

[CONTRIBUTION FROM THE DEPARTMENT OF NUTRITION, HARVARD SCHOOL OF PUBLIC HEALTH, AND THE DEPARTMENT OF BIOCHEMISTRY, HARVARD MEDICAL SCHOOL]

## Two Complex Salts of Choline and Copper Chloride and their Activity as Catalysts of Fat Oxidation<sup>1</sup>

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Two stable complex salts of choline and copper chloride have been prepared and characterized as salts of the higher copper chloride anions,  $\text{CuCl}_3^-$  and  $\text{CuCl}_4^{2-}$ . One which crystallized as brick-red plates was found to be choline copper trichloride,  $[(\text{CH}_3)_3\text{NC}_2\text{H}_4\text{OH}]\text{CuCl}_3$ ; the other which crystallized as bright yellow needles was characterized as choline copper tetrachloride,  $[(\text{CH}_3)_3\text{NC}_2\text{H}_4\text{OH}]\text{CuCl}_4$ . Neither of these salts is stable in aqueous solvents but in "dry reaction" were found to be superior to copper sulfate and copper chloride as catalysts in the oxidation of corn oil.

Earlier studies using the rat as the experimental animal<sup>3</sup> showed that the effects of high copper diets on growth were related to the level of choline in the diet. Increasing either the copper or choline interfered with growth when the diet contained approximately 0.1% copper sulfate. Similar results have been obtained with chicks and in these studies it was noted that the high copper-choline diets rapidly became rancid and in time the presence of a red crystalline deposit in the diets was seen.<sup>4</sup> The concurrent rancidity and deposition of red crystals suggested a chemical interaction of choline and copper in the diets as the causative factor. Two compounds have been prepared and characterized as complex salts of choline and copper chloride. Evidence is presented that these salts have the necessary catalytic activity and are apparently responsible for the rapid development of rancidity in these diets.

### Experimental

An indication that a reaction between these two salts was possible was obtained from the sequence of colors resulting when finely powdered copper sulfate was dusted into moist choline chloride. The color changed from the blue of the sulfate through green to yellow and the reaction finally resulted in the production of a brick red material. This red compound was insoluble in 95% ethanol and hence could be purified by repeated extraction with this solvent to remove the unreacted copper sulfate and choline chloride. Ethanol was found to be the best solvent although glacial acetic acid could be used. Copper nitrate gave the same reaction and because of its greater solubility in 95% alcohol, it was used in further studies of the reaction.

When choline chloride and copper nitrate were dissolved in 95% ethanol in molar ratios greater than 2:1, a mixed precipitate resulted and the solution turned a dark green. This residue was composed of a mixture of red and yellow crystals which were very hygroscopic and difficult to wash even in cold absolute alcohol. Multiple extraction with boiling 95% alcohol separated two fractions, the residue consisting of brick red plates, and the soluble fraction which upon cooling quickly deposited bright yellow needles. The insoluble red compound was very difficult to recrystallize and was best purified by repeated extraction. The yellow crystals could be recrystallized from hot 95% ethanol containing a few drops of HCl.

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(3) N. Kaufmann, J. A. Cartaya, P. L. White, D. M. Hegsted and T. D. Kinney, *J. Nutrition*, **46**, 433 (1952).

(4) P. L. White, D. M. Hegsted and J. Mayer, unpublished.

In order to study the color changes which preceded compound formation the visible absorption spectra of various mixtures of copper nitrate and choline chloride were measured in 95% ethanol. Solutions 0.005 molar in copper nitrate and containing choline chloride in concentrations to give molar ratios of 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 were employed. The Beckman quartz spectrophotometer, model DU, with 1-cm. cells was used. As seen in Fig. 1 the shift in the wave length of absorption maximum of the hydrated copper ion from 795 to 880  $\mu$  was suggestive of compound formation and further was similar to the changes in the position of the maximum occurring with the formation of the coordinated copper chloride anions.<sup>5</sup> To demonstrate that the shift and the color sequences noted earlier were due to the formation of complex copper chloride anions, solutions of copper nitrate in the presence of HCl in molar ratios similar to those employed above, but in the absence of choline, were studied spectrophotometrically. As seen from Fig. 2, a similar shift in the wave length of maximum absorption occurred. It also seemed evident from the greater optical density of the copper-choline mixtures that the choline had stabilized the higher  $\text{CuCl}_2$  anions and that the two unknown salts must have been stable choline-copper chloride complexes.

The compounds were found to contain 5.06 and 6.35% nitrogen for the red and yellow compounds, respectively, but no nitrate nitrogen. The chloride content was 37.57 and 33.85%, respectively, for the red and yellow salts. These data indicated that the chloride from the choline had displaced the nitrate anion of the copper salt and promoted formation of copper chloride complexes. This was substantiated by the failure to demonstrate any compound formation from copper nitrate and choline nitrate in the absence of chloride. Synthesis of both compounds was then achieved by the utilization of copper chloride and choline chloride. The yellow compound in 95% ethanol exhibited an absorption maximum at 875  $\mu$ ; no attempt was made to prevent dissociation of the complex by the addition of HCl. Since the red compound was insoluble in this solvent, no absorption spectrum could be obtained.

The red compound was prepared by dissolving 20 g. of copper chloride trihydrate and 16.4 g. of choline chloride in 200 ml. of hot 95% ethanol. The solution was cooled and allowed to stand in the cold until maximum production of red crystals was obtained. The solution was filtered with suction and the crystals washed five times with hot 95% ethanol, two times with cold absolute alcohol and dried *in vacuo* over  $\text{P}_2\text{O}_5$ .

The red compound, purified by extraction to a uniform melting product (m.p. 166.5–168.5° dec., uncor.) was found to have the following composition which agrees favorably with the formula  $[(\text{CH}_3)_3\text{NC}_2\text{H}_4\text{OH}]\text{CuCl}_3$ . Found: Cu, 23.41; Cl, 37.57; C, 21.92; H, 5.33; N, 5.06. Theory: Cu, 23.19; Cl, 38.80; C, 21.92; H, 5.11; N, 5.11.

The yellow compound was prepared by dissolving 22.4 g. of copper chloride trihydrate and 48 g. of choline chloride in 300 ml. of hot 95% ethanol with the aid of a few drops of concentrated HCl. In some preparations the red compound was formed in small amounts which necessitated a filtration through a steam-heated filter. The filtrate was subsequently cooled very slowly in a jacket of hot water to promote the production of good crystals. Since they are quite

(5) G. Spacu and I. G. Murgulescu, *Z. physik. Chem.*, **A170**, 70 (1934).

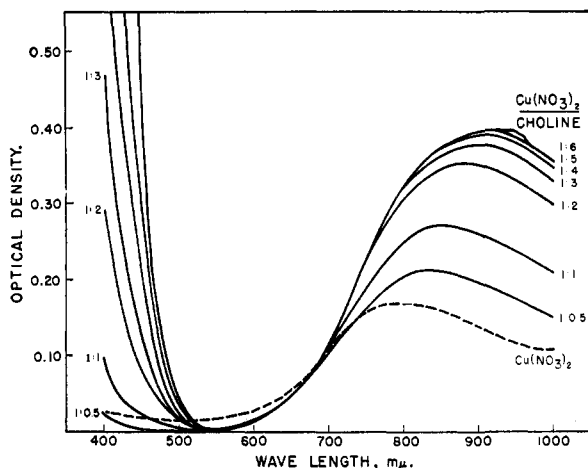


Fig. 1.—Absorption spectra of  $5 \times 10^{-3}$  molar copper nitrate in the presence of varying concentrations of choline chloride.

hygroscopic, the crystals were filtered rapidly, washed with cold absolute alcohol, transferred quickly and dried over  $P_2O_5$  *in vacuo*.

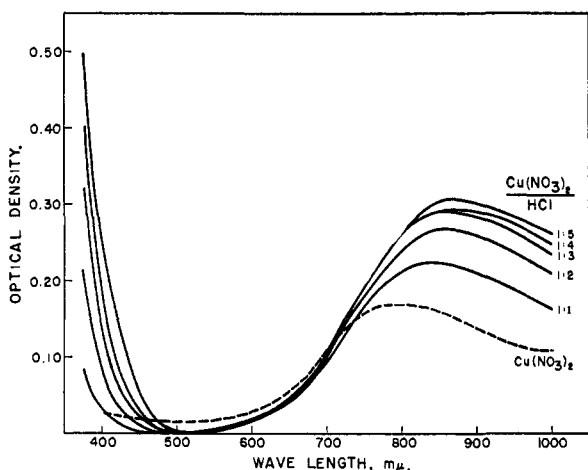


Fig. 2.—Absorption spectra of  $5 \times 10^{-3}$  molar copper nitrate in the presence of varying molar concentrations of hydrochloric acid.

The recrystallized yellow compound (m.p. 223.0–224.5° dec., uncor.) was found to have the following composition which agrees with the formula  $[(CH_3)_3NC_2H_4OH]_3CuCl_4$ . Found: Cu, 15.37; Cl, 34.30; C, 29.04; H, 6.77; N, 6.78. Theory: Cu, 15.46; Cl, 33.85; C, 29.31; H, 6.96; N, 6.35.

Sidgwick<sup>6</sup> and Hüchel<sup>7</sup> have recently summarized the literature concerned with copper chloride complex salts. These authors report that thirty-three such compounds containing  $CuCl_2$  anions have been described: twenty-two containing  $CuCl_4^{-2}$ ; nine  $CuCl_3^{-1}$ ; and the two remaining contained  $CuCl_5^{-3}$ . The majority of the copper chloride complexes described were inorganic salts of potassium, sodium and cesium. However, complex copper chloride salts with organic cations are known. Brintzinger and Hease<sup>8</sup> have described several complex copper salts of ethanolamine and chloroethylamine.

Of particular interest to this Laboratory was the apparent

(6) N. V. Sidgwick, "The Chemical Elements and their Compounds," Vol. I, Oxford at the Clarendon Press, London, 1950.

(7) W. Hüchel, translated by L. H. Long, "Structural Chemistry of Inorganic Compounds," Elsevier Publishing Co., Inc., New York, N. Y., 1950.

(8) H. Brintzinger and B. Hease, *Z. anorg. allgem. Chem.*, **248**, 351 (1941).

increase in the catalytic activity of copper after stabilization of the higher halide salt with choline. In order to study this catalytic activity in more detail it was necessary to avoid aqueous media as well as non-polar solvents. To duplicate the conditions of the experimental rations, corn oil was dispersed on cellulflour in the presence of various catalysts and the production of rancidity followed by the determination of peroxide values. The systems used were prepared as follows: from 0.1 to 0.25 part of corn oil was ground in a mortar with 1 part of cellulflour and then the salts to be tested were mixed in, generally in amounts equimolar with  $CuCl_2$  which was added to give a concentration equal to 0.1% of the corn oil. Large batches were prepared and appropriate aliquots equivalent to 0.1 or 0.25 g. of corn oil were weighed into individual waxed paper candy-cups for incubation. The samples were analyzed for peroxide value and iodine number without prior extractions since the cellulflour did not interfere with the determinations.

Figure 3 shows a comparison of the catalytic effect of copper chloride with that of the red and yellow compounds on the oxidation of corn oil at room temperature. The yellow compound shortened the induction time of the oxidation and catalyzed an oxidation superior to that produced by the red compound, which in turn was superior to copper chloride. There was a corresponding decrease in the iodine number of the corn oil; in the case of the yellow compound the iodine number decreased from 119 to 67 within 7 days. In the presence of the red compound, the iodine number decreased to 91 within the same period of time. In contrast, the sample containing  $CuCl_2$  showed a decrease to 107, and the control had an iodine number of 117 at the end of 7 days.

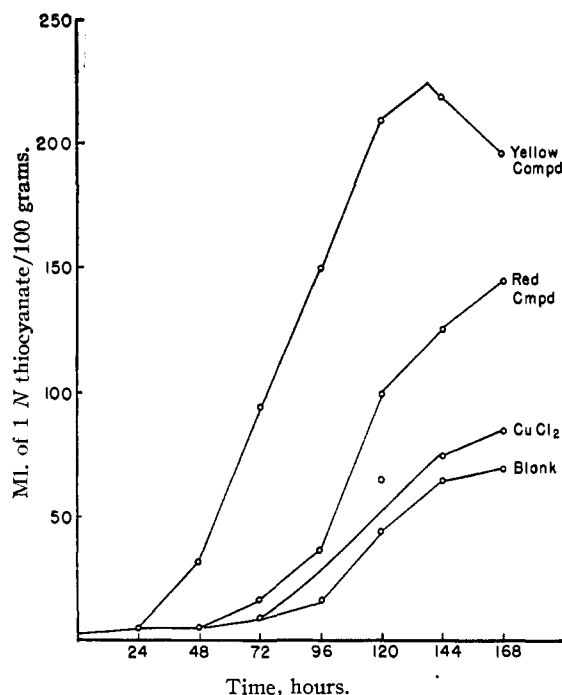


Fig. 3.—Peroxide formation in corn oil in the presence of various copper compounds.

Similar experiments conducted at 56° showed a much accelerated rate of reaction. In one such experiment the two complexes were compared with  $CuSO_4$  and  $CuCl_2$  with and without choline chloride, all in equimolar amounts. The choline was added in amounts equivalent to that found in the yellow compound. The induction periods in the presence of these compounds or combinations of them are shown in Table I. The time listed is the period elapsed before any significant increase in peroxide value was observed.

The complex copper choline salts (red and yellow compounds of Table I) proved to be far superior oxidation catalysts. Whereas a 72-hour induction period was required for the control sample, to which no additions were made, the presence of the red and yellow compounds reduced this period to 3 and 6 hours, respectively. In contrast, induc-

TABLE I

TIME REQUIRED FOR THE INITIATION OF FAT OXIDATION IN THE PRESENCE OF SEVERAL DIFFERENT CATALYSTS

System: cellufLOUR, 2 g.; corn oil, 0.5 g.;  $\text{CuCl}_2$ , 0.5 mg.; other compounds equimolar with  $\text{CuCl}_2$ ; temperature,  $56^\circ$ ; solvent for peroxide determination, chloroform

Addition to corn oil-cellufLOUR mixture	Induction period, <sup>a</sup> hours
None	72
Choline	80
$\text{CuSO}_4$	55
$\text{CuCl}_2$	36
$\text{CuSO}_4$ plus choline	30
$\text{CuCl}_2$ plus choline	18
Red compound	3
Yellow compound	6

<sup>a</sup> Time elapsed before a significant increase in peroxide value was observed.

tion periods of 55 and 36 hours were observed in the samples containing copper sulfate and copper chloride. The fact that the samples containing the copper salts plus choline exhibited shorter induction periods than those samples containing only the copper indicated that some compound formation had occurred in the former.

That compound formation could occur under these conditions is shown more conclusively by the results of the experiment reported in Table II which shows, in addition to the increased catalytic activity of the copper choline compounds, that under the conditions of the experiment such compounds can be formed from the two salts. Hydrochloric acid added to the corn oil-cellufLOUR-copper chloride systems in equimolar amounts with the choline chloride did not accelerate the oxidation over that amount caused by copper

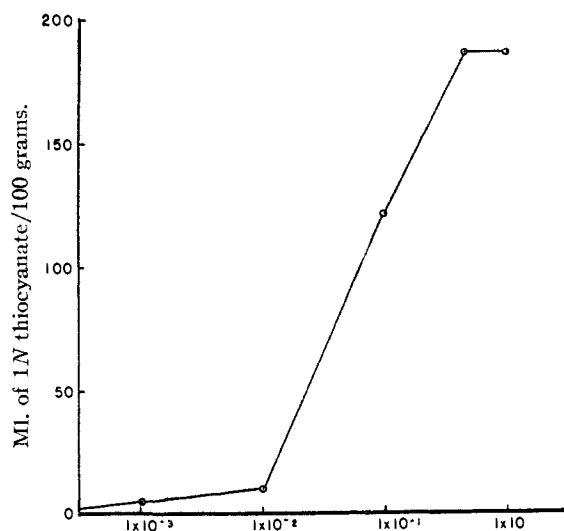


Fig. 4.—Effect of catalyst concentration on peroxide formation in corn oil at  $56^\circ$ .

chloride alone. This would indicate that the copper tri- and tetrachloride anions were not formed, or more probably if formed were not stable because of the lack of appropriate cations.

TABLE II

THE OXIDATION OF CORN OIL DISPERSED ON CELLUFLOUR IN THE PRESENCE OF VARIOUS CATALYSTS<sup>a</sup>

Incubation time at $60^\circ$ , hr.	Blank	$\text{CuCl}_2^b$	$\text{CuCl}_2$ +choline	Red <sup>c</sup> compd.	Yellow <sup>d</sup> compd.
0	1.5	2.4	1.4	1.6	2.0
3	2.4	2.7	3.3	4.6	23.6
6	3.1	4.3	6.5	14.9	132
9	4.1	6.8	16.0	85.5	214
12	5.2	10.8	383	187	191
24	8.0	26.4	276	132	62.3

<sup>a</sup> Peroxide values reported as ml. of 1 N thiosulfate per 100 grams of oil. <sup>b</sup> 0.1% weight of the oil. Other catalysts added in equimolar amounts. <sup>c</sup> Choline-copper trichloride. <sup>d</sup> Dicholine-copper tetrachloride.

In order to determine the minimum concentration of compounds needed to catalyze the type of reaction described in conjunction with the experimental chick rations, corn oil, 0.100 g., and cellufLOUR, 1.9 g., were mixed with varied amounts of the yellow compound (0.001 to 1.0 mg.) and incubated at  $56^\circ$ . Figure 4 is a plot of the reaction after 9 hours showing the peroxide value at this time in the presence of the different amounts of yellow compound. It shows that oxidation was catalyzed in the presence of the compound in a concentration as low as 0.01% of that of the corn oil. This concentration is much lower than that theoretically formed in the experimental chick rations and indicates that the compound in low concentrations could have initiated the rancidity observed, at least in the absence of anti-oxidants. It should be pointed out that only the red compound was found in the diets; the presence of the yellow compound as such was never observed. However, the hygroscopic nature of the latter and the difficulty encountered in its crystallization could account for the failure to find it in the diets.

### Discussion

Experiments with corn oil, cellufLOUR and the copper compounds have shown that the choline-copper chloride complex salts are capable of catalyzing fat oxidation and as such could have been responsible for the rapid development of rancidity in the experimental diets. Although copper salts, such as the sulfate and chloride, also are capable of catalyzing fat oxidation, the complex choline copper salts are far superior in their catalytic action. Whether the toxicity of the diet to the chick<sup>4</sup> was a result of the rancidity of the diet, induced by the salts, or of the ingestion of the choline-copper salts *per se* must await more direct evidence. Experiments designed to investigate this phase of the problem will be the subject of a later publication.

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