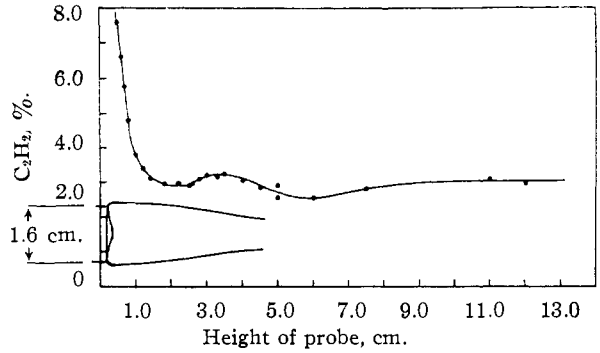

Fig. 1.—Variation of acetylene concentration with height above burner port compared to an outline of the inner cone.

of acetylene in the cooled products. The results are given in Table I.

TABLE I

Composition of feed gas, methane, %	Feed gas flow rate, cc./sec.	Composition dry off gas, C_2H_2, %	Dry off gas flow rate, cc./sec.	Methane converted to acetylene, %
56.3	45.3	2.67	50.4	5.30
56.55	45.05	3.05	50.0	5.92
57.05	44.7	3.60	48.6	6.90

Discussion

On the basis of an equilibrium calculation of the products of a flame of 56.9% methane the amount of acetylene formed should be vanishingly small; therefore, it is evident from the relatively large amounts of acetylene that are found even in the cooled products that equilibrium with the elements is never established in the flame.

The sharp drop in acetylene concentration which occurs as the probe is raised is considered to be due to the decomposition of the acetylene formed in the combustion zone to give solid carbon and hydrogen. The subsequent rise in acetylene concentration is due to a competing reaction which is not clearly understood. It is doubtful if this rise is due to spurious effects of the probe, since the time taken to withdraw the sample varied widely with the diameter of the probe orifice without seeming to affect the reproducibility of the results. The second minimum is also unexpected, but the most reasonable explanation is that further reactions involving acetylene are taking place at quite low temperatures.

The author wishes to thank Dr. Richard S. Brokaw for his assistance and advice during the progress of the research.

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Interactions of Metal Ions with the Sulfhydryl Group of Serum Albumin

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RECEIVED MAY 2, 1952

It has been shown by Hughes[1,2] that the sulfhydryl group of serum albumin combines with mercuric ion and by Benesch and Benesch[3] that it forms complexes with silver. It seems pertinent to describe some experiments which demonstrate that other metals such as copper, zinc, cadmium and lead also form albumin mercaptides. These interactions occur even under acidic or neutral conditions where –SH groups may compete with other side chains of albumin, such as those of histidine, for metallic ions.[4]

In aqueous solutions, between *p*H 5 to 8, containtaining 0.2 *M* sodium acetate or sodium nitrate, an unusual absorption band appears at 375 mμ when bovine serum albumin[5] is added to cupric ions[6] (Fig. 1). It has been possible to prove that a copper–sulfhydryl interaction is responsible for this

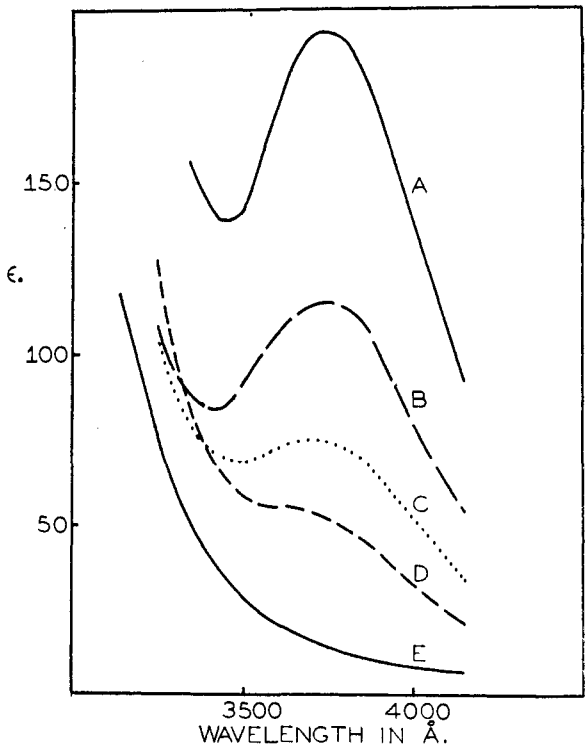

Fig. 1.—Effect of various metallic ions on absorption of copper–albumin complex: A, 0.003 *M* Cu^{++} and 0.0003 *M* bovine serum albumin, *p*H 6.0; B, same as A but with 0.003 *M* Zn^{++}; C, same as A but with 0.003 *M* Cd^{++}; D, same as A but with 0.003 *M* Pb^{++}; E, same as A but with 0.0003 *M* Hg^{++}. Solution A was made up to 0.2 ionic strength with sodium nitrate; the peak is slightly lower if sodium acetate is used. Solutions B to E were made up to 0.2 ionic strength with sodium acetate. The molecular extinction coefficients, ε, were calculated from the equation: $\log I_0/I = \epsilon cd$, where I_0 is the intensity of the light emerging from the pure protein solution, I the intensity of the light emerging from the metal ion–protein solution, c the molar concentration of the metal ion, and d the cell thickness in centimeters.

(1) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1836 (1947).

(2) W. L. Hughes, Jr., *Cold Spring Harbor Symposia on Quantitative Biology*, **14**, 79 (1950).

(3) R. Benesch and R. E. Benesch, *Arch. Biochem.*, **19**, 35 (1948).

(4) E. Barbu, J. Lessiau and M. Macheboeuf, *Bull. soc. chim. Biol.*, **31**, 1254 (1949), have shown that treatment of proteins with strong alkali produces sulfhydryl groups which then combine with copper.

(5) This band has not been found with the samples of human serum albumin at our disposal.

(6) Attention should be drawn to the presence of an absorption band in this same region, as well as in the visible range, in the natural copper-protein, oxyhemocyanin.

peak by three experiments. (1) Addition of one mole of mercuric chloride per mole of albumin completely abolishes the 375 mμ band. (2) Addition of one mole of salyrganic acid, an organic mercurial which reacts with sulfhydryl groups,[7] completely abolishes the band. (3) Addition of one mole of silver nitrate removes the 375 mμ band almost completely. In view of the fact that serum albumin has been shown to have slightly less than one sulfhydryl group[1-3] per protein molecule, and since this –SH group reacts with mercurials as well as with silver ion before other side chains do,[2,3] it is clear that the disappearance of the copper absorption band at 375 mμ must be due to displacement of cupric ion from a mercaptide linkage.

With the establishment of the copper–sulfhydryl linkage, the 375 mμ band can be used as an indicator for the detection of other protein mercaptides. By means of this method, it has been found that one mole of Zn^{++} per mole albumin produces a detectable displacement of Cu^{++} from its sulfhydryl linkage, 10 moles of Zn^{++} a reduction of approximately 30% in the absorption at 375 mμ (Fig. 1) and 100 moles of Zn^{++} a reduction of about 80%. Even 100 moles of Zn^{++}, however, causes a drop of only a few per cent. in the intensity of the copper–albumin absorption near 700 mμ.[8] Thus Zn^{++} is not very effective in displacing Cu^{++} from side chains on the protein other than –SH.

Similar experiments have been carried out with Cd^{++} and with Pb^{++} as competing ions (Fig. 1). These indicate that the order of affinity for the sulfhydryl group of bovine albumin is: $Pb^{++} > Cd^{++} > Zn^{++}$.

Substantial formation of the zinc mercaptide occurs at a total Zn^{++} concentration as low as 0.003 *M*, even when this cation is competing with copper at an equivalent concentration. Interactions of zinc with the sulfhydryl group of albumin would not be distinguishable in equilibrium–dialysis experiments from binding by other side chains. Binding constants calculated on the assumption that only specified residues of the protein are active must take into account the preferential formation of the metal mercaptide.

These investigations were assisted by grants from the Office of Naval Research (Project No. NR124-054) and from the Carnation Company.

(7) The use of this compound was suggested by Dr. R. Benesch.

(8) I. M. Klotz and H. A. Fiess, *J. Phys. Colloid Chem.*, **55**, 101 (1951).

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Amino Acid Contamination in Preparations of Hog Blood Group Substances[1]

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RECEIVED MAY 12, 1952

In a previous communication,[2] some observations on the amino acid content of the dialyzable portion of mild acid hydrolysates of blood group substances were reported. Application of the chromatographic procedures of Sanger[3a,b] and Blackburn[4] to the dinitrophenyl (DNP) derivatives of the amino acids in the dialysate showed the presence of 0.07 to 0.7% free amino acids consisting of aspartic acid, glutamic acid, lysine, serine, threonine and glycine which were thought to represent a portion of the blood group substance proper. More recent studies have shown the necessity for re-evaluating this work.

In an effort to determine the dependence of the previously reported amino acid liberation on *p*H and time of hydrolysis, portions of a hog blood group substance were heated at *p*H 1.5, 1.9 and 2.3 for various periods of time. The free aspartic acid in the dialysate was found to reach a maximum value of around 0.2 mg. per 100 mg. of blood group substance for all three *p*H's. However, almost 0.1 mg. of aspartic acid per 100 mg. of the blood group substance was found in the control experiments in which the materials were dialyzed directly without being subjected to acid *p*H or heating, thus indicating the presence of substantial amounts of amino acid impurities. This was further borne out in the behavior of the other five amino acids which also appeared in significant amounts in the control dialysates.

A reinvestigation of several hog materials used in previous work[2] showed that they too were contaminated to the extent of about 0.4% with the same six free amino acids.

Thus all previous results are subject to considerable error depending on the extent of contamination of the particular substance with free amino acids. Moreover, the bulk of the 22–25% of amino acids known[5,6] to be present in the blood group substances is not liberated by such mild hydrolysis although the blood group activity is destroyed. More work will be necessary before the role of the amino acids in the structure of the blood group substances can be elucidated.

(1) Aided by grants from the United States Public Health Service and the William J. Matheson Commission.

(2) H. Van Vunakis and E. A. Kabat, THIS JOURNAL, **73**, 2977 (1951).

(3) (a) F. Sanger, *Biochem. J.*, **39**, 507 (1945); (b) R. R. Porter and F. Sanger, *ibid.*, **42**, 287 (1948).

(4) S. Blackburn, *ibid.*, **45**, 379 (1949).

(5) K. Landsteiner and R. A. Harte, *J. Expll. Med.*, **71**, 551 (1940).

(6) W. T. J. Morgan and H. K. King, *Biochem. J.*, **37**, 640 (1943).

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Mannich Reactions Involving the Use of Acetaldehyde, Monochloroacetaldehyde and Dichloroacetaldehyde[1]

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RECEIVED JUNE 23, 1952

Mannich and co-workers[3] have described the condensation product obtained from dimethylamine hydrochloride, formaldehyde and acetaldehyde. They showed that an aldol condensation had ac-

(1) Published with the approval of the Monograph Publications Committee, Oregon State College, as Research Paper No. 209, School of Science, Department of Chemistry.

(2) This article is based on a thesis submitted by William D. Schaeffer in partial fulfillment of the requirements for the degree of Master of Science at Oregon State College, June, 1952.

(3) C. Mannich, B. Lesser and F. Silten, *Ber.*, **65**, 378 (1932).