Table VII. Effect of Copper Ions on Partition of Copper Oxinate between Oil and Water

$Cu(OX)_2$, Concn., M	$Cu(Ac)_2$, Concn., M	Transmittance of Light by Xylene Fraction at 425 mμ, %
4×10^{-5}	0	68
4×10^{-5}	4×10^{-4}	89
4×10^{-5}	8×10^{-3}	98

$Cu(Ox)_2$, toward the half chelated metal species, $Cu^{+}Ox$, as required by the equilibrium equations. If, as proposed (*2*), this change occurs and the ionic 1 to 1 chelate that becomes the increasingly more prominent species with increasing metal ion concentration is less able to exert its toxicity by its difficulty in penetrating the fatty, cell membrane, the toxicity of the solution would indeed by suppressed by metal ions. Those metals which have a greater affinity for oxine, as measured by their stability constants, would be most efficient in reversing the toxicity. As has been seen, this is the case.

Evidence for the existence of the 1 to 1 chelate has been given by Albert (*1*) by titration and the stability constant measured. However, since the proposed mode of action is based upon this entity, a special experiment has been run to give further evidence of the presence of this form and to demonstrate whether it is produced from the 2 to 1 chelate with increasing concentration of metal ions.

Copper oxinate, 2 to 1 chelate, was dissolved in xylene and divided into three portions. Each portion was partitioned between the xylene and an aqueous medium. In the first extraction the aqueous medium was water alone; in the second portion it was water containing 10 equivalents of cupric acetate; and in the third portion it was water with 200 equivalents of cupric acetate. The quantity of 2 to 1 chelate remaining in the xylene phase was indicated by the light transmittance measured spectrophotometrically at 425 mμ. If the copper ions resulted in an increased quantity of the half chelate at the expense of the 2 to 1 chelate, and the 1 to 1 chelate were preferentially soluble in the aqueous phase, the quantity of the fully chelated copper in the xylene would be reduced. The data in Table VII show the gradual increase in transmitted light as the concentration of copper acetate was increased. Since the pH was unchanged, it can be assumed that the 2 to 1 chelate was converted to the 1 to 1 chelate, which was transferred to the aqueous extract. There is an equilibrium between the two chelates and even with 10 equivalents of copper, the data exhibit the presence of 2 to 1 chelate in the oil fraction. With 200 equivalents of copper ion, however, the 2 to 1 chelate was virtually absent. The 2 to 1 chelate cannot be considered to be dissociated to the water-soluble oxine anion and copper cation, because the oxine anion concentration is strongly suppressed by the high concentration of metal ions.

In a recent paper (*8*), Goksøyr reports different absorption bands as he adds copper ion to sodium dimethyldithiocarbamate, which he attributes to the 1 to 1 and 2 to 1 complexes of copper dimethyldithiocarbamate. From his biological studies he concludes that the 1 to 1 copper complex is the toxic entity.

The fact that metals such as nickel and aluminum, that are not known to have physiological functions in fungus metabolism, produce reversals indicates that the reversals are not related to specific chemical activity within the living tissues. It is possible that the effective reversal of oxine obtained with ferrous iron (*10, 18*) might be the result of oxidation to ferric iron in the solution, the latter having a very high stability constant.

The parallel reversal, with excess oxine, has been explained by the hypothesis (*2*) that the 1 to 1 chelate is the true toxic species, that it is produced by equilibrium from the 2 to 1 chelate, and that it is free to react with other complexing agents as exist in the living cell. Because the charge-carrying 1 to 1 chelate cannot readily penetrate the cell, it has been proposed (*2*) that the 2 to 1 chelate enters the cell where it is in equilibrium with the toxic 1 to 1 chelate. With an excess of oxine, however, dissociation of the 2 to 1 chelate is inhibited and a reversal of toxicity occurs. Similarly, an excess of other complexing agents counteract the fungitoxicity of copper oxinate, as in the case of citric acid.

Summary and Conclusions

The fungitoxicity of copper oxinate (copper-8-quinolinolate) is progressively reversed with increasing concentrations of copper, nickel, and iron ions. Reversal of the fungitoxicity of copper oxinate with excess oxine (8-quinolinol) has also been shown.

The reversal with excess metals is due to the suppression of the cell-penetrating 2 to 1 chelate, whereas the reversal with excess oxine is due to the suppression of the toxic 1 to 1 chelate within the cell according to the requirement of the equilibrium equations.

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Quantitative Determination of Terminal Methionine, Leucine, and Lysine in Raw and Toasted Soybean Oil Meal–Correction

In the article on "Quantitative Determination of Terminal Methionine, Leucine, and Lysine in Raw and Toasted Soybean Oil Meal" [S. W. Fox, Carol Warner, and T. L. Hurst, AG. AND FOOD **3,** 704 (1955)] the headings for leucine and methionine are interchanged in Table IV. S. W. Fox