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.

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

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

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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.

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

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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-

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(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.

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