Finally, it may not be superfluous to recall the fact that many substances, of great interest by reason of their physiological properties, have been found to possess a lactone structure. Cantharidin, anemonin, aesculin, digitoxin, helenin, santonin, picrotoxin, strophanthin, sedanolid, artemisin, are examples, and it seems not unlikely that there may be some intimate causal relation between the mobility of the lactone ring and the observed physiological activity.

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SPECTROPHOTOMETRIC STUDY OF COPPER COMPLEXES AND THE BIURET REACTION.

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CONTENTS.—I. Introduction. II. Absorption in the ultraviolet: (*a*) Technic; (*b*) Results. III. Absorption in the visible spectrum: (*a*) Technic; (*b*) Results. IV. General discussion. V. Summary.

I. Introduction.

In the first paper[1] on the configurations of the copper complexes and their relation to the biuret reaction, it was found that the copper complexes of amino derivatives and other similar substances could be divided, according to their color, into three classes. (1) Blue, (2) purple—called "semi-biuret"— and (3) red—called "biuret." The first class was also characterized by the fact that there were in each complex *2 nitrogen groups so placed*, that by forming "stable" rings,[2] *they could combine with the copper*. The complexes of the *second class had 3 such groups* and the *third class had 4*.

All told, 114 substances were found which fitted in this classification, of which 89 were studied by the authors. In short, all of the data found pointed to but one explanation, *i. e.*, that the copper in the red complexes was the central atom and that 4 nitrogen groups were combined with it coördinately in the sense used by A. Werner. The nitrogen groups could be either amino, imino, imide, or amide. As the coördination number of copper is four, the purple complexes probably contain besides 3 nitrogen atoms, one aquo or hydroxy group attached to the copper. The bluish tint of these purple complexes is undoubtedly due to the latter groups.[3] Similarly the blue complexes may have two hydroxy groups attached to the copper.

That the nitrogen plus copper is not a red producing chromophore and that the red colors are produced, simply by removing the blue produc-

[1] P. A. Kober and K. Sugiura, *Am. Chem. J.*, **48**, 383 (1912); also original communications, *8th Intern. Congr. Appl. Chem.*, **6**, 165 (1912).

[2] 4, 5, or 6 membered rings.

[3] See discussion on page 471.

ing hydroxy or aquo groups, yielding the true color of cupric copper in a nonhydrated condition seems probable, but cannot be decided definitely yet. What conditions are necessary, either in a nitrogenous substance or in the solvent, to permit such coördinated copper compounds to be possible and to be stable in aqueous solutions has already been considered in a previous paper.[1]

The theory has already proven to be useful, as a new complex of copper and biuret was found, with its help, to exist in solution, and whose isolation and analysis published subsequently by Ley and Werner[2] proved it to have the composition then formulated. The next steps in establishing this theory are: (1) to show that the absorption spectra agree with the macroscopical observations, that these colors can be divided into three classes and only three, and that the absorption spectra of a class are practically identical, and (2) to show that it really is the nitrogen groups themselves and not groups associated with the nitrogen, as for example some have supposed, oxy or hydroxy groups, that produce these colors. As the title indicates this paper deals mostly with the absorption spectra of these complexes, but certain points of the second step are also brought out.

II. Absorption in the Ultraviolet.

(*a*) **Technic.**—The Hilger quartz spectrograph[3] size *c*, was used in connection with a sparking outfit[4] consisting of 1/4 k. w. rotary converter, transformer (giving about 15,000 volts) and condenser, which gave a strong spark between the electrodes. As the details of the arrangements have appeared in another paper[5] they are not given here.

(*b*) **Results.**—The following photographs,[6] Fig. 1, show that the absorption is general and that variations in the constitution of these complexes cannot be followed by quantitative ultraviolet spectroscopy.

The marked general absorption in the ultraviolet, in harmony with the observations of Ley and Legge,[7] is due, no doubt, to the copper in the complex. Whether the quantitative work in the ultraviolet may be of more service in this connection cannot be stated now, but since the classification of the complexes depends on the variation of the visible spectrum, quantitative measurements in this region were then made.

[1] *Loc. cit.*

[2] *Ber.*, **46**, 4040 (1913). The authors state that they had isolated the compound before the appearance of Kober and Sugiura's paper. The results however for "external reasons" were not published till more than a year later.

[3] Made by Adam Hilger, Ltd., London, England.

[4] Made by Clapp-Eastham Co., Cambridge, Mass.

[5] Kober, *J. Biol. Chem.*, **22**, 433 (1915).

[6] We are indebted to Mr. Walter Eberlein, for making these photographs (taken March 7–9, 1914).

[7] *Ber.*, **38**, 70 (1915).

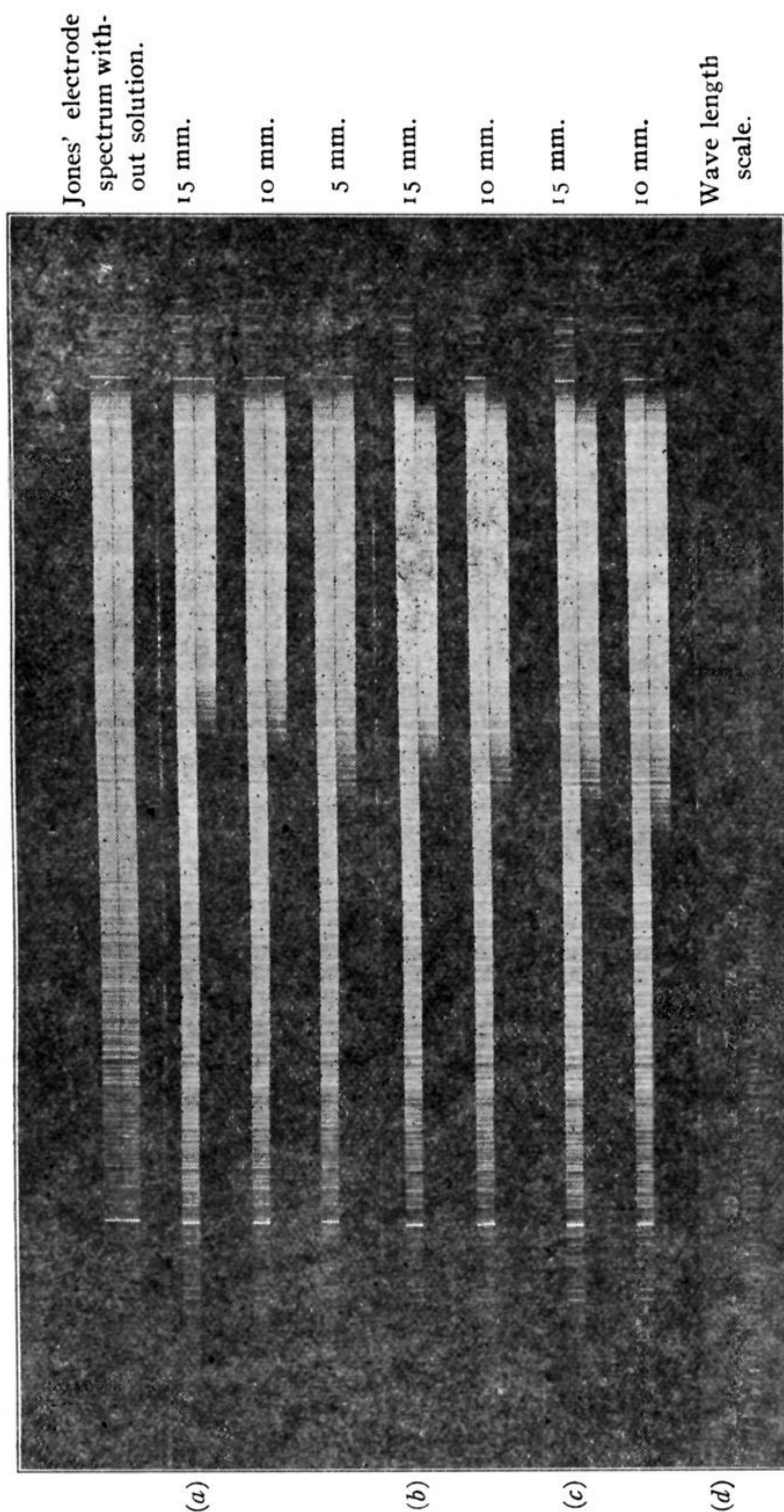


Fig. 1.

(*a*) Blue complex; alanyl-glycin copper (0.110 g. CuO in 100 cc.).
(*b*) Purple complex; alanyl-leucyl-glycin copper (0.062 g. CuO in 100 cc.).
(*c*) Red complex; N-amino-capronyl-diglycyl-glycin copper (0.054 g. CuO in 100 cc.).
(*d*) Wave length scale. Made especially to fit this instrument.

III. Absorption in the Visible Spectrum.

(*a*) **Technic.**—As the apparatus used represents economy in instruments, was simple and effective, a description may be worth while. A Hilger[1]

[1] Made by Adam Hilger, Ltd., London, England.

sector photometer and an ordinary high dispersion spectroscope[1] comprise the essential parts of the apparatus. The photometer was designed for work in the ultraviolet, according to the photographic method of Victor Henri. It had originally a speed of 120 revolutions per minute, but by suitable gearing, this was increased to over 2000 revolutions[2] per minute, so that when the spectrum was seen in the eye-piece, not the slightest trace of vibration and flicker was obtained.

As may be seen in Fig. 2, the light from a Nernst glower, was divided, by means of two prisms, into two paths, the upper going through a cali-

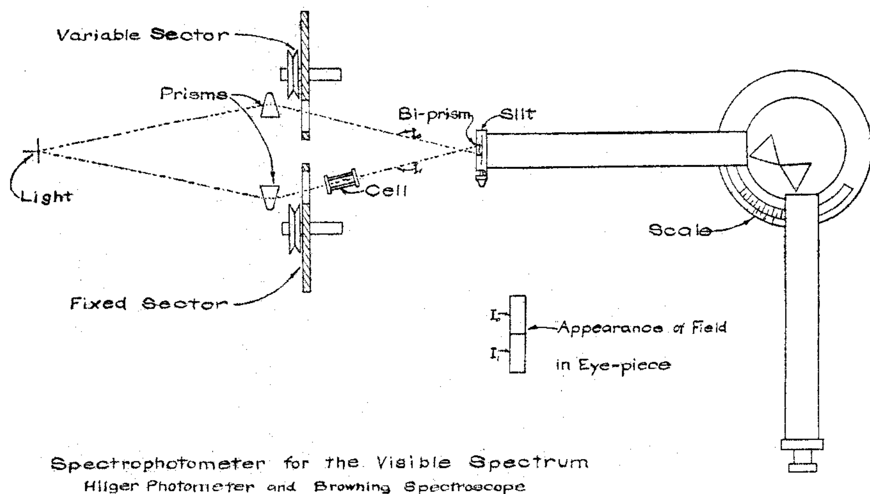


Fig. 2.

brated variable sector direct to the spectroscope. This path represents the light entering the medium (I_o). The lower path passes through a fixed sector, whose aperture is equal to the full aperture of the variable, after which it goes through the absorbing medium in the cell, into the spectroscope. This path represents the transmitted light (I_1). The two paths produce with the aid of a bi-prism[3] two spectra in close juxtaposition and by putting a narrow slit (made from stiff paper) in the eyepiece, a small section of each spectrum is seen and a field resembling that of an ordinary wedge colorimeter is obtained.

After a solution was placed in the cell, the variable sector was changed

[1] Made by John Browning, London, England; kindly loaned to us by Dr. J. T. W. Marshall, for which we wish to express our thanks.

[2] To prevent vibrations to the spectroscopic table, the photometer was mounted on sections of rubber, made by cutting down rubber stoppers to about 1/4 inch thickness.

[3] The sleeve with slit and bi-prism, belonging to the quartz spectrograph for quantitative work in the ultraviolet, were put into the place of the original sleeve and slit, as the two sleeves were practically of the same size.

step by step, from full aperture to 90, 80, 70, 60, 50, 40, 30, 20 and 10% transmission of light. At each step the eye-piece was moved over the whole visible spectrum and the spectroscope scale was read at each point where the two spectral sections were of equal intensity. Thus in 10 settings, the whole absorption curve was quantitatively determined.

The cell was a Schmidt and Haensch 25 mm. polariscope tube, with screw ends, so that the solutions were not open to contamination from the air, *especially from carbon dioxide*, during the measurements. When the two instruments were in position, with distilled water in the cell, the two spectra showed equal intensity throughout.

The basis of this photometric method, Talbot's law of Rotating Sectors, while doubted occasionally in the past, has been shown by E. P.

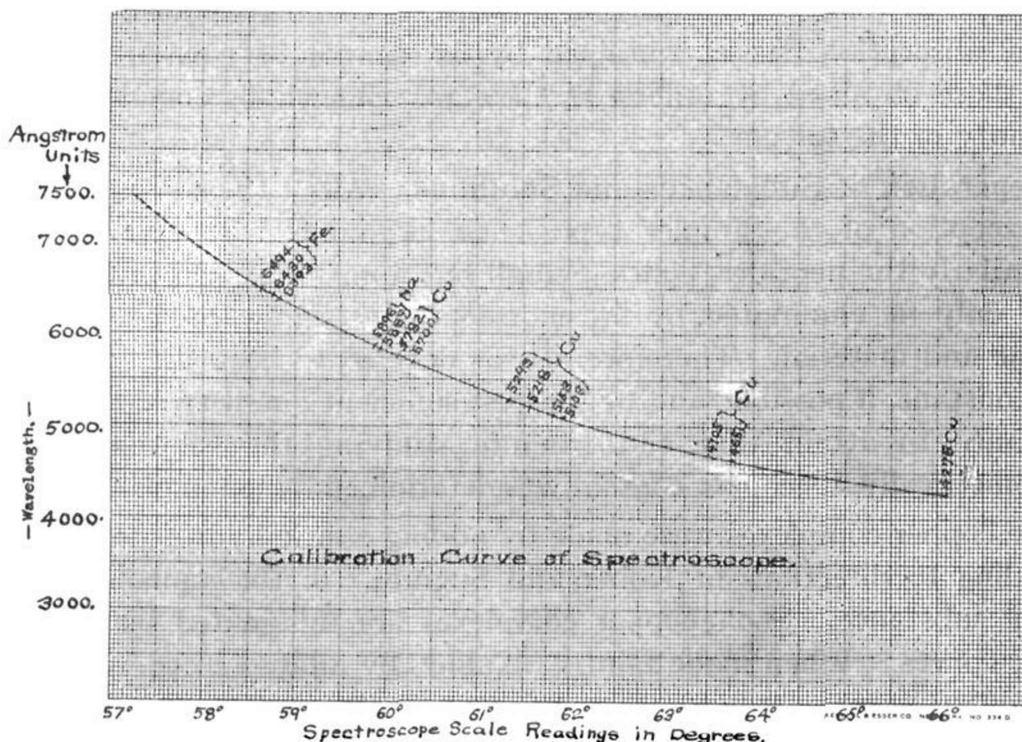


Fig. 3.

Hyde[1] and others, to be accurate within experimental error, and is now fairly well accepted by photometrists.

The wave lengths corresponding to the readings of the spectroscope scale were determined by noting the position of some standard lines in the spectrum of the iron and copper arc and the sodium flame. These readings were then plotted with the wave lengths, and from the curve obtained, Fig. 3, any scale reading could be expressed at once in Ångström Units.

The photometric results of this paper are probably only accurate to within 2%, as not more than two readings were made on an average for

[1] Bureau of Standards, *Bull.* **26**. For white light he found Talbot's law to hold within 0.2% and for colored lights the accuracy was not far from that figure.

each point, although the usual photometric precautions of working in a dark room and shielding the eyes from any glare, were observed. Taking the maximum absorption of the glycine and ammonium complex of copper as standards, our results compare favorably with those obtained with other instruments. Using the formula $\epsilon = 1/m.d \log I_0/I_1$, where m equals the gram molecules of substance, d equals the thickness of absorbing medium in centimeters, I_0 the light entering the medium, and I_1 the transmitted light, we found the molecular absorption coefficient ϵ for the glycin copper complex to be 40. Using a Koenig-Marten spectrophotometer, Ley and Legge[1] found 42. For the ammonium copper complex, we found the coefficient to be 51. Whereas Ley and Legge, and Hantzsch,[2] also using a Koenig-Marten spectrophotometer, found it to be 49.6 to 50.0. The molecular absorption coefficients for other points on the absorption curves, obtained by us, cannot be compared directly with those obtained by others, as they are somewhat dependent on the alkalinity of the solvent.[3]

Preparation of Substances and Solutions.—All of the substances[4] with the exception of the simpler types such as amino acids and ammonia, particularly the peptides, contain small but appreciable impurities, which could only be eliminated by an enormous and unrewarded expenditure of time and material. Therefore, it was decided to keep the copper content constant, if possible, and to add just sufficient quantity of the organic substance to attain that end. Before studying the solution spectrophotometrically, its copper content was determined by iodometric titration.

The copper complexes were made by adding cupric hydroxide, prepared according to directions of Kober and Sugiura,[5] to the aqueous solution of the substance. Such copper complexes are designated by "*neutral*" in the curves. When made faintly alkaline, by adding 20 cc. of "buffer" solution[6] ($C_H = 10^{-8.3}$) to 30 cc. of copper complex solution, they are designated as "*buffer*."[7] When made strongly alkaline by adding from 3 to 10 cc. of normal carbon dioxide free sodium hydroxide, to 25 cc. of copper complex solution, the curves are designated as "alkaline."

Previous work in the quantitative study of absorption in the visible

[1] *Ber.*, **48**, 70 (1915).

[2] Hantzsch and Robertson, *Ibid.*, **42**, 2135 (1909).

[3] See discussion on p. 463.

[4] Taken from the collection of amino acids, peptides and their derivatives purchased from the estate of the late Dr. A. H. Koelker, who made and gathered most of the substances, while working in the Fischer, Abderhalden, and Johns Hopkins Laboratories.

[5] THIS JOURNAL, **35**, 1557 (1913).

[6] *Ibid.*, 1558.

[7] The lack of a potentiometer prevented us from determining accurately the OH concentrations in the final solutions.

has been limited to the measurement of the absorption coefficients at certain wave lengths[1] and only recently Henri measured and plotted the whole absorption curve in his ultraviolet work. For the reader these tables of absorption coefficients are difficult to compare, although for accuracy this method of presentation is excellent. As curves, accurate to within the limits of experimental error, can be constructed, we propose to give all our data in the form of curves. It will present the results more simply for the average reader, while those interested in the details can by little effort, obtain all the accuracy possible, because the accuracy of the curves will not be affected by their reduction in the Journal.

(*b*) **Results.**—The results shown in the following figures, 4, 5, 6, 7 and 8, were obtained at room temperature and are given without comments, as these are brought out in the discussion.

IV. General Discussion.

The first point to which attention may be directed, is that the amount and nature of the absorption of a given complex is somewhat dependent on the concentration of hydroxyl ions. As may be observed, in the tetrapeptide complexes, 5*c*, 6*c* and 7*a*, the weaker alkali only faintly develops the red color, while in the stronger alkali, the red color is most fully developed. On the other hand, in the tripeptides the reverse is true. In the curves 5*a*, 5*b*, 6*a* and 6*b*, the weaker alkali is the most favorable for the development of the purple or semi-biuret color, and the stronger alkali shifts the color towards the blue. The variations in color of the blue dipeptide complexes, with the alkalinity of the solvent, were not studied, spectrophotometrically, owing to the lack of opportunity, but we know that macroscopically there is no change of color, with weakly or strongly alkaline solution. In the amino-acid blue complexes, there is no visible change with weak alkali, but in strong alkali most of the copper is precipitated.[2]

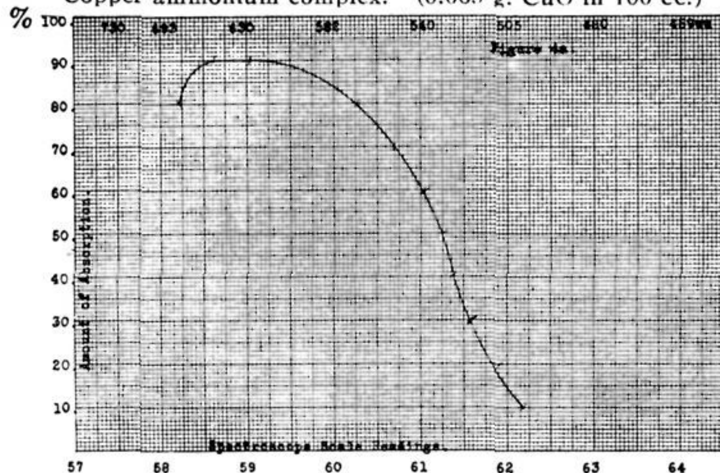
From this we can safely conclude that each class of complexes, or at least each complex, has a certain concentration of OH ions at which the maximum development of red color is obtained.

The results give three types of absorption curves: (1) *Blue complexes:* the absorption beginning at about 480 $\mu\mu$ and reaching a number maximum at 630 $\mu\mu$, as in curves, 4*a*, 4*b*, 4*c* and those marked "neutral" in 5*a*, 5*b*, 5*c*, 6*a*, 6*b*, 6*c* and 7*a*. (2) *Purple or semi-biuret complexes:* absorption beginning at about 459 $\mu\mu$ and reaching a maximum at 540 $\mu\mu$, as in curves marked "buffer" in 5*a*, 5*b*, 6*a* and 6*b*. (3) *Red or biuret com-*

[1] See Hantzsch, Ley and others, *Loc. cit.*

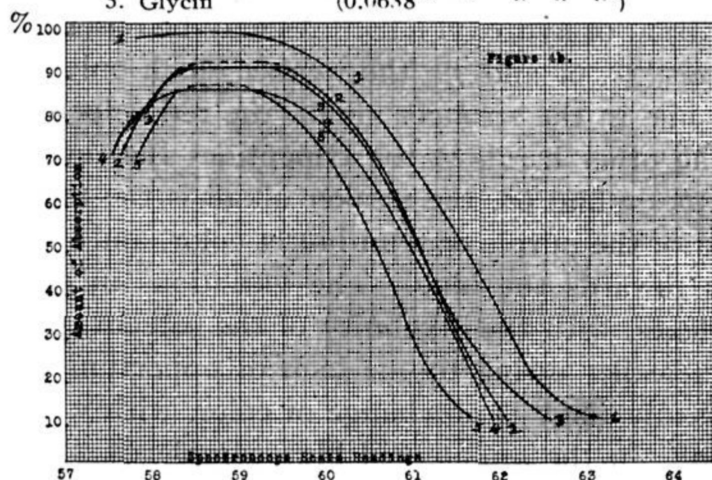
[2] Kober and Sugiura, THIS JOURNAL, **35**, 1572 (1913), showed that the stability, or inversely the precipitability, of the complexes, under certain conditions is in the same order; tetrapeptide complexes precipitate Cu on an average 9.2%; tripeptides, 10.8%; dipeptides, 17.3%; amino acids, 86.5%.

Copper ammonium complex. (0.063 g. CuO in 100 cc.)



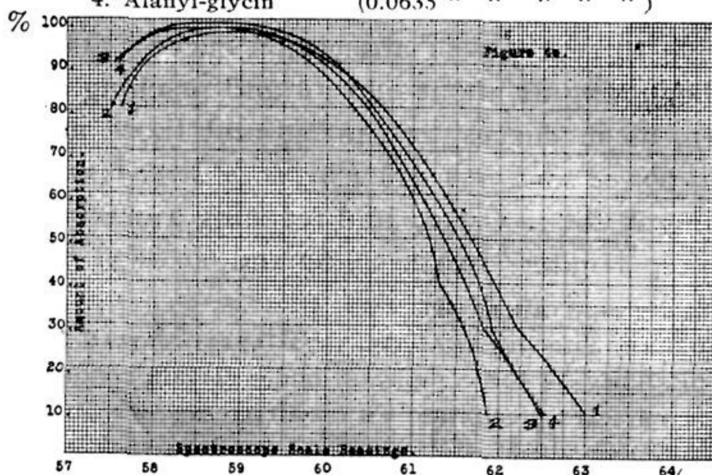
Amino acid copper complexes.

1. Histidin di-HCl (0.0637 g. CuO in 100 cc.)
2. Glutamic acid (0.0633 " " " " ")
3. *Aspartic acid* (0.0617 " " " " ")
4. Alanin (0.0635 " " " " ")
5. Glycin (0.0638 " " " " ")



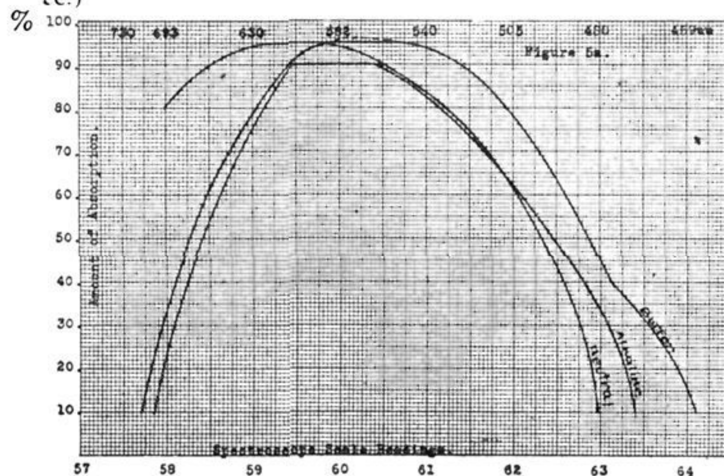
Dipeptide copper complexes.

1. Glycyl-tryptophane (0.0637 g. CuO in 100 cc.)
2. Glycyl-leucin (0.0640 " " " " ")
3. Amino-butyl-glycin (0.0636 " " " " ")
4. Alanyl-glycin (0.0635 " " " " ")

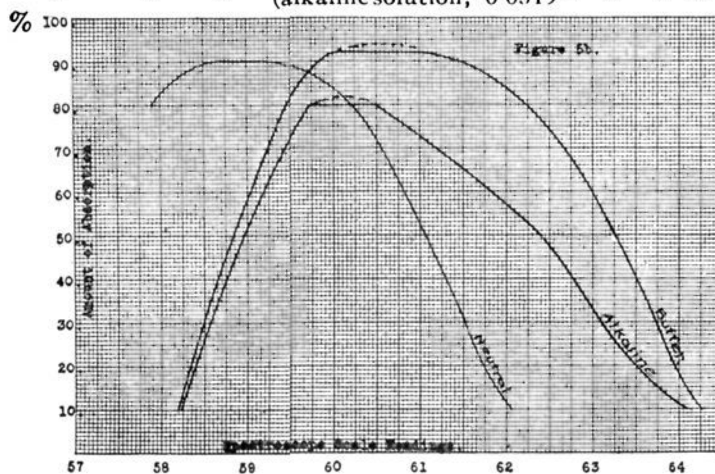


(1) *Tripeptide complexes.*

Alanyl-glycyl-glycin copper (neutral solution; 0.0640 g. CuO in 100 cc.)
Alanyl-glycyl-glycin copper (buffer solution; 0.0316 g. CuO in 100 cc.)
Alanyl-glycyl-glycin copper (alkaline solution; 0.0316 g. CuO in 100 cc.)

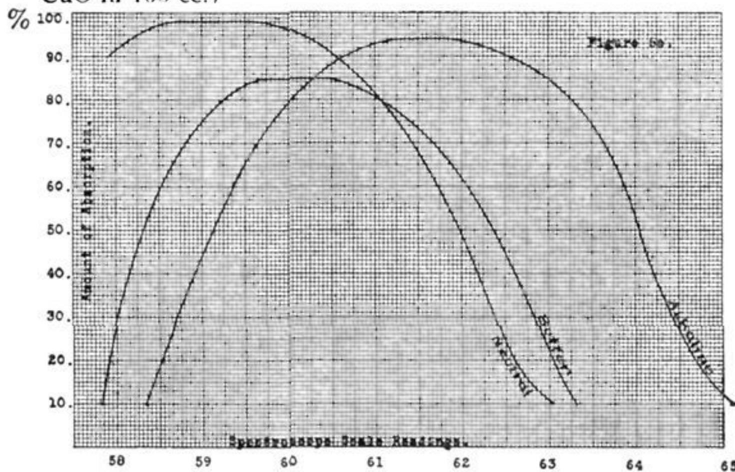


Di-glycyl-glycin copper (neutral solution; 0.0527 g. CuO in 100 cc.)
" " " (buffer solution; 0.0319 " " " " ")
" " " (alkaline solution; 0.0319 " " " " ")



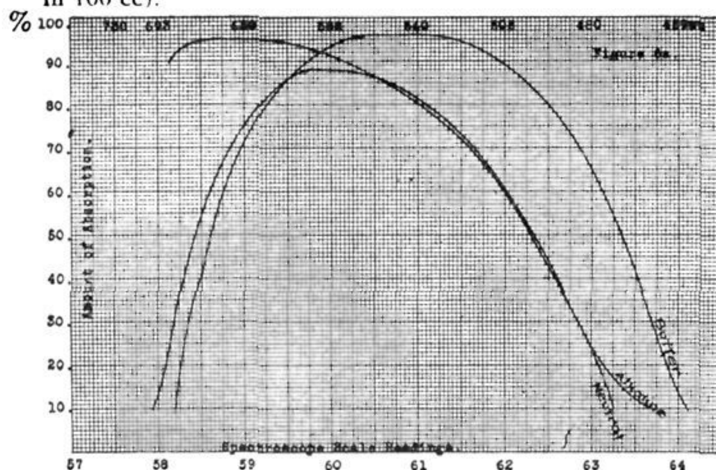
(2) *Tetrapeptide complexes.*

N-amino-caproyl-di-glycyl-glycin copper (neutral solution; 0.0629 g. CuO in 100 cc.)
N-amino-caproyl-di-glycyl-glycin copper (buffer solution; 0.0311 g. CuO in 100 cc.)
N-amino-caproyl-di-glycyl-glycin copper (alkaline solution; 0.0310 g. CuO in 100 cc.)

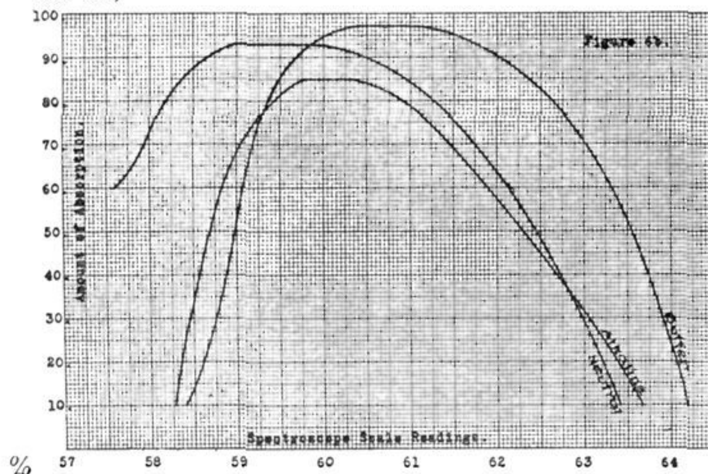


(1) *Tripeptide complexes.*

Amino-butyl-glycyl-glycin copper (neutral solution; 0.0633 g. CuO in 100 cc.)
Amino-butyl-glycyl-glycin copper (buffer solution; 0.0317 g. CuO in 100 cc.)
Amino-butyl-glycyl-glycin copper (alkaline solution; 0.0317 g. CuO in 100 cc).

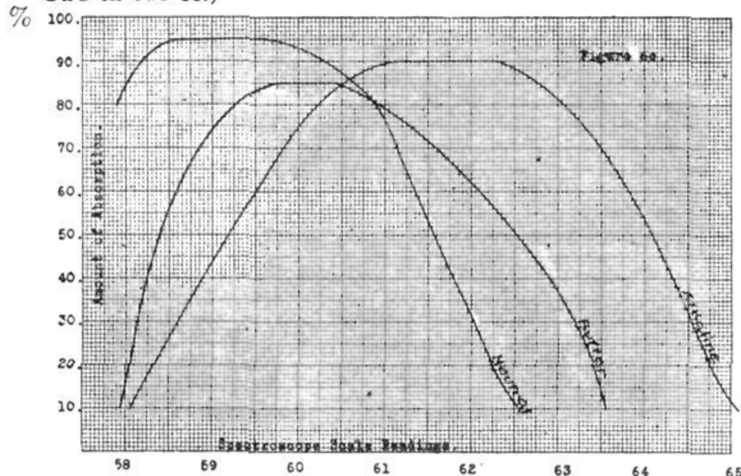


Leucyl-alanyl-glycin copper (neutral solution; 0.0639 g. CuO in 100 cc.)
Leucyl-alanyl-glycin copper (buffer solution; 0.0311 g. CuO in 100 cc.)
Leucyl-alanyl-glycin copper (alkaline solution; 0.0318 g. CuO in 100 cc.)



(2) *Tetrapeptide complexes.*

Amino-butyl-di-glycyl-glycin copper (neutral solution; 0.0634 g. CuO in 100 cc.)
Amino-butyl-di-glycyl-glycin copper (buffer solution; 0.0318 g. CuO in 100 cc.)
Amino-butyl-di-glycyl-glycin copper (alkaline solution; 0.0319 g. CuO in 100 cc.)

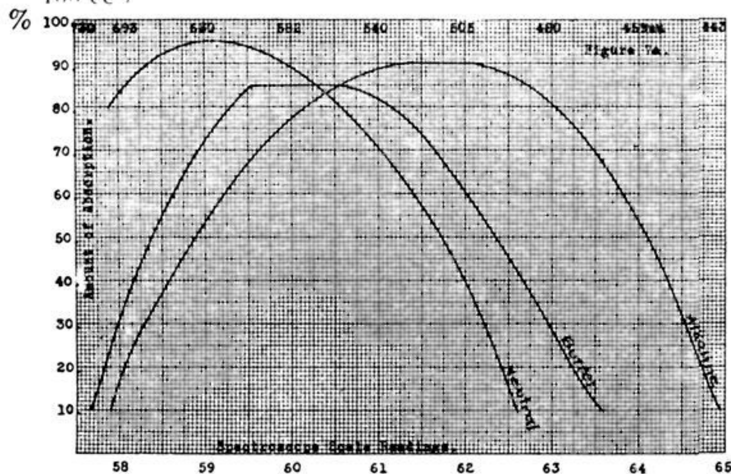


(2) *Tetrapeptide complexes.*

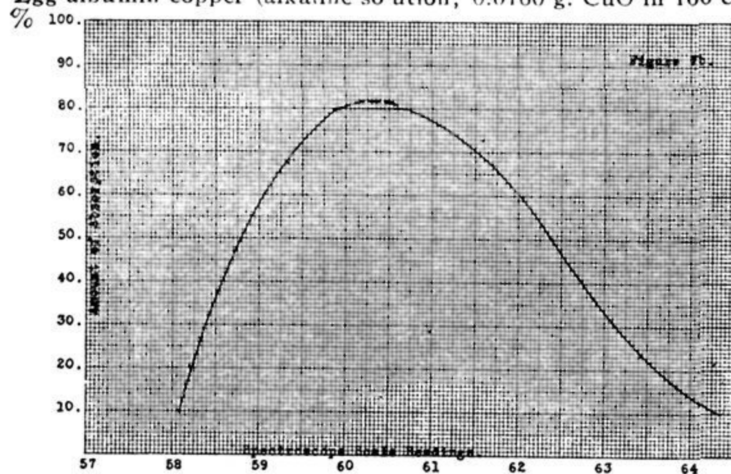
Alanyl-di-glycyl-glycin copper (neutral solution; 0.0630 g. CuO in 100 cc.)

Alanyl-di-glycyl-glycin copper (buffer solution; 0.0316 g. CuO in 100 cc.)

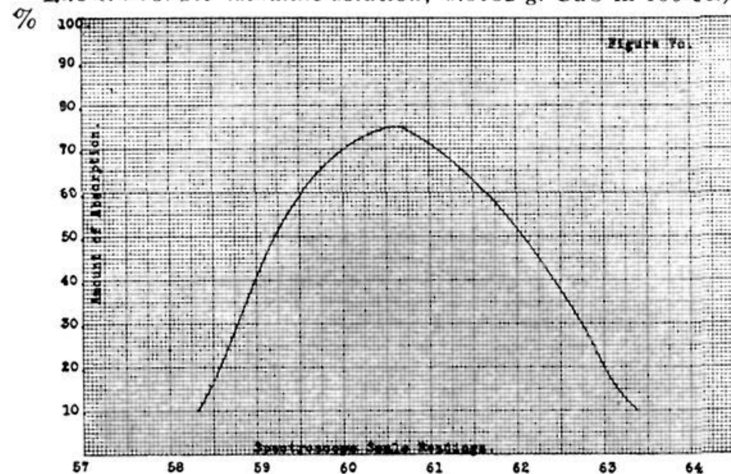
Alanyl-di-glycyl-glycin copper (alkaline solution; 0.0317 g. CuO in 100 cc.)



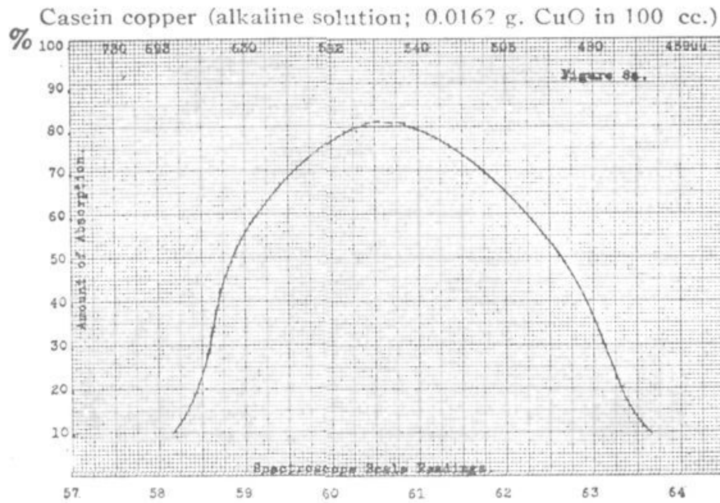
Egg albumin copper (alkaline solution; 0.0160 g. CuO in 100 cc.)



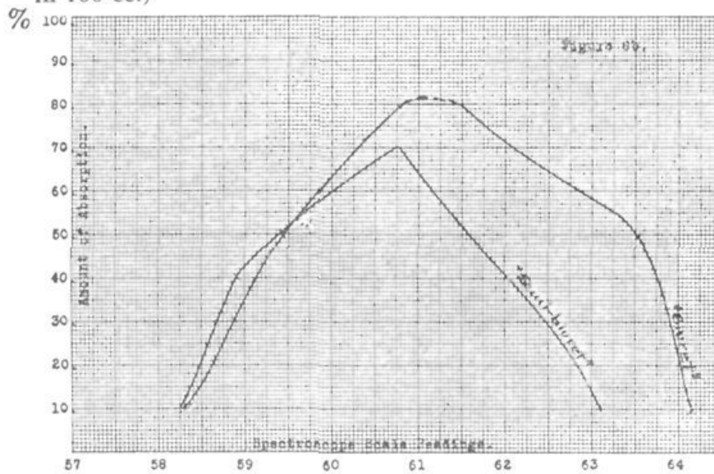
Edestin copper (alkaline solution; 0.0162 g. CuO in 100 cc.)



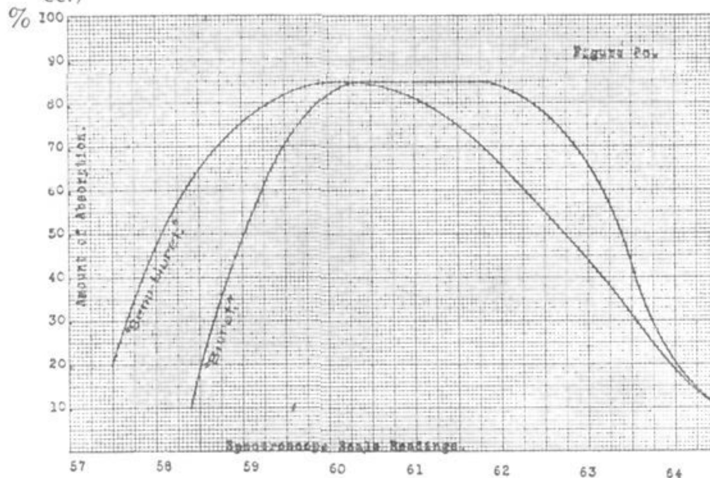
Casein copper (alkaline solution; 0.0162 g. CuO in 100 cc.)



Witte peptone with copper ("semi-biuret") (alkaline solution; 0.0166 g. CuO in 100 cc.).
Witte peptone in excess ("biuret") (alkaline solution; 0.0162 g. CuO in 100 cc.)



Cu(biuret)$_2$ ("biuret") (alkaline solution; 0.0628 g. CuO in 100 cc.)
Cu(biuret)$_1$ ("semi-biuret") (alkaline solution; 0.0628 g. CuO in 100 cc.)



plexes: absorption beginning beyond 443 $\mu\mu$ and reaching a maximum at 505 $\mu\mu$, as in curves marked alkaline in 5*c*, 6*c* and 7*a*, 8*c*.

The results with diglycyl-glycine also show that in the "buffer" solution the semi-biuret color is as strong as with the other tripeptides, corroborating spectrophotometrically a former observation.[1] Therefore, E. Fischer's statement that this peptide does not give a biuret reaction and is an exception to the other tripeptides, is shown to be incorrect. This error is a result of the fact that he used and recommended strong alkali for biuret reactions. *No doubt negative biuret reactions with other substances, which theoretically ought to be positive, are often due to unfavorable amounts of alkali used.*

Although there are variations due to unfavorable alkalinity and to some extent to impurities, yet the results show three types and only three types of absorption.

Biuret Reaction of Proteins.—In 7*b*, 7*c*, 8*a* and 8*b*, the absorption curves of a number of protein complexes are shown. Similarly as with the peptides, a suitable amount of protein was treated with an *excess of cupric hydroxide*, but in a strongly alkaline solution. After filtering and estimating the copper, the absorption was determined.

As the results show, the curves are practically the same as were obtained with the tripeptides, except the apparent absorption is less. On calculating the molecular absorption coefficient, however, for wave length 540 $\mu\mu$, the amount of absorption with the alkaline protein complexes is found to be about the same as with the tripeptide complexes in "buffer" solution, *i. e.*, they give a semi-biuret color.

TABLE I.

	% Absorption.	Coefficient.		% Absorption.	Coeff.
Alanyl-glycyl-glycine	94	122	Casein	79	136
Diglycyl-glycine	93	116	Egg albumin	77	128
Leucyl-alanyl-glycine	97	152	Edestine	71	108
Amino-butyl-glycyl-glycine	96	140			
Av.,	95	133		76	124
			Peptone (semi-	64	188
			(biuret)	80	140

With Witte peptone, the results are less certain, as the substance is a crude and colored mixture, and may contain interfering substances. It will also be observed that an error of 1% in the measurement of absorption at these points is multiplied from 4 to 10 times in the molecular absorption coefficient. The greatest accuracy in determining the coefficient, is obtained by keeping the absorption at 50%.

These results support, if they do not prove two points: (1) *that the so-called "biuret reaction," a test used for the past half century for detecting*

[1] THIS JOURNAL, **35**, 1571 (1913).

proteins qualitatively, is no other than a complex formation with copper and, therefore, as far as color formation is concerned, no decomposition of the protein is involved.[1] (2) That the protein configurations are such that permit only *3* nitrogen groups to form rings with copper; and, therefore, the protein molecule must be aggregated, and is not in the form of long free chains or branches of peptides or conjugated amino acids. This is supported by other facts; Kober and Sugiura[2] showed that these native proteins give no test for free amino acids, and Van Slyke and Birchard[3] showed that most of the amino groups—and, therefore, also the carboxyl groups—are conjugated.

The biuret reaction made in this way, *i. e.*, saturating the protein substance with copper, and determining the nature and the amount of absorption as well as the amount of copper combined, may increase the value of the copper technic[4] in studying the constitution of proteins and their derivatives. Thus, for example, it may be possible by estimating the absorption at wave lengths which are characteristic of each type, to determine in mixtures how much of each type is present. For example (1) wave length 693 $\mu\mu$ possibly could be used for estimating the amount of blue or dipeptide complexes. (2) Wave length 560 $\mu\mu$ could possibly be used for both the purple (tripeptide) and the red (tetrapeptide), while (3) wave length 455 $\mu\mu$ might be used for the latter alone. Subtracting the amount of copper found by (3) from that found with (2), would give the amount of tripeptide complexes.

Some Evidence For these Configurations.—It is reasonably certain, that the direct cause of these red color reactions with copper has nothing to do with the hydrocarbon part of the substance, but is due either to the oxygen or nitrogen groups, or to both. It is of utmost importance for practical purposes, aside from the general interest in these complexes, to know to what groups in the substance this coloration is due. The amount of color in the biuret or semi-biuret complexes is about 13 times as strong as the color of ordinary copper salts, such as the blue sulfate.

If the color were due to the oxy or hydroxy groups alone, would this agree with the following facts? (1) That copper compounds of substances containing no nitrogen, but oxy or hydroxy groups, such as glucose, fructose, lactose, galactose, maltose, glycerol, and lactic acid *are quantitatively* and tartaric, citric, and oxalic acid, *from 50 to 90% decomposed*, in a weakly alkaline solution[5] in which all complexes of amino acids, peptides and other

[1] The strong alkali usually used for making this test does no doubt cause hydrolysis of the protein, but this is purely incidental.

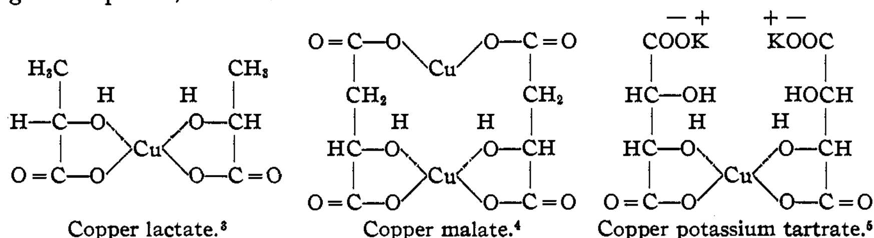
[2] This Journal, 35, 1580 (1913).

[3] *J. Biol. Chem.*, 16, 539 (1914).

[4] This Journal, 35, 1563–4 (1913).

[5] Kober and Sugiura, *Ibid.*, 35, 1562 (1913).

protein derivatives are *perfectly stable.*[1] Similarly that stability of these oxy and hydroxy complexes in strong alkali[2] is not at all comparable to those of the peptide or protein complexes. (2) That thus far no red colored complexes of copper, in aqueous solutions, have ever been found containing only oxygen groups. The specificity accorded heretofore to the biuret reaction for proteins is in harmony with that fact. (3) That a number of copper complexes of oxy or hydroxy substances, which have been studied and which have the same possible ring configuration as these nitrogen complexes, are *blue.*



Copper lactate.[3] Copper malate.[4] Copper potassium tartrate.[5]

To sum up: *oxy or hydroxy complexes of copper, no matter what configuration they may possess, are all bue or green, and never red, and are characterized by their relative instability in alkaline solutions.*

That the red colors are due to nitrogen groups, the following facts tend to prove: (1) Substances possessing *no oxygen and only nitrogen* give a red biuret reaction, *e. g.*, diguanidine copper.[6] (2) Complexes containing *a deficiency of oxygen groups* (more than two groups whether it be oxygen or nitrogen, are necessary for these biuret colors) but sufficient nitrogen groups, give a red biuret reaction, as for example dicyanodiamidine copper,[7] semi-biuret copper,[8] 21 crystalline complexes of Tschugaeff,[9] and others, all of which contain two oxygen and three or four nitrogen groups. (3) Finally, *the great parallelism between the number of nitrogen groups available for the combination with copper, and the red colors;* that the amount of red color in a complex is a function, as is shown in this paper spectrophotometrically, of the number of nitrogen groups—up to four—attached to the copper atom. Thus 68 amino acids and peptides, 21 chloro and bromo derivatives, studied by Kober and Sugiura, 25 amino substances prepared by Emil Fischer and collaborators (not included by K. and S.),

[1] THIS JOURNAL, **35**, 1551–7 (1913).

[2] *Ibid.*, 1563 and 1572.

[3] Paul Calame, *Z. physik. Chem.*, **27**, 401–20 (1908).

[4] *Ibid.*

[5] Kahlenberg, *Z. physik. Chem.*, **17**, 590 (1895).

[6] Tschugaeff, *Ber.*, **40**, 1977 (1907).

[7] Tschugaeff, *Ibid.*, **40**, 1977; **38**, 2904; **37**, 1480.

[8] Kober and Sugiura, *Am. Chem. J.*, **48**, 402 (1912); Ley and Werner, *Ber.*, **46**, 4040 (1913).

[9] Tschugaeff, *Loc. cit.*

21 complexes of Tschugaeff mentioned above *give 135 different complexes which seem to be in harmony with this theory of biuret reaction.* This list is by no means complete.

Having thus shown what seems fairly conclusively, that the red color of these copper complexes is due to the presence of nitrogen groups alone, the next step is to find what the other details of these complexes are, and how the complexes are formed. Since experimental evidence on all points is not available, and on some points evidence is wholly lacking, our discussion on these questions, as well as our reply to recent criticism will be reserved for a subsequent paper.

V. Summary.

1. The absorption in the visible spectrum, of copper complexes of amino acids, peptides and proteins was studied quantitatively, in neutral and alkaline solutions.

2. From the nature and the amount of absorption, considerable support was found for the theory of biuret reaction proposed by Kober and Sugiura.

3. The biuret reactions with proteins seems to be nothing more than ring formations of the copper with the nitrogen groups.

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THE PHOSPHORIC ACID IN STARCH.

BY JOHN H. NORTHROP AND J. M. NELSON.

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Up to the present time no definite evidence has been brought forth as to whether the phosphoric acid present in starch is in chemical combination or not. Ford,[1] Fernbach,[2] Fernbach and Wolff,[3] Malfitano,[4] Malfitano and Moschkoff,[5] Fouard,[6] Thomas,[7] Grewzewska[8] and others, employing a variety of methods such as precipitation with alcohol, acetone, freezing or dialysis, etc., all were unable to completely free the starch from its accompanying phosphorus.

Samec,[9] seems to have shown quite conclusively that the starch granules are not homogeneous and that the phosphorus is associated with the exterior of the grains (amylopectin). He further states that the phosphoric acid and the amylopectin are chemically combined in the form of an amylophosphoric acid, since the only way to account for the decrease

1 *J. Soc. Chem. Ind.*, **23**, 414 (1904).

2 *Compt. rend.*, **138**, 428 (1904); **155**, 617 (1912).

3 *Ibid.*, **140**, 1403 (1905).

4 *Ibid.*, **143**, 400 (1906).

5 *Ibid.*, **150**, 711 (1910).

6 *Ibid.*, **144**, 501 (1907); **146**, 285 (1908).

7 *Biochem. Bull.*, **3**, 407 (1914).

8 *Compt. rend.*, **152**, 785 (1911).

9 *Kolloidchem. Beihefte*, **3**, 123 (1911); **4**, 133 (1912); **5**, 141 (1913); **6**, **23** (**1914**).