THE EFFECT OF SUPPLEMENTATION OF A BASAL PIG RATION WITH ETHYLENEDIAMINETETRAACETATE, IRON AND COPPER ON THE COPPER LEVEL OF THE LIVER AND SPLEEN

by

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TABLE OF CONTENTS

INTRODUCTION

Many studies have been performed on the role of copper in the mammalian body and on deficiencies connected with this metal. In the body copper is related to important functions as the synthesis of hemoglobin and the activation of certain enzymes systems. Deficiency conditions are characterized by a specific anemia and a typical lameness in some species. Workers have noticed the influence of other nutrients in the diet on the absorption of copper. In particular, relationships exist between copper and metals as iron, zinc and molybdenum.

Chelating agents have been used with success in plant nutrition. Smaller amounts of trace metal have given the same effect when added together with a chelating agent than high amounts of trace metal added alone to the soil. In animal nutrition the chelating agent calcium disodium ethylenediaminetetraacetate, EDTA, is the most commonly used. The effect of this non-biological agent on the absorption and metabolism of trace metals varies with numerous factors in the body.

Recently attention has been paid to copper and its effects when fed in supplemental amounts to swine. The animals show better utilization of feed and higher rate of weight gain when fed higher levels of copper than normally needed. The noticed effect of copper on weight gain could

be of great practical use. The overall composition of the diet must, however, be taken into consideration. By adding a chelating agent lesser amounts of copper or other trace metals may prove to be beneficial.

In the experiment reported here, varying amounts of copper, iron and the chelating agent EDTA were added to a control ration. In addition, a low copper and iron ration was studied. Therefore, the purpose of the present study was to determine the influence of the level of these three substances in the rations on the concentration in three lobes of the livers and the spleens.

REVIEW OF LITERATURE

Role of Copper in Mammalian Biological Systems

The association of copper with a definite function in the animal body was shown first by workers at the University of Wisconsin (1, 2, 3). This work was initiated in 1924 by E. B. Hart et al. (1), and consisted of studies on nutritional anemia and factors affecting the hemoglobin regeneration. Rabbits were used in this original work. When the animals were placed on a diet of whole milk at weaning time, they developed an anemia characterized by a low hemoglobin content and erythrocyte count of the blood. Inorganic iron in the form of ferric oxide added to the basal diet did not correct this condition unless accompanied

by fresh cabbage or an alcoholic extract of cabbage or yellow corn. These extracts were free from iron but were supposed to contain some inorganic substance concerned in the building of hemoglobin. The necessity of copper as a supplement to iron for hemoglobin formation in swine was established in 1932 (4). When iron was added as ferric chloride to the whole milk diet of anemic pigs kept under restricted conditions, there was a small temporary improvement. A rapid and a complete recovery was obtained only after copper was supplied in addition to iron.

Copper Deficiency. Teague et al. (5) noticed that pigs on a copper deficient diet in addition to dietary anemia developed an unusual leg condition. The hocks became extremely flexed and forced the animal to assume a sitting position. The forelegs showed various types and degrees of crookedness. By the tenth week some pigs were unable to stand. Copper deficient puppies developed a similar lameness (6). In both experiments copper was shown to be of therapeutic value.

A deficiency of copper has not been demonstrated in adult humans as copper is widely distributed and easily supplied with the food (7). However, reduced copper levels were found in a metabolic disorder, Wilson's disease, with increased urinary excretion of the metal (8). A combined deficiency of copper and iron was observed in some children

with nutritional anemia. This anemic condition was relieved when copper was given in addition to iron (7).

Metabolism of Copper. The complete significance of the functional role of copper is not completely understood. It is known that this metal is concerned in erythropoiesis and that it is important in the formation of bone as well as maintaining the myelin of the central nervous system (9). Copper is present in the blood in at least 4 different fractions: 2 in the serum and 2 in the erythrocytes (10). Red cell copper content tends to be constant (11). The plasma copper, on the other hand, changes under a great variety of circumstances (11). In man, as much as 96% of the plasma copper is bound tightly to the alpha-2-globulin, ceruloplasmin (9, 10). The smaller part of the plasma copper is loosely bound to albumin and is probably the means of copper transport (11). In the erythrocytes, copper is present in a labile form and as a component of the erythrocyte copper protein, erythrocuprein (10). The amount of copper in the erythrocytes of copper deficient pigs is reduced and the life span of these erythrocytes in the circulation is shortened (12). Schultze et al. (13) suggested that rapid continued hematopoiesis cannot take place unless the copper content of the blood is maintained above a minimum level. This level should be approximately 20 µg/100 ml of the blood of the pigs.

In the mammalian body, copper activates or is a constituent of such enzymes as uricase, tyrosinase, glutathione and cytochrome oxidase. Enzymatic activity of uricase appears to be related to the presence of copper in the enzyme (14). Uric acid is oxidized by uricase to allantoin, which is an end product found in the urine of many mammals but not in that of man (15). Tyrosinase requires copper for its activity of oxidation of tyrosine to melanin (16). Glutathione is the coenzyme of glyoxalase which acts as a respiratory carrier of oxygen (15). The content of glutathione in the livers of pigs was decreased in copper deficiency and normal in iron deficiency (17). Cytochrome oxidase is of general importance in cellular oxidations. The reduced form gives rise to water in its capacity of reducing oxygen so that the latter may combine with hydrogen atoms (15). Copper is essential for the function of cytochrome oxidase (17, 18). In copper deficient swine, the activity of cytochrome oxidase in the heart tissue was decreased 8 times and in the liver 3 times. Schultze (19) found in experiments with rats a close relation between the cytochrome oxidase activity of the bone marrow and its ability to form hemoglobin and erythrocytes.

Copper is found in the various tissues with the highest amount in the liver (20). When labeled copper was injected

in rabbits, it was rapidly taken up by the liver and then firmly bound to a protein for storage (21). Copper is also found in the spleen, muscles and bones. Most of the physiological copper is returned to the feces via the biliary circulation, but some urinary excretion occurs as well (22).

Effect of Copper on Iron Metabolism. In copper deficient swine, there are several abnormalities in the metabolism of iron (23, 24). Swine made deficient in both copper and iron showed no increase in plasma iron after iron therapy, but an increase took place when copper was given as well (9). These observations indicated that copper favored iron absorption from the gastrointestinal tract. Copper is also essential in the mobilization of iron from the tissues and the conversion of inorganic iron into hemoglobin (11, 23, 25). When iron was administered orally to animals deficient in both iron and copper, the iron was stored in the liver and other tissues and was not utilized for hemoglobin synthesis. If copper was then administered, iron was rapidly mobilized from the tissues and converted into hemoglobin.

Metabolism of Iron. The influence of copper on iron metabolism is of interest as iron plays a key role in the most vital processes of the mammalian body (26). The human body normally contains 4-5 g iron (27). Of this, 60-70%

is present in the form of circulating hemoglobin. Its main function is the transport of oxygen to the sites where it is required. In the muscle this oxygen is accepted by another heme protein, myoglobin (3-5% of total iron). Some of the most important heme containing enzymes are the cytochromes, catalase, and peroxidase. Myoglobin delivers its oxygen further to the cytochrome system and other energy producing systems of the cell. Catalase and peroxidase are present in nearly all tissues. Their exact physiological functions are not clear. Presumably, they serve to prevent toxic accumulation of peroxides within the cells (28).

The intestinal mucosal cells contain a protein, apoferritin, which combines with iron to form ferritin (28). Iron is taken up by the cells until all apoferritin is converted into ferritin. No more iron is absorbed until the ferritin has given up some of its iron to plasma. Absorbed iron passes through mucosal cells directly into the blood stream, where it forms a complex with a beta-1-globulin. As such, the iron is transported to various parts of the body. Iron is stored chiefly in the 2 forms ferritin and hemosiderin (26, 29). The liver contains the highest concentration and the greatest part of the total storage iron. The spleen and the bone marrow contain considerable amounts also.

Interrelationship of Copper and Iron. Copper appears

to have a depressing effect on iron storage in the liver (30, 31, 32). In an experiment with pigs, the concentration of copper in the liver increased corresponding to an increased copper intake from the diet (30). The iron concentration decreased even though the intake of iron was the same for all groups. Wintrobe et al. (11) observed in iron deficient swine a 4-fold increase in the copper content of the liver as compared with the normal, even though all the animals were fed the same amount of dietary copper. These findings indicate that a reverse relationship exists between the iron and copper concentration in the liver.

The levels of copper found in the spleen was reported as being considerably lower than that in the liver (31). The copper content in the spleen was increased when the iron content in the ration was increased. On the other hand, when copper was increased and the iron level remained at the same high level, the amount of copper in the spleen did not change.

Influence of Other Factors on Copper Metabolism

Zinc and Molybdenum. The metabolism and the absorption of copper from the intestinal tract depend upon numerous factors in addition to the dietary concentration of the metal. These other factors become especially important when the toxic level of copper and the interactions of copper with other nutrients is considered. In particular,

dietary relationships exist between copper, zinc, and molybdenum (33, 34). Zinc was noticed to cause a decrease in the level of liver copper (32). When pigs received 250 ppm copper with no added zinc, they developed symptoms of copper toxicity. When 100 ppm zinc was added to copper supplemented rations, there was an improvement in growth rate. Supplements of copper was shown to overcome an anemia caused by high levels of dietary zinc (33). In an experiment with rats, molybdenum significantly decreased the influence of the dietary copper upon Zn^{65} uptake when there was an excess of copper in the diets (33).

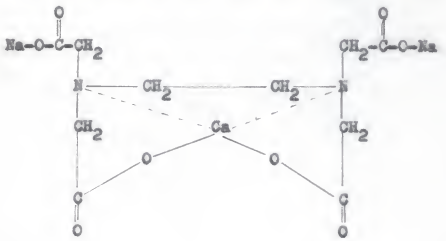
Protein. The level of protein may be of importance on the absorption of copper. The effect of the protein level in the diet fed to rats was greater than the effect exerted by the levels of zinc with respect to a toxic level of copper (35). The action of protein appeared to be both in the intestinal tract and in the liver where there was an increased elimination of copper as the concentration approached a toxic level.

Ethylenediaminetetraacetate. Recently, a non-biological compound, ethylenediaminetetraacetic acid (EDTA), has been studied in experiments with absorption of trace metals in both plant and animal systems. The successful treatment of a nutritional disorder on field grown citrus fruit with iron-EDTA stimulated great interest in the use of chelating

agents for supplying metals to plants (36). Jacobsen published in 1951 (37) that the essential iron could be maintained in solution by use of the organic complex EDTA. When used as a dormant season soil application, as little as 2 ounces of actual iron in this organic form per tree performed what 15 ounces per tree had failed to do (38). Use of chelates of copper, zinc, and manganese also was reported (39, 40, 41).

EDTA is a white crystalline, odorless, and non-poisonous powder (42). It is sparingly soluble in water, insoluble in acids and common organic solvents, but soluble in ammonia solutions. The compound has ability to form complexes especially with di- and trivalent ions (42, 43).

Experiments with animals using EDTA have been performed. EDTA was used successfully in the treatment of lead poisoning and in radioactive metal poisoning (44, 45, 46). Calcium disodium EDTA is the form most commonly used (45).



When calcium disodium EDTA is administered to an animal in lead poisoning, lead replaces calcium in the molecule to form lead disodium EDTA because of the greater stability of this complex (45). Data from an experiment with humans gave the same result indicating that calcium disodium EDTA administration increased the lead excretion (43). The combination of lead in the form of EDTA complex markedly decreases the toxicity of the lead.

It is known that in the animal body during absorption ferric (Fe^{+++}) ions become reduced to the ferrous (Fe^{++}) form. Metal ions vary in the stability of complexes formed with EDTA and in a descending scale from strong to weak chelates. The order is: Fe^{+++}, Cu^{++}, Zn^{++}, Fe^{++}, Mn^{++}, Cu^{+}, and Mg^{+} (39). Therefore, in the body Cu^{++} would take priority in the chelating agent. Patients with nephrotic syndrome were found to excrete increased quantities of copper and iron in the urine (47). A single intravenous injection of EDTA resulted in no appreciable increase in the excretion of copper but was associated with a striking increase in the excretion of iron.

How EDTA acts in the animal body as a chelating agent during absorption is not understood. In 1961 Schanker et al. (48) studied the effect of EDTA on the intestinal absorption of several lipid insoluble compounds. In the experiments with rats, the absorption of 2 neutral molecules, mannitol-1,6 C^{14} and inulin C^{14}, was less than 2% when administered alone

but with EDTA the absorption was 7-11%. These investigators suggested that EDTA as a chelating agent increased the sizes of the membrane pores, or by widening the spaces between the epithelial cells through removal of calcium ions in the intestinal wall.

Effects of Copper Supplementation

Fattening Swine. As copper has been found to have an influence on weight gain, numerous investigators have studied the effect of supplemental copper on the basal diet (49, 50, 51, 52). Growing swine showed an improved rate of gain from high levels of copper supplementation with maximum growth response to copper sulfate being obtained at the 0.1% (250 ppm) level. An improvement in the efficiency of food utilization was also found which resulted in reduction of total food consumed from weaning to bacon weight (49, 53, 54). With this high level, the length of the period of slow growth after weaning was reduced (55).

Copper Poisoning. There is marked individual variation among pigs in their ability to tolerate high levels of dietary copper (56). Wallace et al. (57) reported that copper levels of 250 ppm proved toxic. In a group of 10 pigs, all but 3 pigs showed signs of copper poisoning. In another experiment, 1 out of 10 fed 250 ppm copper added to the basal ration died and upon autopsy exhibited signs

of copper poisoning (58). The use of copper supplementation is thus associated with some danger of copper toxicity. Yet, the specific level of dietary copper which may be considered toxic has not been a matter of complete agreement.

PROCEDURE

In order to study the effect of diet on the copper level of liver and spleen, pigs were fed rations containing various amounts of dietary copper, iron, and in some cases, a chelating agent. The data of chemical analyses performed on the liver and spleen samples were used to compare the effects produced by the different diets.

Samples Examined in the Experiment

History of the Animals. The Department of Animal Husbandry raised the pigs used for this study. Barrows and gilts (20 each), averaging 18.4 kg and representing Duroc, Poland China, and crossbred Duroc-Poland China were divided into 5 lots so that sex and breed were equally represented in all lots. The pigs were penned with galvanized wire. The feed was pelleted, the drinking water softened and fed free choice in separate wooden troughs. These precautions were taken to prevent ingestion of iron and copper by the pigs from sources other than the ration. The pigs were fed the ration presented in table 1.

TABLE 1.

Rations Fed

Lot I:	**Control ration a/**		
	Sorghum grain (milo)	358.3	kg
	Soybean oil meal	43.1	kg
	Meat scraps	22.7	kg
	Alfalfa meal	22.7	kg
	Iodized salt	2.3	kg
	Vitamin A	400000	IU
	B-Complex vitamin (Merck 58-A)	0.2	kg
	Aurofac 1.8 - 1.8 b/	2.3	kg
	$MnSO_4.H_2O$	0.1	kg
Lot II:	Control ration plus 0.1% $CaNa_2EDTA$		
Lot III:	Control ration plus 0.5% $CaNa_2EDTA$		
Lot IV:	Low iron and copper ration consisting of		
	Ground corn	306.2	kg
	Dried skim milk	158.3	kg
	Ground limestone	2.3	kg
	Iodized salt	2.3	kg
	Vitamin A	400000	IU
	B-Complex vitamin (Merck 58-A)	0.2	kg
	Aurofac 1.8 - 1.8 b/	2.3	kg
	$MnSO_4.H_2O$	0.1	kg
	Contains 20 ppm iron and 5 ppm copper		
Lot V:	Control ration plus $FeSO_4$ to make 1311 ppm iron of ration and $CuSO_4$ to make 118 ppm copper of ration		

a/ Contains 88 ppm iron and 15 ppm copper

b/ Commercial aureomycin and vitamin B_{12}

Processing. Animals were individually taken off feed at 91 kg live weight and slaughtered after holding for 24 hours. Hemoglobin level was determined on blood taken at slaughter. The livers and spleens were removed with a stainless steel knife, weighed and sectioned on a wooden table top to avoid contamination from metals. The liver was placed on the table, gall bladder side up with the distal ends of the lobes toward the meat cutter. The lobes were designated 1, 2, and 3 from left to right as shown in figure 1. A 1 cm slice was removed longitudinally from the center of each liver lobe. From each spleen a 1 cm slice was cut longitudinally, avoiding the midline and adhering tissues. The samples were wrapped individually in aluminum foil, labeled with the pig number, immediately frozen on a plate freezer at -23° C. and then stored at -12° to -18° C. until used.

Method of Analysis

From each animal 4 samples (the liver lobes 1, 2, and 3 and the spleen) were analyzed. Selection of the order in which the samples were analyzed was accomplished by using a table of random numbers.

Sample Weights. A 2x2 cm^2 of glassine paper was weighed for each of the 4 copper standards, 4 reagent blanks, 1 reference blank and 6 samples to be used for each

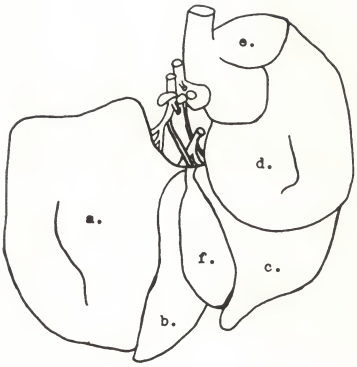


Fig. 1. Diagram of the pig liver showing the order in which the lobes were designated as 1, 2 and 3. a. Left lateral lobe, lobe 1; b.,c. Left and right central lobes respectively, lobe 2; d. Right lateral lobe, lobe 3; e. Caudate lobe, not tested; f. Gall bladder.

sample. The 6 samples were cut from a frozen liver lobe or spleen at random to allow as unbiased and as complete a sampling of the organ as possible. Each sample (between 0.11-0.16 g) was placed on the previously weighed square of glassine paper and weighed using an electric analytical balance.

Chemical Analysis. All samples, blanks and standards were subjected to a wet ash digestion process and analyzed for copper by a modification of the method described by Rice and Grogan (60) for spectrophotometric determination of copper using oxalyldihydrazide.

1. Digestion. One milliliter of 2 µg copper standard was added to each of 4 calibrated 52 x 200 mm test tubes. One milliliter of deionized distilled water was added to the tubes designated as blanks and to those containing the weighed tissue samples. One milliliter of concentrated sulfuric acid followed by 1 ml of perchloric acid were pipetted into each tube. The tubes were placed in a sandbath and heated. When the solution was clear (2½-3 hours), the tubes were removed, covered with plastic film and allowed to cool.

2. Titration. The strongly acid solution was diluted with 2 ml of deionized distilled water, allowed to cool for a short while and then a pinch of citric acid crystals and a 2 mm^2 of Congo red indicator paper were added to each tube.

A pinch of disodium EDTA was added to the tube used as a reference blank. Each tube was titrated to the red of Congo red indicator paper using ammonium hydroxide solution saturated with oxalyldihydrazide. Then, an additional 2 ml of this solution was added.

3. Color production and determination of absorbancies. One-half milliliter of a 50% cold acetaldehyde solution was added and the resulting solution was diluted with deionized distilled water to a 15 ml volume and mixed. The tubes were allowed to stand at room temperature for at least 30 minutes. Using a wave length of 542 mu, the optical densities for each of the reagent blanks, copper standards, and the unknowns were read against the blank containing EDTA. A Beckman DU spectrophotometer with 1 cm silica absorption cells was used. The color is stable for at least several hours (60).

Maintenance of Glassware. With the exception of pipettes and burettes, glassware was washed with detergent and water, rinsed 9 times with tap water, rinsed 9 times with deionized distilled water and inverted on a clean paper towel. The glassware was covered with linen towels while drying and then each tube was covered with plastic film to protect from contamination. After use, the pipettes and burettes were rinsed with tap water, and placed in potassium dichromate cleaning solution for a few

hours or overnight. They were removed, rinsed 9 times with tap water, 9 times with deionized distilled water, and placed tip down on clean linen towels. A linen towel was used to protect them from contamination as they were allowed to dry.

Calculation. The following formula was used for calculating the quantity of copper per gram of pork liver or spleen:

$$\frac{2\ \mu g\ (O.D.\ \text{of unknown} - \text{mean } O.D.\ \text{of blank})}{\text{mean } O.D.\ \text{of standard} - \text{mean } O.D.\ \text{of blank}} = \mu g/g \text{ tissue sample weight (g)}$$

Statistical Analysis

All data were subjected to analysis of variance and least significant differences were calculated when appropriate. The analyses used were:

Source of variation	D/F
Liver:	
Lots	4
Lobes	2
Animals in the same lot	32
Lots x lobes + lobes x animals in the same lot	72
Samples	555
Total	665
Spleen:	
Lots	4
Animals in the same lot	32
Samples	185
Total	221

RESULTS AND DISCUSSION

Liver Copper Levels

The results of the chemical determination of liver copper are presented in table 2. The average copper level found in the liver of pigs fed the control ration, Lot I, was 13.6 μg/g. Copper concentration in the livers of the low copper and iron group, Lot IV, was 7.2 μg/g and varied significantly ($P<0.05$) from the control group, Lot I. Milk rations or low copper and iron rations were shown to produce anemia in animals as early as 1925 by Hart et al. (1, 2, 3, 4, 5). The copper level in the livers of pigs on the ration with high copper and iron, Lot V, was 9.0 μg/g, and was not significantly different from Lot I.

The level of copper and iron in the ration was reported to influence the copper and iron levels in the livers (30, 31, 32). When supplemental copper (118 ppm) and iron (1311 ppm) were added to the ration at a ratio of 1:11, Lot V, the liver copper level was decreased but not significantly over that of the level found on the control ration, Lot I. The ratio of copper (15 ppm) and iron (88 ppm) in the control ration was 1:6. In another study with pigs (31) with copper (66 ppm) and iron (661 ppm) at 1:10 ratio, the liver copper level was similar to the liver copper level of the control ration. The control ration in both studies was identical. The higher amount of copper

TABLE 2

Mean values of copper concentrations in livers of pigs fed varying rations

Lot	No. of Samples	Ration	Lobes 1	Lobes 2	Lobes 3	Lot Means[1]
			µg/g	µg/g	µg/g	µg/g
I	126	Basal	11.5	14.8	14.5	13.6
II	144	Basal + 0.1% EDTA	12.6	13.0	13.9	13.2
III	108	Basal + 0.5% EDTA	14.9	19.5	16.7	17.0
IV	144	Low copper and iron	6.9	7.7	7.0	7.2
V	144	High copper and iron	9.0	8.2	9.8	9.0
Lobes means			11.0	12.6	12.4	

[1]Least significance difference, $P < 0.05$

144,144 = 4.9
144,126 = 5.0
144,108 = 5.3
126,108 = 5.4

and iron in the present study could account for the depressed copper levels in the livers of Lot V over that of Lot I and the differences in the results from the two experiments. When the copper to iron ratio was 1:44, the copper level in the liver was very highly significantly lower from that of the control ration (31). The amounts fed were copper 15 ppm and iron 661 ppm. The very low level of copper in the liver was reported as a result of the imbalance of copper to iron in the ration.

The highest amount of copper, 17.0 μg/g, was found in the liver of pigs with 0.5% EDTA added to the control ration, Lot III, table 2. In comparing this level with the copper concentration in the low copper and iron ration, Lot IV, and the high copper and iron ration, Lot V, the differences were significant ($P < 0.05$). The copper level from Lot III, 0.5% EDTA, was not significantly different from Lot II, 0.1% EDTA, or that for Lot I, the control ration. The addition of 0.1% EDTA to the control ration, Lot II, resulted in a copper level of 13.2 μg/g and was significantly different only from the low copper and iron ration, Lot IV. The level of copper in the livers of Lot I and II were similar, which indicates that EDTA at the 0.1% level has no effect on copper absorption or its metabolism in the liver or both.

It is known that in the animal body ferric iron (Fe^{+++}) is reduced to ferrous iron (Fe^{++}) during absorption (39).

Then according to the chelating scheme, cupric copper (Cu^{++}) would be preferentially complexed with EDTA and thus be favored in the absorption process. As copper was chelated by EDTA, Lot III, because of a preferential valence state, the amount of copper absorbed was greatly increased as indicated by the high level of copper in the liver and which could cause a decrease in liver iron resulting in anemia (60). An inverse relationship is reported to exist between copper and iron (30, 31, 32). As the metal, copper or iron, was increased in the ration, the other metal, copper or iron, decreased in the liver. Another possibility is that once EDTA is absorbed into the body, it can complex with iron and increase its excretion. This would have support in that 2 animals died in Lot III (60). The cause of death was reported as anemia and pneumonia. In an experiment with humans, having nephrotic syndrome, an injection of EDTA resulted in an increased iron excretion and an increased copper retention (47). EDTA was injected into humans and animals in the treatment of lead poisoning (43, 44, 45). The lead complexed with EDTA and was excreted.

Variation Among Liver Lobes

The copper concentration was lower in Lobe 1 than in Lobe 2 or 3 in each lot except Lot V, table 2. When correction for animal variation was made, no significant difference was found between the copper levels in the 3 lobes.

Therefore, copper is stored fairly even in the liver.

Spleen Copper Levels

The copper levels found in the spleens were considerably lower than the levels found in the livers, table 3. The spleen copper level of pigs on the control ration, Lot I, was 1.5 μg/g. Those pigs on the lowest copper and iron ration, Lot IV, had a copper level of 1.4 μg/g, and those pigs on the highest copper and iron intake, Lot V, 1.5 μg/g. The differences between these levels were not significant. Neither was the level in the spleens of those pigs that had 0.1% EDTA added to the control ration, Lot II, (1.6 μg/g) significantly different from Lots I, IV and V. With 0.5% EDTA added to the control ration, Lot III, the copper concentration in the spleen was lowered. The amount found was 1.0 μg/g and varied significantly ($P<0.05$) from the levels of spleen copper found in pigs fed the other rations.

With a decrease or increase of copper and iron in the ration, Lot IV and Lot V, the amount of copper found in the spleen did not differ from that of the control ration. This is in contrast to another study with pigs (31). The addition of iron only to the control ration caused a very highly significant increase in the spleen copper concentration. The ratio of copper to iron was 1:44 and was considered to be imbalanced. However, the spleen copper level was not increased further when both copper and iron were added to

TABLE 3

Mean values of copper concentrations in spleens of pigs fed varying rations

Lot	No. of Samples	Ration	Mean[1]
			µg/g
I	42	Basal	1.5
II	48	Basal + 0.1% EDTA	1.6
III	36	Basal + 0.5% EDTA	1.0
IV	48	Low copper and iron	1.4
V	48	High copper and iron	1.5

[1]Least significant difference, $P<0.05$

48,48 = 0.3
48,42 = 0.3
48,36 = 0.3
42,36 = 0.4

the control ration. The ratio of copper to iron was 1:10 (31). There was no effect of 0.1% EDTA, Lot II, on spleen copper as compared to the control, Lot I, table 3. The decreased spleen copper level in Lot III is presumably caused by the addition of 0.5% EDTA to the control ration. The depressing effect of the high amount of EDTA on the copper level in the spleen may interfere with hemoglobin catabolism and be a contributing factor to the anemia in the pigs which died.

Liver and Spleen Copper Levels

When low copper and iron are fed, Lot IV, the copper level in the liver is decreased, table 2, but remained the same as the control group in the spleen, table 3. The addition of copper and iron to the control ration, Lot V, had a depressing instead of an elevating effect on the liver copper level but did not influence the spleen copper level. The ratios of copper to iron are quite close together, Lot I, 1:6; Lot IV, 1:4; and Lot V, 1:11; but the amount of the metals in the rations in ppm are quite different, Lot I, 15:88; Lot IV, 5:20; and Lot V, 118:1311. The results from Lot IV and Lot V suggest that the liver copper level seems to be more sensitive to the amount of copper and iron in the ration compared with the spleen copper level.

With 0.1% EDTA added to the control ration, Lot II,

there was no effect on the copper level either in the liver or in the spleen as compared with the control group, Lot I. The greatest variance on the copper concentration was observed with 0.5% EDTA added to the control ration, Lot III. The level of liver copper was significantly increased ($P<0.05$) but the level of spleen copper was significantly decreased ($P<0.05$). Under the influence of the non-biological substance, EDTA, the amount of copper in the liver, the main storage depot, was increased. The copper concentration probably reached a toxic level in the liver as 2 animals died in Lot III. The decreased copper level in the spleen in Lot III would indicate that copper was complexed with EDTA and that less copper was available for the rest of the hematopoietic system including the spleen. Anemia was reported as the cause of death of 2 pigs in Lot III. Another possibility is that EDTA could interfere with the hemoglobin catabolism in the spleen as indicated by the low copper concentration found.

Animal Variation

The variation of the copper levels in the liver lobes and the spleens of the animals fed the same diet was very highly significant ($P<0.001$). It is shown by the high and low mean values for copper recorded for the liver lobes and the spleens in each lot (table 4) and for individual animals (Appendix, table 7). The fact that these pigs were

TABLE 4

High and low mean copper values within lots for livers and spleens of pigs fed varying rations

Lot	Ration	Liver lobes[1]			Spleen[1]
		1	2	3	
		µg/g	µg/g	µg/g	µg/g
I	Basal	2.1-21.7	0.3-31.6	2.9-35.9	-1.7-3.9
II	Basal + 0.1% EDTA	6.6-31.6	6.5-29.8	6.7-35.4	-0.3-4.7
III	Basal + 0.5% EDTA	8.0-22.6	9.9-32.4	9.9-26.3	0.0-2.3
IV	Low copper and iron	3.5-11.7	5.2-13.8	3.9-11.5	0.3-3.1
V	High copper and iron	4.7-18.9	3.4-19.7	5.4-17.3	-0.3-4.1

[1]Least significant difference, $P < 0.001$

Duroc, Poland China and crossbred Duroc-Poland China and included both sexes could account for some of the variation among the animals.

SUMMARY

Forty pigs representing Duroc, Poland China and crossbred Duroc-Poland China were divided into 5 lots at weaning time. Lot I was fed a control ration containing 15 ppm copper and 88 ppm iron. A chelating agent, EDTA, was added to Lot II at the level of 0.1% and to Lot III at the level of 0.5%, both of which also contained 15 ppm copper and 88 ppm iron. Lot IV was the low copper and iron ration and contained 5 ppm copper and 20 ppm iron. To Lot V 118 ppm copper and 1311 ppm iron were added. At the weight of 91 kg the pigs were slaughtered and samples removed from each of the 3 liver lobes and the spleen for copper analysis.

The copper level in the livers in Lot V was decreased as compared with Lot I but the difference was not significant. The lowered copper level could be the result of the depressing effect of the high amounts of copper and iron added to Lot V. Copper concentration in the livers in Lot IV, the milk ration or low copper and iron ration, was still lower than that of Lot V and varied significantly from the level of copper found in the livers of pigs in Lot I. The addition of 0.5% EDTA to the control ration gave the highest

level of copper found in the livers and was significantly different from Lot IV and Lot V, but not from Lot I and Lot II. With 0.1% EDTA added to the control ration, Lot II gave only significant difference compared with the low copper and iron ration, Lot IV. The level of copper found in the spleen was considerably lower than the amount found in the liver. The addition of 0.5% EDTA to the diet lowered the copper concentration significantly over the levels of copper in spleens on other rations. The other rations did not have any significant effect on the copper levels in the spleen.

A wide variation of copper concentration of both liver and spleen was observed among the animals throughout the experiment. This could account for the differences in breed and sex of the pigs. When correction for animal variation was made, no difference was found between the copper levels in the 3 liver lobes.

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APPENDIX

TAB

Feedlot and carcass

Animal number	Breed	Sex[1]	Initial weight	Slaughter weight
			lb	lb
Lot I				
80	Poland	G	54	204
81	Duroc	G	40	211
82	Duroc	B	35	209
83	Poland	B	31	202
84	Duroc	G	32	207
85	X-bred	B	38	210
86	X-bred	G	35	143
Average:			38	198
Feed efficiency: 3.40 lb feed/lb gain				
Lot II				
88	Duroc	G	56	217
89	Poland	G	65	202
90	X-bred	G	48	203
91	Duroc	B	33	200
92	Poland	G	29	209
93	Duroc	B	35	201
94	Poland	B	25	210
95	X-bred	B	33	208
Average:			41	205
Feed efficiency: 3.25 lb feed/lb gain				

[1]G, gilts; B, barrows.

LE 5

data for each animal

Average daily gain	Liver weight	Spleen weight	Hemoglobin
lb	g	g	%
1.40	1410.5	141.5	16.8
1.60	1442.6	92.8	14.9
1.63	1348.4	67.4	11.6
1.50	1674.2	107.8	14.6
1.29	1411.0	117.6	15.7
1.10	1589.6	82.5	15.5
0.66	982.9	85.4	13.2
1.36	1408.5	99.3	14.6
1.73	1161.7	95.0	12.1
1.20	1427.4	131.0	9.7
1.28	1399.4	133.6	11.9
1.38	1332.5	119.7	-
1.41	2032.4	154.5	12.8
1.17	1259.5	106.3	11.6
1.30	1886.0	121.4	9.8
1.12	1401.4	83.9	11.8
1.33	1487.5	118.2	11.4

TABLE 5

Animal number	Breed	Sex[1]	Initial weight	Slaughter weight
			lb	lb
Lot III				
96	Duroc	G	52	198
97	Poland	B	61	201
98	Poland	B	45	202
99	Duroc	G	32	212
100	X-bred	B	36	200
101	X-bred	G	40	201
Average:			44	202
Feed efficiency: 3.57 lb feed/lb gain				
Lot IV				
104	Duroc	G	38	204
105	Poland	B	49	205
106	Duroc	B	41	200
107	X-bred	B	43	200
108	Poland	B	48	204
109	X-bred	G	31	211
110	Duroc	B	40	210
111	Poland	B	40	204
Average:			41	205
Feed efficiency: 2.87 lb feed/lb gain				

[1] G, gilts; B, barrows.

(cont'd.)

Average daily gain	Liver weight	Spleen weight	Hemoglobin
lb	g	g	%
1.28	1289.5	96.0	8.4
1.16	1673.5	140.7	10.5
1.23	1684.0	159.0	11.4
1.27	1472.2	124.7	10.6
1.05	1556.3	104.2	9.9
0.99	1587.0	182.8	9.6
1.16	1543.8	134.6	10.1
1.78	1117.4	119.2	16.3
1.68	1370.7	154.2	17.7
1.71	1310.0	89.1	15.8
1.57	1661.2	102.4	12.0
1.36	1919.5	168.3	13.0
1.49	1694.3	127.1	16.6
1.26	1612.1	117.7	12.3
1.21	1408.0	168.9	16.1
1.51	1308.6	130.9	15.0

TABLE 5

Animal number	Breed	Sex[1]	Initial weight	Slaughter weight
			lb	lb
Lot V				
112	Poland	B	63	208
113	Duroc	G	56	203
114	Duroc	B	34	204
115	Poland	B	32	206
116	Duroc	G	29	208
117	X-bred	B	35	202
118	Poland	G	33	207
119	X-bred	G	40	213
Average			40	206
Feed efficiency: 3.22 lb feed/lb gain				

[1]G, gilts; B, barrows.

(concl.)

Average daily gain	Liver weight	Spleen weight	Hemoglobin
lb	g	g	%
1.84	1345.1	123.7	17.8
1.58	1336.0	81.2	15.3
1.70	1111.0	71.9	16.6
1.63	1568.9	134.8	-
1.67	1384.1	82.9	16.0
1.56	1290.9	99.2	19.2
1.53	1563.9	120.8	16.0
1.43	1496.0	107.2	17.0
1.62	1387.0	102.7	16.8

Ranges and means of copper in micrograms for the liver lobes

Animal number	Liver			
	Lobe 1		Lobe 2	
	Range	Mean	Range	Mean
	µg	µg	µg	µg
Lot I				
80	2.1- 6.0	4.2	0.3- 6.5	3.1
81	9.8-12.7	11.0	11.2-17.4	14.0
82	13.3-19.2	16.5	21.9-26.2	23.8
83	6.5- 8.5	7.6	7.1- 9.9	8.7
84	15.5-21.7	17.4	22.0-31.6	26.0
85	7.0-11.2	8.6	9.0-11.2	10.2
86	12.0-19.7	15.2	11.0-27.1	17.8
Lot II				
88	9.6-12.7	11.2	9.5-13.3	11.1
89	16.6-31.6	24.1	19.5-29.8	24.0
90	10.1-21.8	17.3	13.9-16.6	15.3
91	6.8-10.2	7.8	7.6- 9.1	8.2
92	6.6- 8.1	7.2	6.5- 9.0	7.7
93	9.1-11.2	10.3	10.6-12.6	11.3
94	10.7-17.0	12.6	13.2-17.7	15.2
95	9.5-11.6	10.4	10.3-12.2	11.2
Lot III				
96	13.2-18.1	15.3	13.7-29.7	19.1
97	8.0-11.9	10.3	9.9-14.5	12.1
98	13.4-22.6	18.1	28.5-32.4	30.8
99	14.9-19.7	18.1	20.5-26.7	23.7

LE 6

and the spleen of individual pigs fed varying rations.

Liver			Spleen	
Lobe 3		Grand mean		
Range	Mean		Range	Mean
µg	µg	µg	µg	µg
3.3- 5.6	4.6	4.0	0.3-1.4	0.9
2.9-23.1	12.1	12.4	0.9-3.4	2.0
16.4-21.0	18.5	19.6	0.3-2.5	1.2
6.3-11.5	9.3	8.5	-1.7-2.5	1.1
24.5-35.9	29.9	24.4	0.8-2.7	1.9
7.1- 9.1	8.3	9.0	1.1-3.9	1.9
15.7-21.6	19.2	17.4	1.1-2.4	1.8
10.3-14.5	12.0	11.4	0.0-4.5	2.1
26.0-35.4	30.5	26.2	-0.3-0.6	0.2
12.0-18.9	15.2	15.9	0.7-2.0	1.6
9.1-10.9	9.8	8.6	3.3-4.7	3.8
6.7-10.9	8.6	7.8	1.4-3.0	2.3
9.0-11.8	10.9	10.8	0.6-2.6	1.9
10.2-14.8	12.7	13.5	0.6-1.8	1.1
8.6-11.8	9.8	10.5	-0.3-1.0	0.2
9.9-13.5	11.5	15.3	0.4-1.3	0.7
11.7-21.5	16.8	13.1	0.6-1.9	1.3
22.0-26.3	24.3	24.4	0.0-1.4	0.7
16.9-22.2	19.1	20.3	0.9-2.2	1.6

TABLE 6

Animal number	Liver			
	Lobe 1		Lobe 2	
	Range	Mean	Range	Mean
	µg	µg	µg	µg
Lot III (cont'd)				
100	12.8-14.5	13.8	14.3-18.0	16.6
101	11.1-17.2	13.8	12.1-16.8	14.6
Lot IV				
104	4.2- 6.4	5.0	7.3- 9.4	8.7
105	6.1-11.7	8.2	5.5- 6.2	6.0
106	6.6-10.5	9.0	10.8-13.8	12.2
107	5.1- 6.8	6.0	5.3- 6.1	5.8
108	3.5- 6.4	5.1	5.5- 6.8	6.2
109	4.8-10.6	6.6	5.2- 6.7	6.3
110	6.1-10.2	8.1	6.0- 8.2	7.3
111	5.7- 9.6	7.4	6.9-11.2	9.1
Lot V				
112	5.8- 8.9	7.7	6.8- 9.8	7.6
113	4.7- 7.5	6.1	3.4- 5.3	4.5
114	8.4-11.1	9.4	4.3- 5.8	5.2
115	5.9- 9.1	7.5	4.7- 6.2	5.3
116	7.5- 9.1	8.3	9.3- 9.9	9.7
117	15.9-18.9	17.0	9.7-19.7	14.8
118	6.0- 7.3	6.6	6.3-10.3	7.6
119	8.7-10.1	9.2	13.0-19.2	15.1

(concl.)

Liver			Spleen	
Lobe 3		Grand mean		
Range	Mean		Range	Mean
µg	µg	µg	µg	µg
12.2-17.1	15.2	15.2	0.0-1.7	0.7
10.4-18.4	12.9	13.8	0.2-2.3	1.3
4.5- 7.5	6.1	6.6	0.5-2.8	1.2
6.6- 7.6	7.0	7.1	0.3-2.6	0.8
8.8-11.5	9.8	10.3	0.9-3.1	1.9
3.9- 7.8	6.6	6.1	0.6-3.0	1.9
5.5- 7.1	6.3	5.9	0.3-2.9	1.6
4.6- 9.0	6.3	6.4	1.0-1.9	1.3
5.9- 7.9	6.8	7.4	0.5-2.1	1.2
5.4-10.6	7.6	8.0	0.6-1.9	1.3
6.7- 9.2	7.7	7.7	1.8-4.1	3.0
7.6- 9.4	8.7	6.4	0.3-2.5	1.5
7.3-10.7	9.2	7.9	0.3-2.3	1.0
5.4- 8.8	7.2	6.7	1.2-1.5	1.4
9.3-11.1	9.8	9.3	1.1-2.0	1.3
12.6-17.3	15.3	15.7	0.0-3.5	1.5
8.4-10.9	9.8	8.0	1.2-2.1	1.7
8.2-12.1	10.5	11.6	-0.3-2.5	1.7

THE EFFECT OF SUPPLEMENTATION OF A BASAL PIG RATION WITH ETHYLENEDIAMINETETRAACETATE, IRON AND COPPER ON THE COPPER LEVEL OF THE LIVER AND SPLEEN

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ABSTRACT

The relationships between copper, iron and ethylenediaminetetraacetate (EDTA) on the performance on pigs were studied. Forty pigs representing Duroc, Poland China, and crossbred Duroc-Poland China were divided into 5 lots at weaning time. Lot I was fed a control ration containing 15 ppm copper and 88 ppm iron. The ratio of copper to iron was 1:6 and was the same for Lot II and Lot III. A chelating agent, EDTA, was added to Lot II at the level of 0.1% and to Lot III at the level of 0.5%. Lot IV was the low copper and iron ration and contained 5 ppm copper and 20 ppm iron (ratio 1:4). To Lot V, 118 ppm copper and 1311 ppm iron were added (ratio 1:11). At the weight of 91 kg, the pigs were slaughtered and samples removed from each of the 3 liver lobes and the spleen for copper analysis. The samples were digested by wet ash and analyzed for copper content by a modified method of Rice and Grogan (1960).

The copper level in the livers in Lot V was decreased as compared with Lot I but the difference was not significant. The lowered copper level could be the result of the depressing effect of the high amounts of copper and iron added to Lot V. Copper concentration in the livers in Lot IV, the milk ration or low copper and iron ration, was still lower than that of Lot V and varied significantly from the level of copper found in the livers of pigs in Lot I. The addition of 0.5% EDTA to the control ration gave the highest

level of copper found in the livers and was significantly different from Lot IV and Lot V, but not from Lot I and Lot II. With 0.1% EDTA added to the control ration, Lot II gave only significant difference compared with the low copper and iron ration, Lot IV. The level of copper found in the spleen was considerably lower than the amount found in the liver. The addition of 0.5% EDTA to the diet lowered the copper concentration significantly over the levels of copper in spleens on other rations. The other rations did not have any significant effect on the copper levels in the spleen.

A wide variation of copper concentration of both liver and spleen was observed among the animals throughout the experiment. This could account for the differences in breed and sex of the pigs. When correction for animal variation was made, no difference was found between the copper levels in the 3 liver lobes.