CLVIII. A METHOD FOR THE ESTIMATION OF SMALL QUANTITIES OF COPPER IN TISSUES.

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THE common microchemical method for the determination of copper in tissues is that devised by Rose and Bodansky [1920], who have investigated the quantitative distribution of this element in mammalia, in fish, and in crustacea. Their method which is colorimetric depends on the production of a blue colour by the interaction of ammonium hydroxide and CuSO_4 in aqueous solution.

Theoretical.

The present method is based on the development of a light green colour, "Scheele's Green," when a solution of copper sulphate is added to a solution of Na_3AsO_3 . The equation representing the reaction is:

"Scheele's Green" is copper hydrogen arsenite.

We found that anhydrous arsenic chloride dissolved in dilute caustic soda is an extremely colour-sensitive reagent. Presumably these two substances react in solution in the following manner:

The Na_3AsO_3 then reacts as shown in equation (1).

Practical.

To prepare the reagent 10 cc. of 10 % NaOH are taken in a boiling tube and 0.1 cc. of anhydrous AsCl_3 added. A white precipitate which disappears on shaking is formed. A second 0.1 cc. AsCl_3 is now added, and the boiling tube is again shaken. The contents of the tube are now diluted by the addition of 10 cc. of distilled water. The reagent is now ready for use.

An experiment is described illustrating the colour proportionality to copper concentration:

0.8472 g. of CuSO_4 was dissolved in distilled water and made up to 1 litre. 3, 4, 5, 6 cc. respectively of this solution were transferred to four graduated cylinders of 15 cc. capacity, and distilled water was added to each cylinder until the volume was 10 cc. Each cylinder was stirred with a short glass rod, which was left in the solution.

Five drops of the AsCl_3 reagent were now added to each cylinder, and the green colour developed instantaneously. The contents of the cylinder containing 3 cc. of standard CuSO_4 solution were transferred to the colorimeter cup, and the other solutions were read against this as standard.

The following table (Table I) gives the results obtained and from these a curve can be drawn showing the relationship between colour intensity and concentration of CuSO_4 . This curve is approximately a rectangular hyperbola.

Table I.

Concentration in 10 cc.	0.0025416 g.	0.0033888 g.	0.0042360 g.	0.0050832 g.
Colorimeter reading	2.32	1.56	1.26	1.08

The determination of the copper content of ox brain was made by this method. The copper present was converted to copper sulphate. To this end, a portion of brain (100 g.) was subjected to a process of moist combustion, and the inorganic residue was extracted with hot water containing a trace of dilute HNO_3 . The filtrate from the extract was evaporated to dryness, taken up with hot dilute HCl , filtered, and the filtrate treated with H_2S . After filtration, the copper sulphide residue was dissolved in hot dilute HNO_3 and the copper converted into oxide by heating on the microburner after evaporation to dryness. Two drops of 0.1 % H_2SO_4 were added and the contents of the basin transferred to a graduated cylinder, and made up to a volume of 6 cc. with distilled water. The standard was made by taking 1 cc. of CuSO_4 solution (1 cc. = 0.0008472 g. CuSO_4) and diluting to 6 cc. The following were the colour readings:

Reading of Unknown = 1.26.

„ Standard = 0.55.

$\therefore$ Weight of CuSO_4 in 100 g. of brain = $\frac{0.55}{1.26} \times 0.0008472 \text{ g.} = 0.0003697 \text{ g.}$

$\therefore$ Percentage of Copper = 0.0000935.

DISCUSSION.

The arsenic chloride reagent here used is sensitive to 1 in 135,000 of CuSO_4 in water. It can be stored in a well-stoppered bottle without appreciable deterioration, and gives the minimum of trouble in manipulation. Its limits of efficiency are, however, circumscribed, the upper limit of CuSO_4 concentration for which it will give reliable results being ($6 \times 0.0008472 \text{ g.}$) in 10 cc. H_2O . If the concentration is very much increased beyond this point, there is a tendency towards sedimentation of a green substance. This can be obviated by the graduated addition of 10 % NaOH solution, but excess of caustic soda is apt to cause decolorisation. Between the limits S and $6S$ (where $S = 0.0008472 \text{ g. CuSO}_4$ in 10 cc. H_2O), the colour is permanent and there is no centrifugable compound present. There is, of course, a colour permanence below the limit S , but the thinness of colour developed under this concentration is such as renders

it not very suitable for colorimetric comparison. Above a concentration 6*S* a nephelometric technique is necessary.

The coloured compound produced is thermolabile. On warming a test tube containing the green coloured substance in concentration ranging between *S* and 6*S* there is an immediate separation of a solid green substance. It is, therefore, important that tissue extracts be treated in the cold with the reagent.

The presence of iron and magnesium is inimical to the straightforward determination of copper, as the salts of these metals interfere with colour development. These two elements are easily separable from copper in the early process of extraction as above described, and the method therefore requires no elaboration here.

REFERENCE.

Rose and Bodansky (1920). *J. Biol. Chem.* **44**, 103.