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## COPPER SALTS IN NUTRITION

JOHN M. EVVARD, V. E. NELSON, AND W. E. SEWELL

A great deal of work has been done on the distribution of copper in plant and animal matter. It is not the intention of the authors to critically review all of the work of a biological nature concerning this element but only such as applies to the problem at hand. Investigations concerning the presence and role of copper in marine life are numerous. Harless<sup>1</sup> found this metal in combination with protein in the blood of *Eledone* and *Helix Pomatia* and concluded that it functioned in a manner similar to that of hemoglobin of higher animals. Fredericq<sup>2</sup> also said that copper plays the same role in the molluscs as iron does in the blood of higher animals. He called the copper protein compound hemocyanin. Since the work of these authors a number of experiments have conclusively demonstrated that hemocyanin functions as a respiratory pigment.

Experimental data bearing on the relation of copper to the nutrition of mammals are relatively limited. The Purdue station<sup>3</sup> reported some work on White Leghorn chickens in which the specific effects on intestinal putrefaction of several substances including copper sulphate were studied. Copper sulphate supplied in the drinking water of chickens at the rate of one part of salt to 400 parts of water decreased the mortality and increased the weight of the lot compared with the check group. Drummond<sup>4</sup> fed peas "greened" with copper in amounts which would supply 1.5 milligrams of copper per day per 100 grams of live weight. The experiment continued for three months at the end of which time the health of the rats appeared good. Growth in the copper and check groups was identical. He found that animals can absorb copper and that part of the absorbed metal was held by the liver until excreted either by way of the urine or feces. A recent and very important work on copper is that by McHargue.<sup>5</sup> He observed the presence of this element in foodstuffs of high nutritive value and he therefore conducted an experiment to determine the effect and role of this and other elements in animal nutrition. A group of rats was fed the following basal ration: Argo cornstarch 54, commercial casein 26, Domino cane sugar 10, Price's lard 5, and Mineral Mixture 5. A second group of rats received, in addition to the basal ration, copper as copper sulphate to the extent of 25 parts of copper per million parts of ration. A third lot of rats was fed the basal ration plus copper as copper sulphate (25 parts copper per

million of ration), manganese in the form of manganese peptonate (100 parts manganese per million of ration) and zinc as zinc lactate (25 parts zinc per million of ration). The check lot made the least growth; the animals on the combination grew best whereas the copper lot was, as far as growth is concerned, midway between the other two groups. The check lot succumbed first, the copper group second, and those on the combination lived longest. Some of the rats developed ophthalmia which was attributed to excess minerals in the ration. The diets employed by McHargue were deficient in vitamins. However, the data are valuable because the deficiency of vitamins was common to all lots.

McHargue concluded that possibly copper has an important biological function in animal metabolism. He says: "Rats confined to a synthetic diet gave results indicating that compounds of manganese more definitely and possibly copper and zinc also have important biological functions in animal metabolism. Moreover it is assumed that the compounds of these elements as they occur in the natural state in green leaves, and seeds of mature plants and the vital organs of animals have a much more active biological potency than could be expected from feeding equal proportions of a crystalline salt of these metals in a synthetic diet."

Albino rats and Poland China gilts were employed in all of our experiments. Three groups of young rats, each group consisting of three males and three females, were selected for uniformity in breeding, health, previous treatment, age, etc. The initial weights of the rats ranged between 49 and 64 grams with an average of approximately 54 grams. The basal ration fed the check group expressed in percentages was as follows: purified casein 18, dextrin 61.3, yeast 12, cod liver oil, fed daily, 5 and salt mixture 3.7. Casein was prepared by washing the commercial product daily with dilute acetic acid for four weeks. Dextrin was made by autoclaving starch moistened with citric acid solution at fifteen pounds pressure for three hours. McCollum and Davis salt mixture number 185 was used. The second group of rats received the basal ration together with 50 parts of copper as crystallized cupric sulphate per million of ration. The third lot received in addition to the basal ration 300 parts of copper as crystallized cupric sulphate per million of ration. Gains, Feed Consumption, and Economy of Feed in the Production of Gains are given in Table I.

Table I is self explanatory. The basal ration was believed prior to this work to have supplied sufficient proteins, vitamins, inorganic constituents and energy for normal growth and general well

Table I—Gains and Feeding Data—First Experiment, Rats

LOT NUMBER	I		II		III	
	CHECK		COPPER 50 PARTS PER MILLION OF RATION		COPPER 300 PARTS PER MILLION OF RATION	
<i>Grams</i>						
Average daily gain per rat	1.78		1.93		2.22	
Relative gains		100.0		108.4		124.7
Feed consumed per rat —average daily	7.74		7.94		8.32	
Relative feed consumption		100.0		102.6		107.5
Feed required for 100 grams gain	434.0		410.7		375.6	
Relative feed requirement		100.0		94.6		86.6

being. The addition of copper to the extent of 50 parts per million of ration increased the daily gain and economy of gain. The third group receiving copper sulphate, 300 parts of copper per million of ration, excelled both of the other groups in the rate of growth and economy of growth measured by feed requirements. It is possible that the basal synthetic ration is not free of copper, but it is to be noted that all groups received the same basal ration and the only variable was cupric sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .

It was observed in the ashing of the animals for analyses that the greater part of the stored copper was confined to the liver. The light blue color, characteristic of copper salts, was very evident in the ash.

Some further experiments with rats were conducted with the assistance of Mr. Edward B. Fraser. Three groups of six rats each, three males and three females, received the following Mixture A: Yellow corn, ground, 65.5, ground oats 10.0, linseed oil meal, old process, 10.0, wheat middlings, standard, 10.0, meat meal tankage "Swift's 60 per cent protein," 4.0, flake salt 0.5, total 100 lbs. self fed. Mixture A: 98 parts, plus 2 parts of mineral mixture constituted the basal ration for the three groups of rats. The mineral mixture consisted of:

Limestone (Over 98 per cent $\text{CaCO}_3$ )	30.60
Bone Meal (65 per cent $\text{Ca}_3(\text{PO}_4)_2$ )	36.50
Hardwood Ashes, Screened	11.75
Meat and Bone (Armour's 45 per cent Protein, 20 per cent $\text{Ca}_3(\text{PO}_4)_2$ )	10.845
Vegetable Char	4.70
Sodium Bicarbonate	4.70
Iron Oxide (technical)	0.29
Iron Sulphate (technical)	0.29
Manganese Sulphate (technical)	0.29
KI	0.035

One lot received this basal ration only, a second group was given crystallized cupric sulphate equivalent to one per cent of the minerals (Basal ration 99.98, plus  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.02) and the third group was fed crystallized cupric sulphate equivalent to three per cent of the minerals (Basal ration 99.94, plus cupric sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.06).

The average daily gains per rat of the three lots were respectively: check 1.55, small amount of copper 1.94 and larger amount of copper 1.73 grams.

The feed requirements for thirty days were as follows: check 548, small amount of copper 488, larger amount of copper 545.

The copper additions increased feed consumption quite markedly. The check rats consumed 8.80 grams per rat daily as contrasted with 10.3 and 10.2 for the small and greater amounts of copper.

Our investigations (with collaboration of C. C. Culbertson and W. E. Hammond) covering the feeding of copper sulphate to swine are still in progress, the animals now entering the second generation. Weanling gilt pigs weighing on the average 48 pounds per head, ten to the group, were started in July, 1927, on native pasture, mostly bluegrass, the basal ration carrying corn grain (mixed in color, mostly yellow), meat meal tankage, cottonseed meal, linseed oilmeal, alfalfa meal, salt, ground limestone (over 98 per cent calcium carbonate), bone meal, and potassium iodide. When on pasture the addition of a small amount of copper sulphate, technical grade, approximately  $5\frac{1}{2}$  grains per head daily, did not produce positive results.

However, during the subsequent winter season covering four months to date, when the gilts were in the breeding yards (dry lots) the two check lots of seven gilts each receiving no copper sulphate made average daily gains of 1.16 and 1.21 pounds per gilt respectively whereas the copper sulphate group (consuming daily a little over 7 grains of the copper salt) increased at the rate of 1.23 pounds, or 3.7 per cent above the average of the checks, slightly excelling in both comparisons. The feed requirements per hundred weight of gain produced were 508 and 486 pounds in the two check groups and 480 pounds when the copper sulphate was added.

In this current long-time experiment the organic grain and supplemental feeds were kept at the same level for all groups, but a mineral mixture was self-fed to each group.

The check lots received Mineral Mixture A, Salt 20, limestone (over 98 per cent calcium carbonate) 39.99, bone meal 39.99 and

potassium iodide .02; total 100 pounds. The copper fed gilts were allowed Mineral Mixture B, composed of Mineral Mixture A, 99 and copper sulphate 1; total 100 pounds. Mineral Mixture A was consumed at the rate of 0.032 and 0.17 pound (average = 0.02+5 pound) per gilt daily during the winter breeding period but where copper sulphate was included the ingestion was larger or .064 pound, over twice as much. Apparently the palatability of the minerals was improved by the incorporation of the copper salt.

Our experiments with swine are being continued with different basal rations, and the results therefrom will be released in due course.

#### SUMMARY

The significance of copper salts in the growth and development of the animal body has been under investigation. It has been found that copper sulphate is of considerable nutritive importance.

Our researches with rats and swine show that these animals make better gains and exhibit a higher food utilization per unit of weight increase when small amounts of copper sulphate are incorporated in the ration. The basal rations used were, in the light of our present knowledge, complete from the standpoint of proteins, vitamins, inorganic constituents and energy.

In the ashing of the rats fed copper sulphate it was observed that the greater part of the stored copper was confined to the liver. The light blue color, characteristic of copper salts, was very evident in the ash.

It is possible that the medicinal and nutritive value of liver and its proper functioning may be somehow related to this element.

Our conclusions to date are that copper fed as copper sulphate plays an important part in the growth (weight and dimensional) of the mammals used.

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