correlations within the balance measures are summarized in Table 33. Dietary Mn level was positively correlated to Mn intake, excretion and Mn retention as percent of intake. However, these correlations were significant ($P<0.01$) only with Mn intake and absolute fecal Mn excretion. Urinary Mn excretion as a percent of intake was significantly ($P<0.01$) negatively correlated to dietary Mn levels. Woerpel and Balloun (1964) and Hill and Holtkamp (1954) reported that retention was highly related to dietary Mn levels. Mn intake and absolute fecal and urinary Mn excretion were all positively correlated to Mn retention as percent of intake and absolute Mn retention. Mn intake was significantly ($P<0.01$) positively correlated to urinary Mn excretion as percent of intake. Absolute Mn retention had high correlations with Mn retention as percent of intake ($r = + 0.85$) and fecal Mn excretion as percent of intake ($r = - .85$). There was a poor correlation between

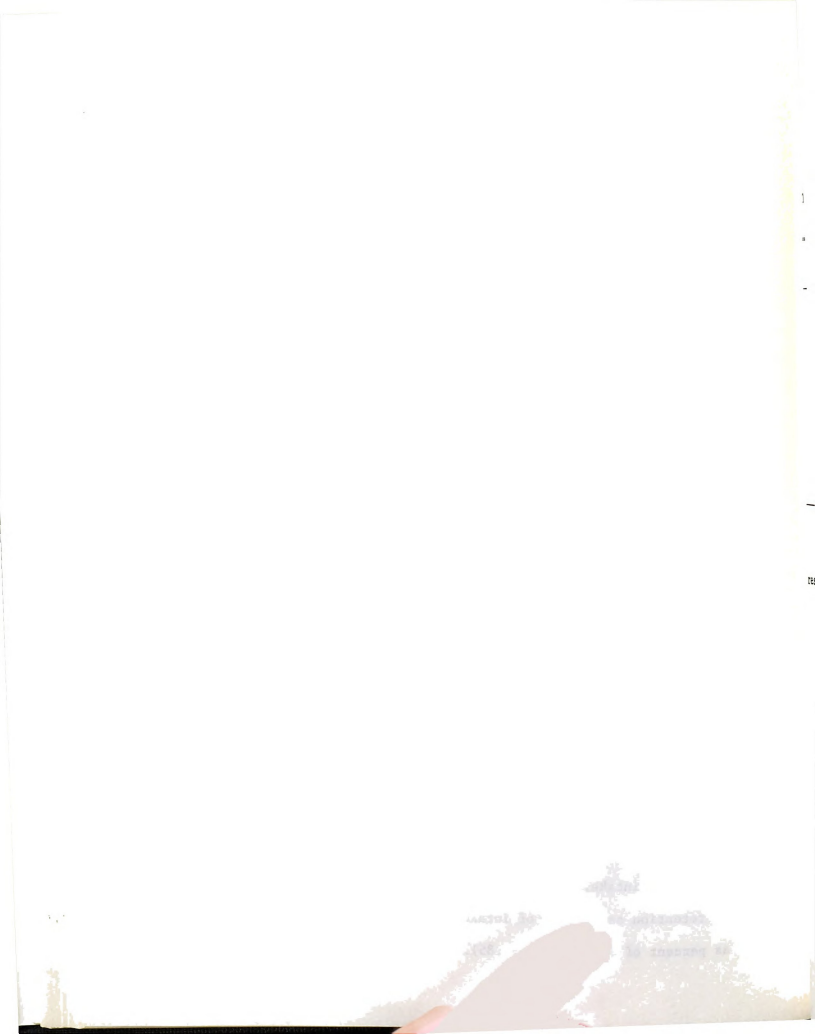


TABLE 32. CORRELATIONS WITHIN THE SERUM PARAMETERS

Period[1]	Comparison[2]	r[3]
9th day	SMn vs SAP	0.31
	SMn vs SMg	-.13
	SMg vs SAP	0.09
21st day	SMn vs SAP	0.31
	SMn vs SMg	0.08
	SMg vs SAP	0.03
28th day	SMn vs SAP	0.57[4]
	SMn vs SMg	0.60[4]
	SMg vs SAP	0.39

[1] Days after the start of the experiment.

[2] SMn, SMg, SAP (Serum Mn, Mg and alkaline phosphatase, respectively).

[3] Correlation coefficient.

[4] Significant ($P<0.05$).

TABL

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RE

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UEPI

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TABLE 33. CORRELATIONS WITHIN THE MN BALANCE MEASURES[1]

	Feed Mn	Mn Intake	FE	UE	RET	REPI	FEPI	UEPI
Feed Mn	1.00	.99[2]	.87[2]	.52	.50	.54	-.52	-.84[2]
Mn intake		1.00	.88[2]	.54	.50	.56	-.54	-.86[2]
FE			1.00	.45	.03	.19	-.17	-.67[3]
UE				1.00	.31	.51	-.50	-.63[3]
RE					1.00	+.85[2]	-.85[2]	-.60[3]
REPI						1.00	-1.00[2]	-.80[2]
FEPI							1.00	.79[2]
UEPI								1.00

[1]Abbreviations are as follows: FE = fecal Mn excretion; UE = absolute urinary Mn excretion; RE = absolute Mn retention; REPI = Mn retention as percent of intake; FEPI = fecal Mn excretion as percent of intake; UEPI = urinary Mn excretion as percent of intake.

[2]Significant at $P<0.01$.

[3]Significant at $P<0.05$.

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absolute fecal Mn excretion and absolute Mn retention. Mahoney and Small (1968) reported a good relationship between fecal Mn excretion and body retention. Mn retention as percent of intake had a perfect ($r = -1.0$) negative correlation with fecal Mn excretion as percent of intake and a highly significant ($P<0.01$) correlation with urinary Mn excretion as percent of intake. Urinary and fecal Mn excretion, both as percent of intake, were significantly ($P<0.01$) positively related. In this study, there was no significant positive correlation between Mn intake and absolute Mn retention or Mn retention as percent of intake, which is contrary to Murty's (1957) findings in sheep and to Mathers' and Hill's (1967) findings in chickens.

Figure 4.1. The effect of dietary Mn concentration on serum Mg concentration.

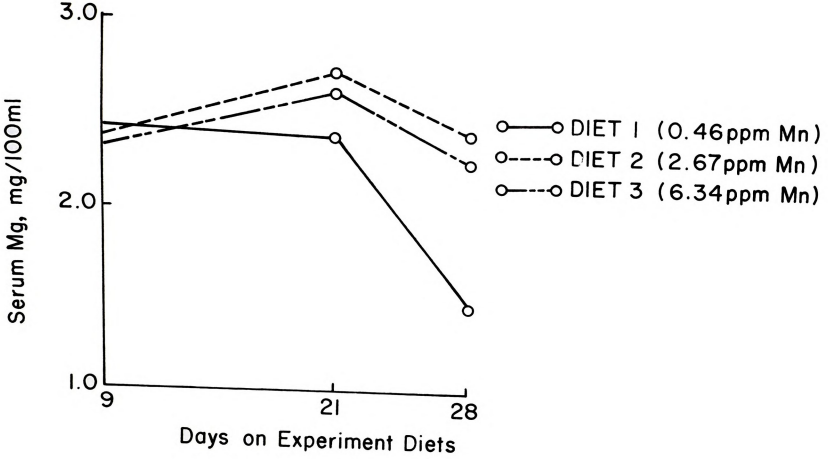
3.0
2.0
1.0
Serum Mg, mg/100ml
9
21
28
Days on Experiment Diets
DIET 1 (0.46ppm Mn)
DIET 2 (2.67ppm Mn)
DIET 3 (6.34ppm Mn)

Figure 4.1.

Figure 4.2. The effect of dietary Mn concentration on serum alkaline phosphatase activity.

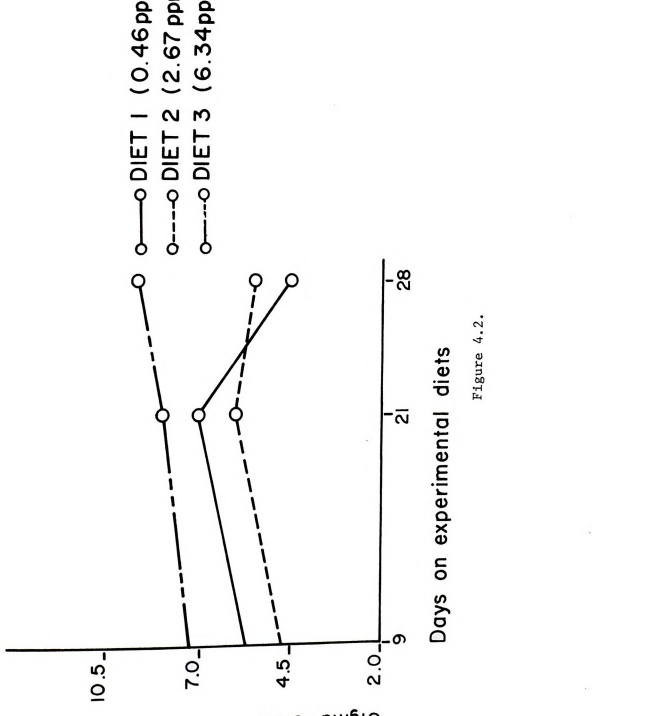
Serum Alkaline Phosphatase
Sigma Units
10.5
7.0
4.5
2.0
9
21
28
Days on experimental diets
DIET 1 (0.46ppm Mn)
DIET 2 (2.67 ppm Mn)
DIET 3 (6.34ppm Mn)

Figure 4.2.

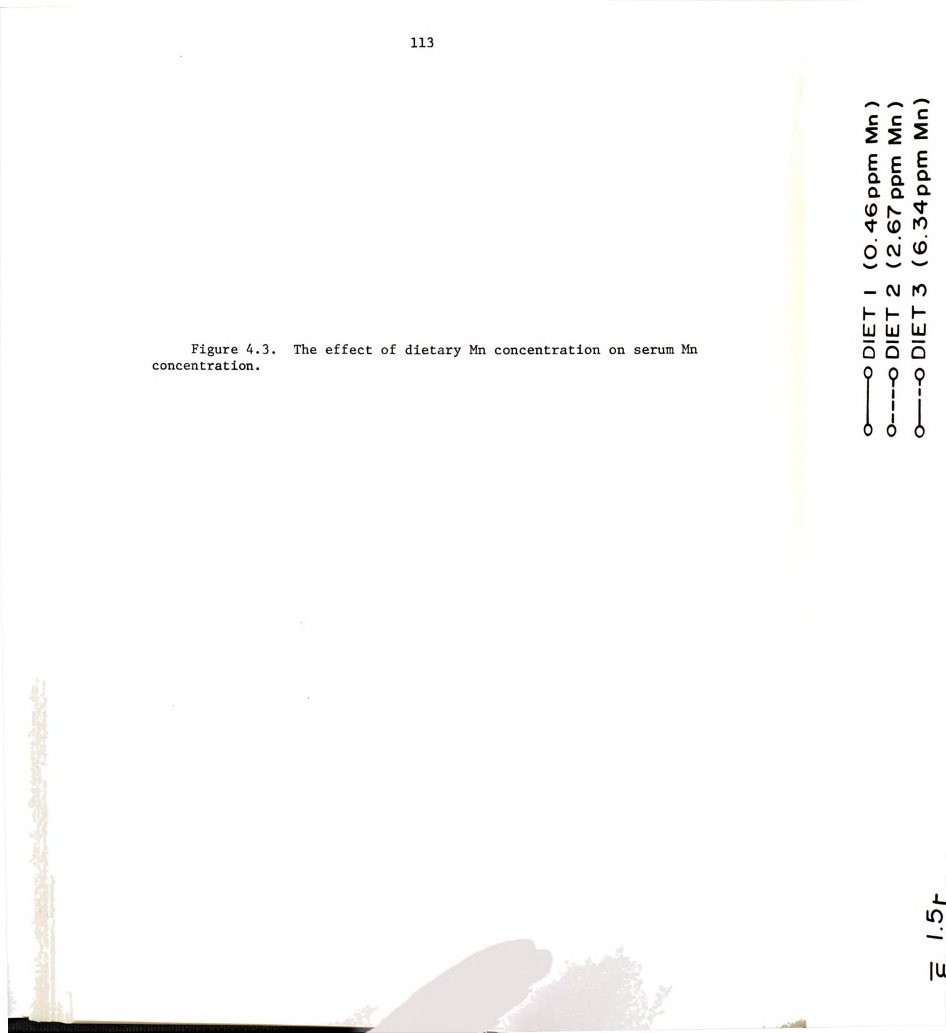

Figure 4.3. The effect of dietary Mn concentration on serum Mn concentration.

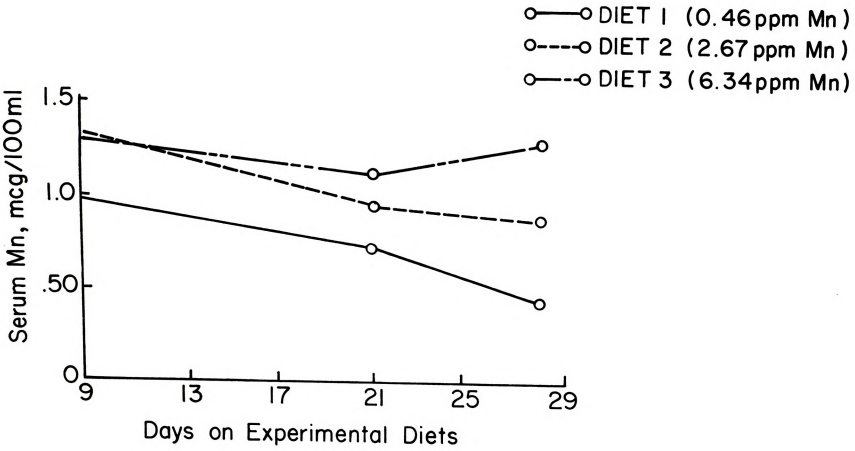
DIET 1 (0.46ppm Mn)
DIET 2 (2.67ppm Mn)
DIET 3 (6.34ppm Mn)
Serum Mn, mcg/100ml
1.5
1.0
.50
0
9
13
17
21
25
29
Days on Experimental Diets

Figure 4.3.

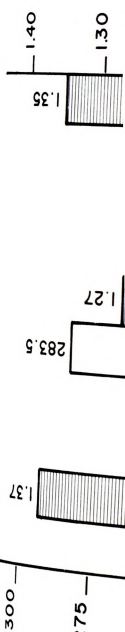

Figure 4.4. The effect of dietary Mn concentration on average daily gain (ADG) and feed efficiency (F/G).

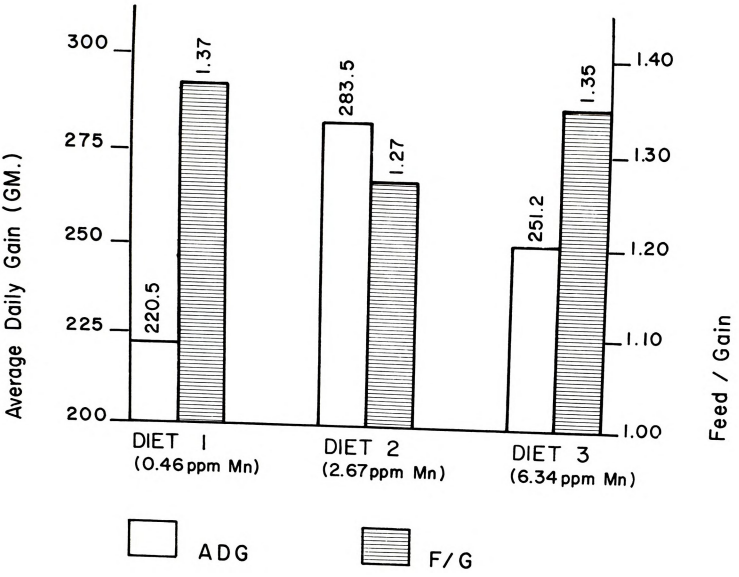
Average Daily Gain (GM.)
300
275
250
225
200
220.5
1.37
283.5
1.27
251.2
1.35
1.40
1.30
1.20
1.10
1.00
Feed / Gain
DIET 1
(0.46ppm Mn)
DIET 2
(2.67ppm Mn)
DIET 3
(6.34ppm Mn)
ADG
F/G

Figure 4.4.

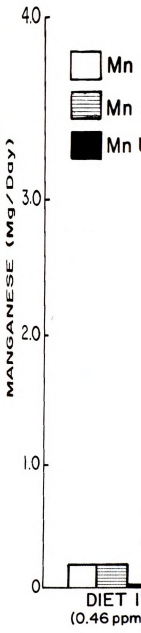

Figure 4.5. Mn balance data, intake, fecal and urinary excretion and retention on different dietary Mn levels.

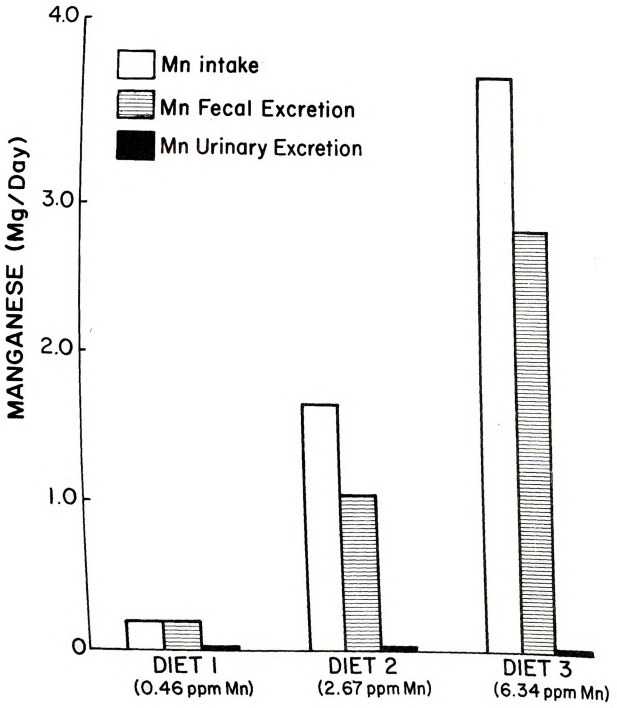
4.0
3.0
2.0
1.0
0
MANGANESE (Mg/Day)
Mn intake
Mn Fecal Excretion
Mn Urinary Excretion
DIET 1
(0.46 ppm Mn)
DIET 2
(2.67 ppm Mn)
DIET 3
(6.34 ppm Mn)

Figure 4.5.

Within the limits
employed, the results
1. Growth rates
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amounts of Mn from eit
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and tissue Mn concentr
3. Regardless of
was recovered in the fe
was minimal and constan
very low dietary Mn lev
excretion resulting in
4. The requiremen
was 3.0 ppm Mn in the d
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maximum gain over longe
5. Serum Mn and s
correlated with Mn inta
were not. Mn retention
had high correlations w
of growth rate than abs
the serum parameters.

CONCLUSIONS

Within the limits of the experimental conditions and procedures employed, the results of this study lead to the following conclusions:

1. Growth rates were equal whether Mn was derived from the 16% corn-soy basal diet or from the same diet supplemented with equal amounts of Mn from either the sulfate or the carbonate or the oxide.

2. Mn from the supplemented diets was more available to the young pig than that from corn and soy alone when assessed by Mn balance and tissue Mn concentrations.

3. Regardless of dietary Mn source, over 90% of the excreted Mn was recovered in the feces; absolute excretion of Mn via the kidneys was minimal and constant irrespective of dietary Mn levels; and on very low dietary Mn levels (0.50 ppm), there was an obligatory Mn excretion resulting in a negative Mn retention.

4. The requirement for maximal daily gain over a 4-week period was 3.0 ppm Mn in the diet of baby pigs but may be higher (3-6 ppm) for maintenance of normal serum Mn concentration (and possibly for maximum gain over longer periods).

5. Serum Mn and serum alkaline phosphatase levels were highly correlated with Mn intake, but average daily gain and feed efficiency were not. Mn retention and fecal Mn excretion, as percent of intake, had high correlations with average daily gain and were better indicators of growth rate than absolute Mn intake, excretion and retention, and the serum parameters.

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7. Mn supplement

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8. Although Mn s

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ratio, regardless of c

6. Mn from the supplemented diets was apparently absorbed in the stomach, cranial small intestine and cecum, whereas Mn from corn and soy was apparently absorbed in the stomach and cecum only. Net cecal absorption of Mn from the basal diet was higher than that of Mn from supplemented diets, and net secretion of Mn into the caudal small intestine on the supplemented diets was higher than on the basal diet.

7. Mn supplementation of a 16 ppm Mn diet did not significantly affect hemoglobin and hematocrit values, serum Ca, Mg, and inorganic P levels, bone physical measurements, breaking strength or related parameters.

8. Although Mn supplementation produced a more normal histologic structure of the epiphysis, it reduced the thickness of compact bone in the region of the diaphysis.

9. There was a significant metabolic interaction between Ca, P and Mn levels in the diets of pigs, which produced changes in bone structure that were distinctly different from those seen in rickets.

It should be emphasized that the deleterious effects on weight gain, feed efficiency and the histology of bone of a low dietary P level (0.35%) from soybean meal were much more pronounced than the effects of excessive dietary levels of Ca and P or an inverse Ca to P ratio, regardless of dietary Mn supplementation.

BIBLIOGRAPHY

Andur, B. H., H. Rill
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Andur, M. O., L. C. N
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Angelico, R., L. Maggi
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Antonova, M. V. 1968.
on immunologica

Antonova, M. V., V. A.
small amounts o
responses. Gig

Archibald, J. G. and J
J. Dairy Sci. 2

Atkinson, R. L., J. W.
Effect of vario
turkeys. Poul.

BIBLIOGRAPHY

Amdur, B. H., H. Rilling and K. Block. 1957. The enzymatic conversion of mevalonic acid to squalene. J. Amer. Chem. Soc. 79: 2646.

Amdur, M. O., L. C. Norris and G. F. Heuser. 1946. The lipotropic action of manganese. J. Biol. Chem. 164: 783.

Anderson, B. M. and H. E. Parker. 1955. The effects of dietary manganese and thiamine levels on growth rate and manganese concentration in tissues of rats. J. Nutr. 57: 55.

Angelico, R., L. Maggiore and M. Quintiliani. 1961. Inorganic constituents in the diet and radiosensitivity. Mortality from X-rays and nitrogen mustard in rats given diets with added Mn. Rend. 1st Super. Sanita, Rome 24: 711.

Angelico, R., M. Manganaro, L. M. Maggiore and M. Quintiliani. 1965. Influence of inorganic constituents of the diets on sensitivity of ionizing radiation and radiometric substances. Ann. 1st Super. Sanita, Rome 1: 689.

Anke, M., H. Joroch, M. Dietrich and G. Hoffman. 1967. Incorporation and distribution of radioactive manganese from different manganese salts in laying hens. Arch. Tierernahtung. 17: 69.

Anke, M. and B. Groppel. 1970. Manganese deficiency and radioisotope studies on manganese metabolism. Proc. 1st Int. Symp. Trace Element Metab. Anim., 1969 p. 133 (C. F. Mills, ed.) Livingstone, Edinburgh.

Anong-Nilubol, M. L., K. Chayawatanangkur and S. Kritalugsana. 1968. Manganese toxication in the human body determined by activation analysis. J. Nucl. Med. 9: 178.

Antonova, M. V. 1968. Effect of different amounts of Mn in the diet on immunological activity. Vop. Pitan. 27: 36.

Antonova, M. V., V. A. Pristupa and V. P. Popov. 1968. Effect of small amounts of trace elements in the diet on immunological responses. Gigiena Sanit. 3: 39.

Archibald, J. G. and J. G. Lindquist. 1943. Manganese in cows' milk. J. Dairy Sci. 26: 325.

Atkinson, R. L., J. W. Bradley, J. R. Couch and J. H. Quinsberry. 1967. Effect of various levels of Mn on reproductive performance of turkeys. Poul. Sci. 46: 472.

Babin, J. A. A., P. I
Complex supple
trace elements
changes in son
144.

Bandemer, S. L., J. A
of manganese i
Poul. Sci. 19:

Barnes, L. L., G. Spe
in the albino
46: 562.

Barren, E. J. 1966.
General proper
acids. Bioche

Belani, S. G., G. M. I
Chronic mangan

Belicenko, R. 1968.
vodstvo 11: 12.

Bentley, O. G. and P.
ration upon dai

Bentley, O. G. and P.
deficiency in t
liver homogenat

Bentley, O. G., C. V.
Certain trace e
selected group
Wisconsin. J.

Bertinchamps, A. J., S
pendence of rou
211: 217.

Blakemore, F., J. A. Ni
high manganese
reference to lac

Bolin, D. W., P. P. Kir
method for the d
as an index. Sc

Bolton, W. 1955. The
into immature pu
plasma and some

Babin, J. A. A., P. N. Gavrilova, P. I. Sidorov and A. E. Lev. 1964. Complex supplements of vitamins of the B group and salts of the trace elements manganese, copper and cobalt in pig feeding and changes in some tissue enzymes. Trudy Saratov. Zooventinst. 12: 144.

Bandemer, S. L., J. A. Davidson and P. J. Schaible. 1940. Availability of manganese in natural and precipitated manganese carbonate. Poul. Sci. 19: 116.

Barnes, L. L., G. Sperling and L. A. Maynard. 1941. Bone development in the albino rat on a low Mn diet. Proc. Soc. Exp. Biol. Med. 46: 562.

Barren, E. J. 1966. The mitochondrial fatty acid synthesizing system: General properties and acetate incorporation into monoenoic acids. Biochem. Biophys. Acta 116: 425.

Belani, S. G., G. M. Umarji, R. A. Bellare and H. C. Merchant. 1967. Chronic manganese poisoning. J. Postgrad. Med. Bombay 13: 116.

Belicenko, R. 1968. Manganese and zinc in broiler rations. Ptice-vodstvo 11: 12.

Bentley, O. G. and P. H. Phillips. 1951a. The effect of low manganese ration upon dairy cattle. J. Dairy Sci. 34: 396.

Bentley, O. G. and P. H. Phillips. 1951b. The effect of manganese deficiency in the chick on the uptake of inorganic phosphate by liver homogenates *in vitro*. Arch. Biochem. Biophys. 32: 338.

Bentley, O. G., G. V. Quicke, J. Kastelic, and P. H. Phillips. 1951. Certain trace elements in the feeds, organs and tissues of a selected group of repeat-breeding cows in Northwestern Wisconsin. J. Dairy Sci. 34: 363.

Bertinchamps, A. J., S. T. Miller and G. C. Cotzias. 1966. Interdependence of routes of excreting manganese. Amer. J. Physiol. 211: 217.

Blakemore, F., J. A. Nicholson and J. Stewart. 1937. Some effects of high manganese content in the diet of animals with special reference to lactation tetany. Vet. Record 49: 415.

Bolin, D. W., P. P. King and E. W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index. Science 116: 634.

Bolton, W. 1955. The effect of injections of estradiol deproprianate into immature pullets upon the manganese content of the blood plasma and some tissues. Brit. J. Nutr. 9: 170.

Bolton, W. 1957. Th
chicks and the
lines of Brown

Bonomi, A. 1966. Ma
plain. Riv. 2

Borg, D. C. and G. C.
rapid exchange
clearance and

Boyer, P. D., J. H. S
manganese defi

Britton, A. A. and G.
turnover on in

Brown, W. O. and K. J
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of fecal endog
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Buccellato, G. 1953.
metabolism of
vitro. Bol. S

Burnett, W. T., R. R.
Radio manganese
dog. Amer. J.

Caputto, R., P. B. McC
manganese and
extracts of ani
1025.

Caskey, C. D., W. D. C
manganese in th

Caskey, C. D. and L. C
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Soc. Exp. Biol.

Caskey, C. D. and L. C
by manganese de
Soc. Exp. Biol.

Caskey, C. D., L. C. N
tal ataxia in c
diet. Poul. Sc

Chatterjee, I. B., N.
lipid cofactor
into L-ascorbic

Bolton, W. 1957. The incidence of head retraction in newly-hatched chicks and the manganese contents of the eggs of some inbred lines of Brown Leghorn fowls. Poul. Sci. 36: 732.

Bonomi, A. 1966. Manganese content of forages produced in the Parma plain. Riv. Zootec. Agric. Vet. 4: 275.

Borg, D. C. and G. C. Cotzias. 1958. Manganese metabolism in man: rapid exchange of Mn^{56} with tissue as demonstrated by blood clearance and liver uptake. J. Clin. Invest. 37: 1269.

Boyer, P. D., J. H. Shaw and P. H. Phillips. 1942. Studies on manganese deficiency in the rat. J. Biol. Chem. 143: 417.

Britton, A. A. and G. C. Cotzias. 1966. Dependence of manganese turnover on intake. Amer. J. Physiol. 211: 203.

Brown, W. O. and K. J. McCracken. 1965. The portion of certain mineral nutrients in the colostomised laying pullet and the determination of fecal endogenous calcium and phosphorus excretion by an isotopic dilution method. J. Agric. Sci. 64: 305.

Buccellato, G. 1953. Possible action on activating carbohydrate metabolism of a compound of pyridoxine and manganese formed *in vitro*. Bol. Soc. Ital. Biol. Spec. 29: 1542.

Burnett, W. T., R. R. Bigelow, A. W. Kimball and C. W. Sheppard. 1952. Radio manganese studies in the mouse, rat and pancreatic fistula dog. Amer. J. Physiol. 168: 620.

Caputto, R., P. B. McCay and M. P. Carpenter. 1958. Requirements of manganese and cobalt for the synthesis of ascorbic acid by liver extracts of animals deprived of tocopherol. J. Biol. Chem. 233: 1025.

Caskey, C. D., W. D. Gallup and L. C. Norris. 1939. The need for manganese in the bone development of the chick. J. Nutr. 17: 404.

Caskey, C. D. and L. C. Norris. 1939. Relative effectiveness of ingested and injected manganese in preventing perosis. Proc. Soc. Exp. Biol. Med. 40: 590.

Caskey, C. D. and L. C. Norris. 1940. Micromelia in adult fowl caused by manganese deficiency during embryonic development. Proc. Soc. Exp. Biol. Med. 44: 332.

Caskey, C. D., L. C. Norris and G. F. Heuser. 1944. A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poul. Sci. 23: 516.

Chatterjee, I. B., N. C. Kar, N. C. Ghosh and B. C. Guha. 1960. On a lipid cofactor involved in the conversion of L-gluconolactone into L-ascorbic acid. Arch. Biochem. Biophys. 86: 154.

Chornock, C., N. B. G
manganese on c
445.

Christiansen, J. B.,
relation betwe
of hen's eggs.

Christiansen, J. B.,
nature of the
rations for th

Chubb, L. G. 1954.
deficient diets
Poultry Cong. S

Combs, G. F., L. C. Nc
tionship of man
ment. J. Nutr.

Cotzias, G. C. 1958.
38: 503.

Cotzias, G. C. 1960.
minerals. Fed.

Cotzias, G. C. 1968.
manganese concer
picture. Neuro

Cotzias, G. C. and J.
the manganese pa
1298.

Crampton, E. W. and L.
2nd edition. Fr

Creek, R. D., C. W. Car
effect of techni
Poul. Sci. 39: 1

Crosby, W. H., J. I. Mu
method for clini
J. 5: 693.

Cunningham, G. N., M. B
dietary levels o
stituents of calv

Curran, G. L. 1954. Ef
hepatic synthesis
210: 765.

Chornock, C., N. B. Guerrant and R. A. Dutcher. 1942. Effect of manganese on calcification in the growing rat. J. Nutr. 23: 445.

Christiansen, J. B., J. G. Halpin and E. B. Hart. 1939. A possible relation between manganese, sunlight and winter hatchability of hen's eggs. Science 90: 356.

Christiansen, J. B., J. G. Halpin and E. B. Hart. 1940. Studies on the nature of the effective supplements for soybean oil meal in rations for the production of hatching eggs. Poul. Sci. 19: 55.

Chubb, L. G. 1954. Further observations on the feeding of manganese deficient diets to growing and breeding chicken. 10th World Poultry Cong. Sect. Papers, Edinburgh.

Combs, G. F., L. C. Norris and G. F. Heuser. 1942. The interrelationship of manganese phosphatase and vitamin D in bone development. J. Nutr. 23: 131.

Cotzias, G. C. 1958. Manganese in health and disease. Physiol. Rev. 38: 503.

Cotzias, G. C. 1960. Metabolic relations of manganese to other minerals. Fed. Proc. 19: 655 (Abstr.).

Cotzias, G. C. 1968. Chronic manganese poisoning clearance of tissue manganese concentrations with persistence of neurological picture. Neurology 18: 376.

Cotzias, G. C. and J. J. Greenough. 1958. The high specificity of the manganese pathway through the body. J. Clin. Invest. 37: 1298.

Crampton, E. W. and L. E. Harris. 1969. Applied animal nutrition. 2nd edition. Freeman, San Francisco.

Creek, R. D., C. W. Carrick, S. M. Hange and H. E. Parker. 1960. The effect of technical grade manganese sulfate on vitamin stability. Poul. Sci. 39: 109.

Crosby, W. H., J. I. Munn and F. W. Furth. 1954. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5: 693.

Cunningham, G. N., M. B. Wise and E. R. Barrick. 1966. Effect of high dietary levels of manganese on the performance and blood constituents of calves. J. Anim. Sci. 25: 532.

Curran, G. L. 1954. Effect of certain transition group elements on hepatic synthesis of cholesterol in the rats. J. Biol. Chem. 210: 765.

Davis, P. N., L. C. N
soybean protei
Nutr. 77: 217.

Dietrich, C. P., M. E
degradation of
and properties
J. Biol. Chem.

Diez-Ewald, M., L. R.
relationship o
Biol. Med. 129

Dyer, I. A. 1960. D
Handbook. Was

Dyer, I. A. 1961. In
of deformed ca
(Abstr.).

Dyer, I. A., W. A. Ca
deficiency in t
31.

Edgar, G. 1942. Expe
1. The effect
Vet. J. 18: 28.

Ellis, G. H., S. E. Sm
Further studies
34: 21.

Erway, L., L. S. Hurle
otolith defects
100: 643.

Everson, G. J. 1970.
tation of the o
Metab. Anim., 1
Edinburgh.

Everson, G. J. and A.
in children. J

Everson, G. J., L. S. H
deficiency in gu

Everson, G. J. and R. E
manganese defici

Fain, P., J. Dennis and
manganese in fee
Amer. J. Vet. Re

Davis, P. N., L. C. Norris and F. H. Kratzer. 1962. Interference of soybean proteins with the utilization of trace mineral. J. Nutr. 77: 217.

Dietrich, C. P., M. E. Silva and Y. M. Michelacci. 1973. Sequential degradation of heparin in *Flavobacterium heparium*. Purification and properties of five enzymes involved in heparin degradation. J. Biol. Chem. 248: 6408.

Diez-Ewald, M., L. R. Weintraub, and W. H. Crosby. 1968. Interrelationship of iron and manganese metabolism. Proc. Soc. Exp. Biol. Med. 129: 448.

Dyer, I. A. 1960. Deformed calves, a nutritional problem. Stockman's Handbook. Washington State University.

Dyer, I. A. 1961. Implications of manganese deficiency in the etiology of deformed calves. Proc. West. Sect. Am. An. Prod. 12: 67 (Abstr.).

Dyer, I. A., W. A. Cassatt, Jr., and R. R. Rao. 1964. Manganese deficiency in the etiology of deformed calves. Bioscience 14: 31.

Edgar, G. 1942. Experiments on copper storage in the liver of sheep. 1. The effect of increased iron, cobalt and manganese. Austral. Vet. J. 18: 28.

Ellis, G. H., S. E. Smith, E. M. Gates, D. Lobb and E. J. Larson. 1947. Further studies on manganese deficiency in the rabbit. J. Nutr. 34: 21.

Erway, L., L. S. Hurley and A. S. Fraser. 1970. Congenital ataxia and otolith defects due to manganese deficiency in mice. J. Nutr. 100: 643.

Everson, G. J. 1970. The effects of manganese deficiency during gestation of the offspring. Proc. 1st Int. Symp. Trace Element Metab. Anim., 1969 p. 125 (C. F. Mills, ed.) Livingstone, Edinburgh.

Everson, G. J. and A. L. Daniels. 1934. A study of manganese retention in children. J. Nutr. 8: 497.

Everson, G. J., L. S. Hurley and J. F. Geiger. 1959. Manganese deficiency in guinea pig. J. Nutr. 68: 49.

Everson, G. J. and R. E. Shrader. 1968. Abnormal glucose tolerance in manganese deficient guinea pigs. J. Nutr. 94: 89.

Fain, P., J. Dennis and F. G. Harbough. 1952. The effect of added manganese in feed on various mineral components of cattle blood. Amer. J. Vet. Res. 13: 348.

Fournier, P. and A. Fo
absorption and
Séances de la S

Frost, G., C. W. Aslin
in manganese de

Gallup, W. D. and L. C
required to pre

Gallup, W. D. and L. C
manganese in th

Gamble, C. T., S. L. H
1971. Manganes
gravid gilt. J

Giessler, H. and M. Ki
copper and mang
Landswirtsch. F

Gilbert, F. A. 1957.
University of O

Gligor, V., A. Tacu, S
Beris. 1966.
and zinc on sow
Zooteh., Buchar

Golding, W. V., P. J.
ference in mang
19: 263.

Gomorri, G. 1942. A
determination f
Lab. Clin. Med.

Grashuis, J., J. J. Le
De Schothorste.

Grummer, R. H., O. G.
The role of man
swine. J. Anim

Gubler, C. J., D. S. T
M. Wintrobe. 1
manganese on th
Med. 86: 223.

Gutowska, M. S. and R.
tion of laying
Sci. 21: 277.

Gutowska, M. S., E. M.
absorption in f

Fournier, P. and A. Fournier. 1972. Effect of ingestion of lactose on absorption and retention of manganese. Comptes. Rendus des Séances de la Société de Biologie 166: 29.

Frost, G., C. W. Asling and M. M. Nelson. 1959. Skeletal deformities in manganese deficient rats. Anat. Rec. 134: 37.

Gallup, W. D. and L. C. Norris. 1939a. The amount of manganese required to prevent perosis in the chick. Poul. Sci. 18: 76.

Gallup, W. D. and L. C. Norris. 1939b. The effect of a deficiency of manganese in the diet of the hen. Poul. Sci. 18: 83.

Gamble, C. T., S. L. Hansard, B. R. Moss, D. J. Davis and E. R. Lidvall. 1971. Manganese utilization and placental transfer in the gravid gilt. J. Anim. Sci. 32: 84.

Giessler, H. and M. Kirchgessner. 1959. Effect of supplements of copper and manganese in pig fattening and poultry rearing. Landswirtsch. Forsch. 12: 159.

Gilbert, F. A. 1957. Mineral nutrition and the balance of life. University of Oklahoma Press.

Gligor, V., A. Tacu, S. Florescu, N. Vermesan, I. Feredean, and L. Beris. 1966. Action of the trace elements cobalt, manganese and zinc on sows and piglets. Lucr. Stiint. Inst. Cercet. Zooteh., Bucharest 23: 57.

Golding, W. V., P. J. Schaible and J. A. Davidson. 1940. A breed difference in manganese requirement of laying hens. Poul. Sci. 19: 263.

Gomorri, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. J. Lab. Clin. Med. 27: 955.

Grashuis, J., J. J. Lehr, L. L. E. Beuvery and Asman-Beuvery. 1953. De Schothorste. Inst. Moderne Veevoeding, Netherlands.

Grummer, R. H., O. G. Bentley, P. H. Phillips and G. Behstedt. 1950. The role of manganese in growth, reproduction and lactation of swine. J. Anim. Sci. 9: 170.

Gubler, C. J., D. S. Taylor, E. J. Eichwald, G. E. Cartwright and M. M. Wintrobe. 1954. Copper metabolism. 12. Influence of manganese on the metabolism of copper. Proc. Soc. Exp. Biol. Med. 86: 223.

Gutowska, M. S. and R. T. Parkhurst. 1942. Studies in mineral nutrition of laying hens. 1. The manganese requirement. Poul. Sci. 21: 277.

Gutowska, M. S., E. M. Parrott and F. A. Slesinski. 1941. Manganese absorption in fowl. Poul. Sci. 20: 379.

Bahn, K. J., D. J. Tu
continuous rad
and manganese

Hakimova, A. M., N. P
thyroid glands
the feed. Gig

Hart, P. C. 1953. M
Diergeneesk. 7

Hart, P. C. 1954. A
in cow livers.

Hartman, R. H., G. Ma
dietary mangan

Hartmans, J. 1970.
Anim., 1969 p

Hawkins, G. E., G. H.
1955. Manganes
levels of calc

Heller, V. H. and R.
perosis in chi

Hendricks, D. E. 196
the baby pig.
Lansing.

Hill, R. 1967. Vitar
Brit. J. Nutr.

Hill, R. M. and D. E.
and thiamine i

Hill, R. M., D. E. Hol
Manganese defic
of equilibrium.

Hill, R. and J. W. Mat
metabolism of t
content of eggs
content. Brit.

Hill, R. and J. W. Mat
tissues of pull
Brit. J. Nutr.

Holtkamp, D. E., R. M.
effect on growt
with some obser
J. Nutr. 41: 30

Hahn, K. J., D. J. Tuma and J. L. Sullivan. 1968. Rapid and simple continuous radiochemical separation of copper, magnesium, zinc, and manganese in biological materials. Anal. Chem. 40: 974.

Hakimova, A. M., N. P. Zelinkova, and E. I. Pacenko. 1969. Changes in thyroid glands of rats given increased amounts of manganese in the feed. Gigiena Sanit. 1: 113.

Hart, P. C. 1953. Manganese content of livers of cows. Tijdschr. Diergeneesk. 78: 929.

Hart, P. C. 1954. An investigation into the content of trace elements in cow livers. Netherlands J. Agric. Sci. 2: 298.

Hartman, R. H., G. Matrone and G. H. Wise. 1955. Effect of high dietary manganese on hemoglobin formation. J. Nutr. 57: 429.

Hartmans, J. 1970. In Proc. 1st Int. Symp. Trace Element Metab. Anim., 1969 p 132 (C. F. Mills, ed.) Livingstone, Edinburgh.

Hawkins, G. E., G. H. Wide, G. Matrone, R. K. Waugh and W. L. Lett. 1955. Manganese in the nutrition of dairy cattle fed different levels of calcium and phosphorus. J. Dairy Sci. 38: 536.

Heller, V. H. and R. Penquite. 1937. Factors producing and preventing perosis in chickens. Poul. Sci. 16: 243.

Hendricks, D. E. 1967. Some factors affecting mineral utilization by the baby pig. Ph.D. thesis, Michigan State University, East Lansing.

Hill, R. 1967. Vitamin D and manganese in the nutrition of the chick. Brit. J. Nutr. 21: 507.

Hill, R. M. and D. E. Holtkamp. 1954. Storage of dietary manganese and thiamine in the rat. J. Nutr. 53: 73.

Hill, R. M., D. E. Holtkamp, A. R. Buchanan and E. K. Rutledge. 1955. Manganese deficiency in rats with relation to ataxia and loss of equilibrium. J. Nutr. 41: 359.

Hill, R. and J. W. Mathers. 1968a. Manganese in the nutrition and metabolism of the pullet. 1. Shell thickness and manganese content of eggs from birds given a diet of low or high manganese content. Brit. J. Nutr. 22: 625.

Hill, R. and J. W. Mathers. 1968b. The manganese content of the tissues of pullets given diets of high or low manganese content. Brit. J. Nutr. 22: 635.

Holtkamp, D. E., R. M. Hill, L. Toll and E. Campbell. 1950. The effect on growth of the level of manganese in the diet of rats, with some observations on the manganese-thiamine relationship. J. Nutr. 41: 307.

Hoogendoorn, H. 1940
Tijdschr. 52:

Howes, A. D. and I. A
effects on man
Sci. 32: 141.

Hughes, E. R. and G.
and manganese

Hughes, E. R., S. T.
tions of manga
207.

Hurley, L. S. 1969.
Nutr. Rev. 27:

Hurley, L. S. and G.
ing reflexes o
Soc. Exp. Biol.

Hurley, L. S., G. J.
deficiency in
66: 309.

Hurley, L. S., G. J.
phosphatase act
ing rats. J.

Hurley, L. S., G. J.
Disproportionat
rats. I. The

Hurley, L. S., J. Gowa
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Hurley, L. S., E. Woot
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Hurley, L. S., E. Woot
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offspring of ma

Hurley, L. S., L. L. T
chondria from ma
ultrastructure.

Insko, Jr., W. M., M.
requirement of
621.

Hoogendoorn, H. 1940. Minerals in poultry feeding. Landbouwk. Tijdschr. 52: 330.

Howes, A. D. and I. A. Dyer. 1971. Diet and supplemental mineral effects on manganese metabolism in newborn calves. J. Anim. Sci. 32: 141.

Hughes, E. R. and G. C. Cotzias. 1961. Adrenocorticosteroid hormones and manganese metabolism. Amer. J. Physiol. 201: 1061.

Hughes, E. R., S. T. Miller and G. C. Cotzias. 1966. Tissue concentrations of manganese and adrenal function. Amer. J. Physiol. 211: 207.

Hurley, L. S. 1969. Nutrients and genes: Interaction and development. Nutr. Rev. 27: 3.

Hurley, L. S. and G. J. Everson. 1959. Delayed development of righting reflexes of offspring of manganese deficient rats. Proc. Soc. Exp. Biol. Med. 102: 360.

Hurley, L. S., G. J. Everson and J. F. Geiger. 1958. Manganese deficiency in rats: Congenital nature of ataxia. J. Nutr. 66: 309.

Hurley, L. S., G. J. Everson and J. F. Geiger. 1959. Serum alkaline phosphatase activity in normal and manganese deficient developing rats. J. Nutr. 67: 445.

Hurley, L. S., G. J. Everson, E. Wooten and C. W. Asling. 1961a. Disproportionate growth in offspring of manganese deficient rats. 1. The long bones. J. Nutr. 74: 274.

Hurley, L. S., J. Gowan and G. Milhand. 1969. Calcium metabolism in the manganese-deficient and zinc-deficient rats. Proc. Soc. Exp. Biol. Med. 130: 856.

Hurley, L. S., E. Wooten and G. J. Everson. 1961b. Disproportionate growth of offspring of manganese-deficient rats. 2. Skull, brain and cerebrospinal fluid pressure. J. Nutr. 74: 282.

Hurley, L. S., E. Wooten, G. J. Everson and C. W. Asling. 1960. Anomalous development of ossification in the inner ear of offspring of manganese-deficient rats. J. Nutr. 71: 15.

Hurley, L. S., L. L. Theriault and I. E. Droosti. 1970. Liver mitochondria from manganese-deficient and pallid mice: Function and ultrastructure. Science 170: 1316.

Insko, Jr., W. M., M. Lyons, and H. H. Martin. 1938. The quantitative requirement of the growing chick for manganese. J. Nutr. 15: 621.

Jacobson, A., S. Barr
and Y. T. Oest
activation ana
metals in bloo

Johnson, S. R. 1940.
Soc. Animal Pr

Johnson, S. R. 1943.
manganese. J.

Johnson, S. R. 1944.
natural foodstu

Johnson, R. R., O. G.
minerals and pr
and chicks fed

Kaellis, E. 1970. Ef
in the rat. Pr

Kanchatnov, V. P. 195
content of food
regions. Gigie

Kanchatnov, V. P. 195
subjects and pa

Kamimura, T. 1938. T
J. Orient. Med.

Kealy, R. D. and T. W.
and interaction
1352.

Keith, T. B., R. C. Mi
Nutritional def
corn, tankage,
pigs. J. Anim.

Kemmerer, A. R., C. A.
relation of many
Chem. 92: 623.

Kent, N. L. and R. A. K
"minor" elements
Biochem. J. 35:

Kolomijceva, M. G. and
different ratios
tective reaction

Konseko, L. G. 1965.
patients with di
Exp. Biol. 24 Tr

Jacobson, A., S. Barr, T. Fields, I. G. Tels, E. Kaplan, P. Gustafson and Y. T. Oester. 1961. Practical application of neutron activation analysis for the determination of protein bound metals in blood serum. J. Nucl. Med. 2: 289.

Johnson, S. R. 1940. Growth on manganese low rations. Proc. Amer. Soc. Animal Prod. p. 325.

Johnson, S. R. 1943. Studies with swine on rations extremely low in manganese. J. Anim. Sci. 2: 14.

Johnson, S. R. 1944. Studies with swine on low manganese rations of natural foodstuffs. J. Anim. Sci. 3: 136.

Johnson, R. R., O. G. Bentley and T. S. Sutton. 1959. Divalent minerals and proteolytic activity of pancreas tissue from rats and chicks fed manganese-deficient diets. J. Nutr. 67: 513.

Kaellis, E. 1970. Effect of manganese ions on thyroidal iodine metabolism in the rat. Proc. Soc. Exp. Biol. Med. 135: 216.

Kamchatnov, V. P. 1953. Relative characteristics of the manganese content of food products in endemic and non-endemic goitre regions. Gigiena Sanit. 2: 33.

Kamchatnov, V. P. 1959. Manganese content of the thyroid in healthy subjects and patients with goitre. Gigiena Sanit. 4: 76.

Kamimura, T. 1938. The influence of manganese on the growth of bone. J. Orient. Med. 29: 25.

Kealy, R. D. and T. W. Sullivan. 1966. Studies on manganese requirement and interactions in the diet of young turkeys. Poul. Sci. 45: 1352.

Keith, T. B., R. C. Miller, W. T. S. Thorp, and M. A. McCarty. 1942. Nutritional deficiencies of a concentrate mixture composed of corn, tankage, soybean oilmeal, and alfalfa meal for growing pigs. J. Anim. Sci. 1: 120.

Kemmerer, A. R., C. A. Elvehjem and E. B. Hart. 1931. Studies on the relation of manganese to the nutrition of the mouse. J. Biol. Chem. 92: 623.

Kent, N. L. and R. A. McCance. 1941. The absorption and excretion of "minor" elements by man. 2. Cobalt, nickel, tin and manganese. Biochem. J. 35: 877.

Kolomijceva, M. G. and F. M. Veznesenskaja. 1968. Significance of different ratios of copper to manganese in the diet for protective reactions of the organism. Vop. Pitan. 27: 77.

Konseko, L. G. 1965. Concentration of trace elements in blood of patients with diabetes mellitus. Fed. Proc. Fed. Amer. Soc. Exp. Biol. 24 Trans. Suppl. T237.

Krieg, R. 1966. Eff
ments in the f
of organs and

Krolak, E. 1968. Ef
fertility and
Arch. Wet. 11:

Lassiter, J. W. and J
diet on certai

Lassiter, J. W., J. D
manganese on s
1st Int. Symp.
Mills, ed.) L

Leach, R. M. 1967.
saccharides.

Leach, R. M. 1968.
plate in the y

Lehninger, A. L. 195
Physiol. Rev.

Lehninger, A. L. 197

Leibholz, J. M., V. C
manganese on b
J. Anim. Sci.

Litricin, V. B. 1967
Acta vet. Belg

Litricin, V. B. and V
of chicken on
Sarajevo 15: 3

Long, C. H., D. E. Ul
phatase in post
activity. Proc

Longstaff, M. and R.
of thin and thi
varying mangane
Trace Element
Livingstone, Ed

Longstaff, M. and R.
ation with othe
istics. Brit.

Lyons, M. 1939. Some
Arkansas Agr.

Krieg, R. 1966. Effect of different amounts of trace element supplements in the feed of White Leghorn hens. 3. Manganese content of organs and tissues. Arch. Geflugelk. 30: 213.

Krolak, E. 1968. Effect of adding manganese salts to the diets on fertility and manganese content of the hair of cows. Polskie. Arch. Wet. 11: 293.

Lassiter, J. W. and J. D. Morton. 1968. Effects of a low manganese diet on certain ovine characteristics. J. Anim. Sci. 27: 776.

Lassiter, J. W., J. D. Morton and W. J. Miller. 1970. Influence of manganese on skeletal development in the sheep and rat. Proc. 1st Int. Symp. Trace Element Metab. Anim., 1969 p. 130 (C. F. Mills, ed.) Livingstone, Edinburgh.

Leach, R. M. 1967. Role of manganese in the synthesis of mucopolysaccharides. Fed. Proc. 26: 118 (Abstr.).

Leach, R. M. 1968. Effect of manganese upon the epiphyseal growth plate in the young chick. Poul. Sci. 47: 828.

Lehninger, A. L. 1950. Role of metal ions in enzyme systems. Physiol. Rev. 30: 393.

Lehninger, A. L. 1970. Biochemistry. Worth Publishers, New York.

Leibholz, J. M., V. C. Speer and V. W. Hays. 1962. Effect of dietary manganese on baby pig performance and tissue manganese levels. J. Anim. Sci. 21: 772.

Litricin, V. B. 1967. Experimental manganese deficiency and perosis. Acta vet. Belgrade 17: 353.

Litricin, V. B. and V. Andrejevic. 1966. Growth of leg and wing bones of chicken on diet deficient in manganese. Veterinaria, Sarajevo 15: 385.

Long, C. H., D. E. Ullrey, E. R. Miller. 1965. Serum alkaline phosphatase in post-natal pig and effect of serum storage on enzyme activity. Proc. Soc. Exp. Biol. Med. 119: 412.

Longstaff, M. and R. Hill. 1970. The matrix and uronic acid contents of thin and thick shells produced by pullets given diets of varying manganese and phosphorus content. Proc. 1st Int. Symp. Trace Element Metab. Anim., 1969 p. 137 (C. F. Mills, ed.) Livingstone, Edinburgh.

Longstaff, M. and R. Hill. 1971. The influence of manganese in association with other dietary components on certain shell characteristics. Brit. Poultry Sci. 12: 169.

Lyons, M. 1939. Some effects of manganese on egg-shell quality. Arkansas Agr. Exp. Sta. Bull. 374.

Lyons, M. and W. M.
embryo. Ky.

McGovern, J. J., A.
crit of capill

Mahoney, J.P. and W.
logical half l
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Marot, R. and M. Dura
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Mathers, J. W. and R.
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Mathers, J. W., M. Lo
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Matrone, G., R. H. Ha
manganese-iron
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Maynard, L. S. and S.
radio-manganese
35: 831.

Mehrolera, M. P., M.
manganese chlor
Assoc. 42: 517.

Mena, I., O. Marin, S.
manganese poiso
Neurology 17: 1

Mildvan, A. S., M. C.
carboxylase: V
J. Biol. Chem.

Miller, E. R., D. E. U
Luecke and J. A
to maturity. I
concentration.

Miller, E. R., D. E. U
J. A. Hoeffer a
the baby pig.

Miller, R. C., T. B. R
Manganese as a
lameness in pigs

Lyons, M. and W. M. Insko, Jr. 1937. Chondrodystrophy in the chick embryo. Ky. Agr. Exp. Sta. Bull. 371.

McGovern, J. J., A. R. Jones and A. G. Steinberg. 1955. The hematocrit of capillary blood. New England J. Med. 253: 308.

Mahoney, J.P. and W. J. Small. 1968. Studies on manganese. The biological half life of radio manganese in man and factors which affect this half life. J. Clin. Invest. 47: 643.

Marot, R. and M. Durand. 1944. Manganese in tumors. C. R. Acad. Sci. 219: 287.

Mathers, J. W. and R. Hill. 1967. Factors influencing the retention of an oral dose of radioactive manganese by the chick. Brit. J. Nutr. 21: 513.

Mathers, J. W., M. Longstaff and R. Hill. 1971. The influence on shell formation of the pre-laying period for which a low manganese diet is given. Brit. Poultry Sci. 12: 179.

Matrone, G., R. H. Hartman and A. J. Clawson. 1959. Studies on a manganese-iron antagonism in the nutrition of rabbits and baby pigs. J. Nutr. 67: 309.

Maynard, L. S. and S. Fink. 1956. The influence of chelation on radio-manganese secretion in man and mouse. J. Clin. Invest. 35: 831.

Mehrolera, M. P., M. G. Chakravarti and R. P. Mangal. 1964. Oral manganese chloride as a hypoglycaemic agent. J. Indian Med. Assoc. 42: 517.

Mena, I., O. Marin, S. Feunzelida and G. C. Cotzias. 1968. Chronic manganese poisoning: Clinical picture and manganese turnover. Neurology 17: 128.

Mildvan, A. S., M. C. Scrutton and M. F. Utter. 1966. Pyruvate carboxylase: VII. A possible role for tightly bound manganese. J. Biol. Chem. 241: 3488.

Miller, E. R., D. E. Ullrey, Inge Ackermann, D. A. Schmidt, R. W. Luecke and J. A. Hoeffer. 1961. Swine hematology from birth to maturity. II. Erythrocyte population, size and hemoglobin concentration. J. Anim. Sci. 20: 890.

Miller, E. R., D. E. Ullrey, C. L. Zutant, B. V. Baltzer, D. A. Schmidt, J. A. Hoeffer and R. W. Luecke. 1962. Calcium requirement of the baby pig. J. Nutr. 77: 7.

Miller, R. C., T. B. Ruth, M. A. McCarty and W. T. S. Thorp. 1940. Manganese as a possible factor influencing the occurrence of lameness in pigs. Proc. Soc. Exp. Biol. Med. 45: 50.

Miller, W. J. 1973.
tion turnover
cadmium, mang
1915 (Abstr.)

Miller, W. J., M. W.
Lassiter. 19
over of ^{54}Mn
practical-typ

Miller, W. J., M. W.
Lassiter, and
rates of radi
dosing in Hol
Sci. 34: 460.

Moinuddin, J.F. and
changes due t
sodium sulfat

Morimoto, H., M. Yosh
fied ration f
purified ratio

Muraleedharan, K. and
prosthogonimid
Indian Vet. J.

Murty, V. N. 1957.
Res. 16C: 120-

Mussehl, F. E. and C.
to a specific

National Research Cou
animals, No. 2
Acad. Sci., Na

Nehrer, G. M., L. P. I
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Newland, H. W. and G.
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Newland, H. W., G. K.
fer of phosphor
dietary mangane

Nielsen, H. N. and D.
deformities in

North, B. B., J. M. Le
metabolism in c

Miller, W. J. 1973. Dynamics of absorption rates, endogenous excretion turnover and homeostatic control mechanisms of zinc, cadmium, manganese and nickel in ruminants. Fed. Proc. 32: 1915 (Abstr.).

Miller, W. J., M. W. Neathery, R. P. Gentry, D. M. Blackmon and J. W. Lassiter. 1973. Fecal excretion, tissue accumulation and turnover of ^{54}Mn after intravenous dosing in Holstein calves fed a practical-type diet. J. Anim. Sci. 37: 827.

Miller, W. J., M. W. Neathery, R. P. Gentry, D. M. Blackmon, J. W. Lassiter, and F. M. Pate. 1972. Distribution and turnover rates of radioactive manganese in various tissues after duodenal dosing in Holstein calves fed a practical-type diet. J. Anim. Sci. 34: 460.

Moinuddin, J.F. and H. W. T. Lee. 1960. Alimentary, blood and other changes due to feeding manganese sulfate, magnesium sulfate and sodium sulfate. Amer. J. Physiol. 199: 77.

Morimoto, H., M. Yoshida and H. Hoshii. 1959. Preparation of a purified ration for chicks. 1. Manganese deficiency in semi-purified ration. Bull. Nat. Inst. Agric. Sci. Japan 18: 15.

Muraleedharan, K. and B. P. Pande. Experimentation infection with a prosthogonimid metacercaria in chicks fed deficient mashes. Indian Vet. J. 45: 641.

Murty, V. N. 1957. Manganese requirements of sheep. J. Sci. Indust. Res. 16C: 120-121.

Mussehl, F. E. and C. W. Ackerson. 1939. The effect of adding manganese to a specific ration for growing poults. Poul. Sci. 18: 408.

National Research Council. 1973. Nutrient requirements of domestic animals, No. 2. Nutrient requirements of swine. 7th ed. Natl. Acad. Sci., Natl. Res. Council. Washington, D.C.

Nehrer, G. M., L. P. Doyle, D. M. Thrasher and M. P. Plumlee. 1956. Radiographic and histopathological findings in the bones of swine deficient in manganese. Amer. J. Vet. Res. 17: 121.

Newland, H. W. and G. K. Davis. 1961. Placental transfer of manganese in swine. J. Anim. Sci. 20: 15.

Newland, H. W., G. K. Davis and H. D. Wallace. 1960. Placental transfer of phosphorus in sows maintained on high and low levels of dietary manganese. Amer. J. Physiol. 198: 745.

Nielsen, H. N. and D. E. Madsen. 1942. Some studies on leg bone deformities in turkeys. Poul. Sci. 21: 500.

North, B. B., J. M. Leichsenring and L. M. Norris. 1960. Manganese metabolism in college women. J. Nutr. 72: 217.

O'Dell, B. L., B. C
cies of milk
73: 151.

Orent, E. R. and E.
manganese in

Panic, B. and L. Ekn
in domestic a

Papavasiliou, P. S.,
liver in regu
Amer. J. Phys

Parker, H. E., F. N.
Hauge. 1955.
with radioact

Pepper, W. F., S. J.
ameomycin on
levels of sal

Pepper, W. F., S. J.
on niacin and
656.

Perla, D. and M. Sanc
B1 and mangane
effects. Proc

Pilla, A. M. 1958.
Ann. Sper. Agr

Plumlee, M. P., D. M.
Parker. 1956.
growth develop
15: 352.

Poll, E., V. Martin,
manganese for
17: 59.

Pollack, S., J. N. Ge
1965. The abs
J. Clin. Inv.

Pontremoli, S., E. Gr
phatase from r
sites measured
597.

Querishi, M. S., I. A.
growing chick

O'Dell, B. L., B. C. Hardwick and G. Reynolds. 1961. Mineral deficiencies of milk and congenital malformations in the rat. J. Nutr. 73: 151.

Orent, E. R. and E. V. McCollum. 1931. Effects of deprivation of manganese in the rat. J. Biol. Chem. 92: 651.

Panic, B. and L. Ekman. 1967. Binding of manganese to serum proteins in domestic animals. Acta Vet. Belgrade 17: 167.

Papavasiliou, P. S., S. F. Miller and G. C. Cotzias. 1966. Role of liver in regulating, distribution and excretion of manganese. Amer. J. Physiol. 211: 211.

Parker, H. E., F. N. Andrews, C. W. Carrick, R. D. Creek and S. M. Hauge. 1955. Effect of manganese on bone formation studied with radioactive isotopes. Poul. Sci. 34: 1154.

Pepper, W. F., S. J. Slinger and I. Motzok. 1952. The effect of ameomycin on the manganese requirement of chicks fed varying levels of salt and phosphorus. Poul. Sci. 31: 1054.

Pepper, W. F., S. J. Slinger, and I. Motzok. 1953. Effect of auromycin on niacin and manganese requirements of chicks. Poul. Sci. 32: 656.

Perla, D. and M. Sandberg. 1939. Metabolic interdependence of vitamin B_1 and manganese. Reciprocal neutralization of their toxic effects. Proc. Soc. Exp. Biol. Med. 41: 522.

Pilla, A. M. 1958. Manganese in relation to growth index of chickens. Ann. Sper. Agrar. 12: 747.

Plumlee, M. P., D. M. Thrasher, W. M. Beeson, F. N. Andrews and H. E. Parker. 1956. The effects of a manganese deficiency upon the growth development, and reproduction of swine. J. Anim. Sci. 15: 352.

Poll, E., V. Martin, M. Dumitrescu and V. Martin. 1967. Importance of manganese for the growth of young birds. Rev. Zovtch. Med. Vet. 17: 59.

Pollack, S., J. N. George, R. C. Reba, R. M. Kaufman and N. H. Crosby. 1965. The absorption of non-ferrous metals in iron deficiency. J. Clin. Inv. 44: 1470.

Pontremoli, S., E. Grazi and A. Accorsi. 1969. Fructose 1, 6-diphosphatase from rabbit liver. XIII. The number of Mn^{++} binding sites measured with $^{54}Mn^{++}$. Biochem. Biophys. Res. Commun. 37: 597.

Querishi, M. S., I. A. Khan and B. H. Schneider. 1963. Manganese in growing chick rations. W. Pakistan J. Agric. Res. 1: 68.

Radoin, L. 1944. C
iron, mangane
of all these
diets contair

Ray, T. W. and L. J.
Effect on oxy
Biol. Med. 51

Rehner, G. and W. St
nickel on liv
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Rehner, G. and H. D.
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Reineke, E. P. and C
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Richards, M. B. 193(
J. 24: 1572.

Robinson, N. W., S. I
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Rojas, M. A., I. A. D
in the bovine.

Rothstein, A., A. Hay
active transpo
J. Gen. Physio

Rozybakiev, M. A. 19
sheep. Trudy

Rubenstein, A. A., N.
induced by man

Rudra, M. N. 1939.
ascorbic acid.

Rudra, M. N. 1944a.

Rudra, M. N. 1944b.
ascorbic acid.

Sahagian, B. M., I. Ha
zinc, manganese
intestine. J.

Sahagian, B. M., I. Ha
involvements of
intestine. J.

Radoin, L. 1944. Comparison of the effect of single deprivation of iron, manganese, iodine and aluminium with that of deprivation of all these trace elements in producing polyneuritis with diets containing B-vitamins. C. R. Soc. Biol. 138: 732.

Ray, T. W. and L. J. Deysach. 1942. Storage of manganese by thyroid. Effect on oxygen consumption of guinea pig. Proc. Soc. Exp. Biol. Med. 51: 228.

Rehner, G. and W. Stelte. 1970. Effect of manganese, cobalt and nickel on liver arginase activity *in vitro* and *in vivo*. Med. Ernahrung. 11: 32.

Rehner, G. and H. D. Cremer. 1970. Assessment of trace element requirement by estimation of enzyme activities. Nahrung. 14: 193.

Reineke, E. P. and C. W. Turner. 1945. The effect of manganese compounds and certain other factors on the formation of thyroxine in iodinated casein. J. Biol. Chem. 161: 613.

Richards, M. B. 1930. Manganese in relation to nutrition. Biochem. J. 24: 1572.

Robinson, N. W., S. L. Hansard, D. M. Johns and G. L. Robertson. 1960. Excess dietary manganese and feed lot performance on beef cattle. J. Anim. Sci. 19: 1290.

Rojas, M. A., I. A. Dyer and W. A. Cassatt. 1965. Manganese deficiency in the bovine. J. Anim. Sci. 24: 664.

Rothstein, A., A. Hayes, D. H. Jennings and D. C. Hooper. 1958. The active transport of magnesium and manganese into the yeast cell. J. Gen. Physiol. 41: 585.

Rozybakiev, M. A. 1966. Effect of manganese on parotid secretion in sheep. Trudy Inst. Fizol. Akad. Nauk Kazahsk. SSR. 10: 85.

Rubenstein, A. A., N. W. Levin and G. A. Elliot. 1962. Hypoglycaemia induced by manganese. Nature 194: 188.

Rudra, M. N. 1939. Role of manganese in the biological synthesis of ascorbic acid. Nature 144: 868.

Rudra, M. N. 1944a. Manganese hunger in animals. Nature 153: 111.

Rudra, M. N. 1944b. Role of manganese in the biological synthesis of ascorbic acid. Nature 153: 733.

Sahagian, B. M., I. Harding-Barlow and H. M. Perry. 1966. Uptakes of zinc, manganese, cadmium and mercury by intact strips of rat intestine. J. Nutr. 90: 259.

Sahagian, B. M., I. Harding-Barlow and H. M. Perry. 1967. Transmural involvements of zinc, manganese, cadmium and mercury by rat small intestine. J. Nutr. 93: 291.

Saltman, P., R. D. Fi
metabolism of
agents. J. Bi

Samofal, T. S. 1961.
of rats on gro
20: 44.

Sandberg, M., D. Perl
vitamin B_1 and
metabolism in
42: 368.

Sandstedt, H. and H.
caused by defi

Sandstedt, H., I. Dyr
ment of cattle

Sawney, P. C. and N.
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17C: 185.

Schaible, P. J. and S
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Shrader, R. E. and G.
manganese defi

Sigma Technical Bulle
alkaline and p
Louis, Mo.

Saltman, P., R. D. Fiskin, S. B. Bellinger and T. Alex. 1956. The metabolism of iron by rat liver slices. The effect of chemical agents. J. Biol. Chem. 220: 751.

Samofal, T. S. 1961. Effect of reduced manganese content in the diet of rats on growth and development of their progeny. Vop. Pitan. 20: 44.

Sandberg, M., D. Perla and D. M. Holly. 1939. Interdependence of vitamin B_1 and manganese. 2. Manganese, copper and iron metabolism in B_1-deficient rats. Proc. Soc. Exp. Biol. Med. 42: 368.

Sandstedt, H. and H. Carlquist. 1951. A form of rickets in pigs caused by deficiency of manganese. Nord. Vet. Med. 3: 1118.

Sandstedt, H., I. Dyrendahl and K. Kjalmars. 1951. Preventive treatment of cattle with manganese. Nord. Vet. Med. 3: 342.

Sawney, P. C. and N. D. Kehar. 1958. Studies on the composition of blood in relation to manganese intake. J. Sci. Indust. Res. 17C: 185.

Schaible, P. J. and S. L. Bandemer. 1942. The effect of mineral supplements on the availability of manganese. Poul. Sci. 21: 8.

Schaible, P. J., S. L. Bandemer and J. A. Davidson. 1938. The manganese content of feedstuffs and its relation to poultry nutrition. Mich. Agr. Exp. Sta. Tech. Bull. 159.

Scott, M. L., M. C. Nesheim and R. J. Young. 1969. Nutrition of the chicken. M. L. Scott & Assoc. Ithaca, N.Y.

Scrutton, M. C., M. F. Utter and A. S. Mildvan. 1966. Pyruvate carboxylase. VI. The presence of tightly bound manganese. J. Biol. Chem. 241: 3480.

Settle, E. A., F. R. Mraz, C. R. Douglas, and J. K. Bletner. 1969. Effect of diet and manganese level of growth, perosis and ^{54}Mn uptake in chicken. J. Nutr. 97: 141.

Shils, M. D. and E. V. McCollum. 1943. Further studies on the symptoms of manganese deficiency in rat and mouse. J. Nutr. 26: 1.

Shrader, R. E. and G. J. Everson. 1967. Anomalous development of otoliths associated with postural defects in manganese deficient guinea pigs. J. Nutr. 91: 453.

Shrader, R. E. and G. J. Everson. 1968. Pancreatic pathology in manganese deficient guinea pigs. J. Nutr. 94: 269.

Sigma Technical Bulletin 104. 1963. Determination of serum acid, alkaline and prostate phosphatase. Sigma Chemical Co., St. Louis, Mo.

Skinner, J. T. and
upon concent
Amer. J. Phy

Skinner, J. T. and
ascorbic and
Amer. J. Phy

Skinner, J. T. and
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Slavin, W. 1968.
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Smith, S. E., M. Me
in the rabbi

Speer, V. C., Calvo
Trace mineral
Anim. Sci. 1

Starodubova, T. V.
during term a
27: 36.

Sullivan, T. J. 1960
rats. Nature

Suso, F. A. and H. M
agents on abs
Poul. Sci. 47

Thiers, R. E. and B.
cellular frac

Thomas, J. W. 1970.
53: 1107.

Tsai, H. C. C. and G.
deficiency on
Nutr. 91: 447.

Tukey, J.W. 1953. T
Princeton Univ

Turton, J. D. 1953.
deficiency. B

Ugnenko, V. K. 1972.
cobalt and man
and manganese

Skinner, J. T. and J. S. McHargue. 1944. Effect of manganese intake upon concentration of bisulfite-binding substance in blood. Amer. J. Physiol. 141: 647.

Skinner, J. T. and J. S. McHargue. 1946a. Supplementary effects of ascorbic and manganese on copper in the synthesis of hemoglobin. Amer. J. Physiol. 145: 500.

Skinner, J. T. and J. S. McHargue. 1946b. Experiments to ascertain the effect of manganese on the synthesis of ascorbic acid in the guinea pig. Amer. J. Physiol. 145: 506.

Slavin, W. 1968. Atomic absorption spectroscopy. Interscience Publishers. New York.

Smith, S. E., M. Medlicott and G. H. Ellis. 1944. Manganese deficiency in the rabbit. Arch. Bioch. 4: 281.

Speer, V. C., Calvon, P. G. Homomeyer and C. C. Culbertson. 1952. Trace minerals for growing-fattening swine fed in drylot. J. Anim. Sci. 11: 12.

Starodubova, T. V. 1968. Manganese balance in boarding school students during term and during summer in a pioneers' camp. Vop. Pitan. 27: 36.

Sullivan, T. J. 1960. Effect of manganese edetate on blood formation in rats. Nature 186: 87.

Suso, F. A. and H. M. Edwards. 1968. Influence of various chelating agents on absorption of ^{60}Co, ^{59}Fe, ^{54}Mn and ^{65}Zn by chickens. Poul. Sci. 47: 1417.

Thiers, R. E. and B. L. Vallee. 1957. Distribution of metals in subcellular fractions of rat liver. J. Biol. Chem. 226: 911.

Thomas, J. W. 1970. Metabolism of iron and manganese. J. Dairy Sci. 53: 1107.

Tsai, H. C. C. and G. J. Everson. 1967. The effect of manganese deficiency on acidic mucopolysaccharides in cartilages. J. Nutr. 91: 447.

Tukey, J.W. 1953. The problem of multiple comparisons. Ditto, Princeton University.

Turton, J. D. 1953. Perosis in duckling complicated by a riboflavin deficiency. Brit. Vet. J. 109: 171.

Ugnenko, V. K. 1972. Effect of different ratios of iodine, copper, cobalt and manganese in the diet on the distribution of copper and manganese in organs of rats. Voprosy. Pitaniya 31: 21.

Ullrey, D. E., E. R
Hoeffer. 19
Serum calciu
inorganic ph

Underwood, E. J. 1
3rd edition.

Urban, T. J. 1959.
manganese fo

Van Reen, R. and P.
duck. J. Nu

Vohra, P. and J. R.
manganese an

Vorob'eva, A. M. K.
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Wachtel, L. W., C.
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Wacker, W. E. C. an
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Watson, L. T., C. B
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Watson, L. T., C. B
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Sci. 49: 154

Watson, L. T., C. B.
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Weed, R. I. and Roth
adult red cel

Weinberg, E. D. 196
secondary bio
12: 436.

Wells, I. C. and C.
choline-defic
Biophys. 112:

Werner, A. and M. An
as a means of
rung. 10: 142

Wessinger, G. D. and
boron compoun

Ullrey, D. E., E. R. Miller, B. E. Brent, B. L. Bradley and J. A. Hoeffer. 1967. Swine hematology from birth to maturity. IV. Serum calcium, magnesium, sodium, potassium, copper, zinc and inorganic phosphorus. J. Anim. Sci. 26: 1024.

Underwood, E. J. 1971. Trace elements in human and animal nutrition. 3rd edition. Academic Press, New York.

Urban, T. J. 1959. The reaction of the rat to different levels of manganese following hepatectomy. J. Nutr. 69: 158.

Van Reen, R. and P. B. Pearson. 1955. Manganese deficiency in the duck. J. Nutr. 55: 225.

Vohra, P. and J. R. Heil. 1969. Dietary interactions between zinc, manganese and copper for turkeys. Poul. Sci. 48: 1686.

Vorob'eva, A. M. K. 1970. Effect of small amounts of copper, manganese on animals. Gigiena Saint. 11: 96.

Wachtel, L. W., C. A. Elvehjem and E. B. Hart. 1943. Studies on the physiology of manganese in the rat. Amer. J. Physiol. 140: 72.

Wacker, W. E. C. and B. L. Valler. 1964. In Mineral metabolism. Vol. IIA (C. L. Comer and F. Bronner, ed.), Academic Press, N.Y.

Watson, L. T., C. B. Ammerman, J. P. Feaster and C. E. Roessler. 1973. Influence of manganese intake on metabolism of manganese and other minerals in sheep. J. Anim. Sci. 36: 131.

Watson, L. T., C. B. Ammerman, S. M. Miller and R. H. Harms. 1970. Biological assay of inorganic manganese for chicken. Poul. Sci. 49: 1548.

Watson, L. T., C. B. Ammerman, S. M. Miller and R. H. Harms. 1971. Biological availability to chicks of manganese from different inorganic sources. Poul. Sci. 50: 1693.

Weed, R. I. and Rothstein, F. 1958. Uptake of Mn^{++} by normal human adult red cells. Fed. Proc. 18: 167 (Abstr.).

Weinberg, E. D. 1964. Manganese requirement for sporulation and other secondary biosynthetic processes of bacillus. Appl. Microbiol. 12: 436.

Wells, I. C. and C. N. Reiny. 1965. Choline metabolism in normal and choline-deficient rats of different ages. Arch. Biochem. Biophys. 112: 201.

Werner, A. and M. Anke. 1960. Trace element content of cattle hair as a means of diagnosing deficiency disorders. Arch. Tierernahrung. 10: 142.

Wessinger, G. D. and J. P. Weinmann. 1943. The effect of manganese and boron compounds on the rat incisor. Amer. J. Physiol. 139: 233.

Wiese, A. C., B. C.
Halpin. 193
J. Biol. Che

Wilgus, H. S., L. C
certain inor
perosis. Sc

Wilgus, H. S., L. C.
manganese an
of perosis.

Wilgus, H. S. and A.
utilization

William, J. P., and
Res. 15: 24.

Wilson, J. G. 1965.
Vet. Rec. 77:

Wilson, J. G. 1966.
Cornwall: Re

Woerpel, H. R. and S
on manganese

Wolbach, S. B. and D
in chicken in
Arch. Pathol.

Zajcev, A. N. 1959.
compounds by

Zerebcov, P. I., V.
trace element
metabolism in
4: 159.

Wiese, A. C., B. C. Johnson, C. A. Elvehjem, E. B. Hart and J. G. Halpin. 1939. A study of blood phosphatase in chick perosis. J. Biol. Chem. 127: 411.

Wilgus, H. S., L. C. Norris and G. F. Heuser. 1936. The role of certain inorganic elements in the cause and prevention of perosis. Science 84: 252.

Wilgus, H. S., L. C. Norris and G. F. Heuser. 1937. The role of manganese and certain other trace elements in the prevention of perosis. J. Nutr. 14: 155.

Wilgus, H. S. and A. R. Patton. 1939. Factors affecting manganese utilization in the chicken. J. Nutr. 18: 35.

William, J. P., and P. R. Noland. 1949. Improved pig rations. Farm Res. 15: 24.

Wilson, J. G. 1965. Bovine infertility: Response to manganese therapy. Vet. Rec. 77: 489.

Wilson, J. G. 1966. Bovine functional infertility in Devon and Cornwall: Response to manganese therapy. Vet. Rec. 79: 562.

Woerpel, H. R. and S. L. Balloun. 1964. Effect of iron and magnesium on manganese metabolism. Poul. Sci. 43: 1134.

Wolbach, S. B. and D. M. Hegsted. 1953. Perosis: Epiphyseal cartilage in chicken in choline and manganese deficiencies in the chick. Arch. Pathol. 56: 437.

Zajcev, A. N. 1959. The balance of manganese taken in different compounds by rats. Vop. Pitan. 18: 77.

Zerebcov, P. I., V. F. Vrakin and N. S. Sevelev. 1970. Effects of trace elements on process of rumen metabolism and nitrogen metabolism in ruminants. Izv. Timirjazev. Sel'skohoz Ada. 4: 159.

APPENDIX

APPENDIX A

TABLE A-1. MICHI

Nutrient	
Vitamin A, m	
Vitamin D_2,	
Vitamin E, t	
Riboflavin	
Nicotinic ac	
Choline chlo	
D-pantothenic	
Vitamin B_{12}	
Zinc	
Manganese	
Iodine	
Copper	
Iron	
Antioxidant[2]	
Carrier (ground ye	

[1]Ten pounds

[2]Butylated

TABLE A-2. MINERAL

Nutrient	
KCl	
KI	
$FeSO_4 \cdot 2H_2O$	
$CuSO_4$	
$COCO_3$	
$MnSO_4 \cdot H_2O$	
$ZnSO_4$	
$MgHCO_3$	
$CaHPO_4 \cdot 2H_2O$	
$CaCO_3$	
Cerelose	
$NaHCO_3$	

[1,2]For 3 and

TABLE A-1. MICHIGAN STATE UNIVERSITY VITAMIN-TRACE MINERAL PREMIX

Nutrient	Amount in 10 lbs of premix[1]
Vitamin A, million	3.0 I.U.
Vitamin D_2, million	0.6 I.U.
Vitamin E, thousand	10.0 I.U.
Riboflavin	3.0 gm
Nicotinic acid	16.0 gm
Choline chloride	100.0 gm
D-pantothenic acid	12.0 gm
Vitamin B_{12}	18.0 gm
Zinc	68.0 gm
Manganese	34.0 gm
Iodine	2.5 gm
Copper	9.0 gm
Iron	54.0 gm
Antioxidant[2]	45.0 gm
Carrier (ground yellow corn) to bring total to 10 lbs	

[1]Ten pounds of premix mixed in 1 ton of feed.

[2]Butylated hydroxyanisole (BHA) and/or butylated hydroxytoluene (BHT).

TABLE A-2. MINERAL MIXTURE USED IN SEMI-PURIFIED EXPERIMENTAL DIETS

Nutrient	Percentage in diet
KCl	10.0
KI	0.0002
$FeSO_4 \cdot 2H_2O$	0.7
$CuSO_4$	0.1
$COCO_3$	0.1
$MnSO_4 \cdot H_2O$	0.0, .009[1/], .027[2/]
$ZnSO_4$	0.4
$MgHCO_3$	2.0
$CaHPO_4 \cdot 2H_2O$	36.0
$CaCO_3$	12.5
Cerelose	13.098, 13.089[1/] 13.071 [2/]
$NaHCO_3$	25.0

[1,2]For 3 and 9 ppm Mn supplementation of the diets 2 and 3.

TABLE A-3. VITAMI

Nutrient
Thiamine monon
Riboflavin
Nicotinamide
Calcium pantot
Pyridoxine HCl
Para-amino ben
Ascorbic acid
D-α-tocopheryl
Inositol
Choline chlorid
Pteroyl glutami
Biotin
Cyanocobalamin
2-methyl-1, 4-na
Vitamin A palmi
Vitamin D_2

TABLE A-4. CLEANING

Polyethylene via
ing solutions:

1. Xylene
2. 95% ethy
3. Distille
4. (0.1N) d
5. Distille

The vials were:

1. Dried in
   to loadi
2. Numbered
   metal roc

TABLE A-3. VITAMIN MIXTURE USED IN SEMI-PURIFIED EXPERIMENTAL DIETS

Nutrient	ppm in diet
Thiamine mononitrate	3
Riboflavin	6
Nicotinamide	40
Calcium pantothenate	30
Pyridoxine HCl	2
Para-amino benzoic acid	13
Ascorbic acid	80
D-α-tocopheryl acetate	10
Inositol	130
Choline chloride	1300
	ppb in diet
Pteroyl glutamic acid	260
Biotin	50
Cyanocobalamin	100
2-methyl-1, 4-naphthoquinone	40
Vitamin A palmitate	1500
Vitamin D_2	12.5

TABLE A-4. CLEANING POLYETHYLENE VIALS

Polyethylene vials were cleaned successively with the following solutions:

1. Xylene
2. 95% ethyl alcohol
3. Distilled deionized water
4. (0.1N) dilute nitric acid
5. Distilled deionized water

The vials were:

1. Dried in a draft oven at 100°C immediately prior to loading
2. Numbered by engraving letters using a stainless metal rod

TABLE A-5. IL MOD

1. Instrument	
Mode of	
Hollow c	
Lamp cu	
Photomul	
P.M. vol	
Slit wid	
Wavelen	
2. Recorder	
Response	
Range	
3. Parameters	
Mode	
Purge ga	
Gas flow	
Dry sett	
Pyrolyze	
Height o	
Analyse	

TABLE A-5. IL MODEL 335 FLAMELESS SAMPLER

1. Instrumental parameters		
Mode of operation	A-B	Channel B
Hollow cathode	45472	H_2 continuum
Lamp current	5mA	#IL 63490
Photomultiplier	R456	20mA
P.M. voltage	620V	
Slit width	320 µm	
Wavelength	279.5	
2. Recorder (full scale)		
Response time	0.5 sec	
Range	100 mV	
3. Parameters for 355		
Mode	Manual	
Purge gas	Argon	
Gas flow rate	10 SCFH	
Dry setting	4 turns	
Pyrolyze setting	5 turns on Analyse Scale	
Height of measurement	2 mm	
Analyse setting	10 turns	