

A preparation made from the small intestine of the dog contained almost as much histaminase as did kidney powder, while analogous preparations from the brain, spleen, and lung showed no oxygen uptake with histamine after 3 to 4 hours' observation. Kidney powder from the guinea pig, cat, human (obtained 2 hours after death), rat, and chicken with histamine showed such slight oxygen consumption that they were not adequate as a source of histaminase in these experiments. Fresh dog kidney in the form of a 200 mg. chunk showed about a 50% increase in oxygen consumption when 1 mg. of histamine was added. Freshly ground up kidney gave a much smaller histaminase effect.

An attempt to split histamine on a blood-charcoal model with the uptake of oxygen was unsuccessful.† Histidine, however, had a definite oxygen uptake on blood charcoal in phosphate buffer solution. Warburg and Negelein<sup>3</sup> in a similar type of experiment have observed the splitting of cystine, leucine, and tyrosine with an uptake of oxygen.

The manometric measurements reported above agree with the physiological and chemical observations of Best and McHenry in most respects. However, they observed (1) decreased inactivation of histamine at pH 10, (2) that the optimum inactivation of histamine occurred at 37° with a destruction of the inactivating enzyme at 60°, and (3) complete inhibition of the inactivating substance with KCN at 0.004, 0.002, 0.001, and 0.0005 molar concentrations.

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### Reactions of Proteins in Liquid Ammonia with Metallic Sodium.

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(Introduced by C. J. Farmer.)

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McChesney and Miller<sup>1</sup> introduced the use of liquid ammonia as a medium for the study of the chemical properties of proteins. They found that proteins were acidic in liquid ammonia and that when they were treated with alkali metals at  $-33.5^{\circ}$  C. or with ammono

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† Choline, acetylcholine, neurine, tyramine, as well as betaine, showed no gas change with the charcoal model.

<sup>3</sup> Warburg, O., and Negelein, E., *Biochem. Z.*, 1921, **113**, 257.

<sup>1</sup> McChesney and Miller, *J. Am. Chem. Soc.* (1931), **53**, 3888.

bases at 80-120° C. for 48-72 hours, they are partially ammonolyzed. We have reported<sup>2</sup> a study of the liberation of hydrogen when sodium is added to liquid ammonia solutions or suspensions of silk fibroin, diketopiperazine, glycyl-alanine, n-methylacetamide, glycollic acid, acetanilide, tyrosine, and alanine. This report includes the results of further investigation on silk fibroin, diketopiperazine, and dipeptides, and investigations on casein, egg albumin and edestin. A chemical examination of the products formed will be carried out later.

When the quantity of hydrogen liberated by a sample of protein, such as silk-fibroin\* and egg albumin having a definite nitrogen content is plotted against the quantity of sodium added, the curve may be divided into 3 distinct parts.

The first part of the curve is characterized either by absorption of sodium by the protein or by the efficient absorption of hydrogen by one part of the protein molecule following the liberation of hydrogen by sodium from another part of the molecule, or both. Any of these events involves reduction of some part of the protein molecule. When 2 sodium atoms are added for each 4 nitrogen atoms, the reduction is equivalent to the addition of 2 hydrogen atoms. The second part of the curve covers a range in which the quantity of hydrogen liberated approximately parallels the quantity of sodium added. The third part of the curve is characterized by the condition where all of the available hydrogen is liberated by the sodium and the protein is saturated with respect to the sodium. The point where the second and third parts of the curve join represents a condition in which 5 sodium atoms have been added for each 4 atoms of nitrogen in the sample and 2 atoms of hydrogen have been formed. The total extent of the reduction of the protein is equivalent to the absorption of 3 hydrogens for 4 atoms of nitrogen. While the curves for silk fibroin and casein are not identical, they have the same general form. Our data for egg albumin and edestin indicate that their curves will have a similar form.

These observations raise 2 questions: (1) What is the origin of the hydrogen that is liberated? and (2) What part of the protein molecule is reduced? The carboxyl groups in a protein molecule are acidic and would be one origin of hydrogen, but they are of minor importance, for the acidity of silk fibroin does not indicate

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<sup>2</sup> Presented before the Organic Division, Am. Chem. Soc., Indianapolis, March, 1931.

\*The silk fibroin was furnished to us by Cheney Brothers, South Manchester, Connecticut.

that there are two carboxyl groups for each 4 nitrogen atoms present. Since certain compounds containing the imide group are acidic in liquid ammonia, as for example n-methyl acetamide, it would suggest the possibility of the imide link in the protein or the diketopiperazine rings, if they are present, as the origin of the hydrogen. An investigation of the acid properties of glycylalanine and glycyglycine has shown that the imide links of these dipeptides are not acidic. Whether or not the imide links in a longer polypeptide chain would be acidic in liquid ammonia or more readily reduced is not known and must be determined. In the case of diketopiperazine, two molecules of diketopiperazine containing 4 imide links will decolorize a solution containing 4 atoms of sodium with the liberation of 0.9 of an atom of hydrogen. Diketopiperazine is, therefore, reduced by the sodium. When an equimolecular mixture of diketopiperazine and glycyglycine is treated with sodium, there is no vigorous reaction of the sodium with the hydrogen of the carboxyl group. For each 4 atoms of nitrogen in the mixture, 4 sodiums react to give one-fourth of an atom of hydrogen or less. If proteins contain diketopiperazine rings as Abderhalden has suggested, then they would be the part of the protein molecule that is reduced by the sodium.

More experimental work must be carried out before we can state definitely what part of the protein molecule furnishes the hydrogen and what part of the molecule is reduced.

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**Some Properties and Reactions of Carbohydrates in Liquid Ammonia.**

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An accurate insight into the intermediary metabolism of carbohydrates is dependent in part upon a knowledge of the structure of glycogen and blood sugar. Water has been and still is the most frequently used medium for the study of the chemical properties of the carbohydrates, particularly of the sugars. It occurred to us that liquid ammonia might also be a solvent for the sugars and a medium for the study of the chemical properties of the polysaccharides. Schmid