TABLE V.

Soil.	Free amido acid nitrogen.	Polypeptide nitrogen.	Soil.	Free amino acid nitrogen.	Polypeptide nitrogen.	Soil.	Free amino acid nitrogen.	Polypeptide nitrogen.
101	0.30	0.56	104	0.40	0.92	108	0.65	1.50
102	0.29	0.88	106	0.24	0.74	Peat	0.071	0.26
103	0.16	0.84	107	0.29	0.71			

Conclusions.

1. The amount of nitrogen precipitated from a neutralized alkali extract of soil varies, in a qualitative way, inversely with the strength of the acid.

2. The amount of humin nitrogen, as found by the Van Slyke method, extracted by dilute alkali from soil is very high when compared to the amounts in proteins.

3. Dilute alkali does not extract any typical class of organic compound from the soil.

4. Before definite conclusions are drawn from the remainder of the Van Slyke results it is believed that analyses on these same plots in future years should be awaited.

5. The amount of amino acid and peptide nitrogen in soil is found to be very small when compared to the amounts of amino acids formed by hydrolysis.

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A DISTURBING FACTOR IN BARFOED'S TEST.[1]

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Barfoed's test has been used extensively in biological chemistry for the detection of reducing monosaccharides. In this connection, it was used to show the difference in the chemical reaction between starch paste and hydrolytic products obtained from starch paste by boiling with dilute hydrochloric acid. It was found that when starch could no longer be detected with iodine it was impossible to obtain the typical glucose reaction with Barfoed's reagent, but that a greenish white precipitate was formed. All mineral acid had been removed from the solution of the hydrolytic products by neutralization with sodium hydroxide and the solution was subsequently made very faintly acid with acetic acid. These experiments seemed to indicate that sodium chloride was the substance that interfered with the reaction. In order to ascertain whether this

[1] Preliminary communication; *Proc. Am. Soc. Biol. Chem.*, **1909**, p. 180. Read before the Medical Research Club, University of Illinois, Chicago, Ill., June 9, 1915.

assumption is correct the following experiments were made: A solution of sodium chloride was boiled with Barfoed's reagent. A greenish white precipitate was formed. When sodium chloride was added to a solution of pure glucose and Barfoed's test was applied to the mixed solution, a greenish white precipitate was formed instead of the red cuprous oxide of the typical Barfoed's test. Since the first part of this work was done, Mathewson,[1] has shown that a great variety of inorganic, as well as organic compounds, interfere with this test.

Interfering Concentration of Sodium Chloride.—A series of tests was made to determine the concentration of sodium chloride which would interfere with the Barfoed's tests in various concentrations of glucose. The tests were carried out as follows: Five cc. of glucose solution, 4 cc. of sodium chloride solution and 1 cc. of Barfoed's reagent were measured into a test tube and the mixed solution was boiled for thirty seconds over a free flame. Table I shows the results of these experiments.

TABLE I.

Percentage concentration of sodium chloride.	Percentage concentration of glucose.					
	0.163.	0.312.	0.625.	1.25.	2.5.	5.
0.0156	Reduction and precipitate					
0.0312						
0.0625		Very faint reduct.				
0.125		Ppt.	Very faint reduct.	Reduct.	Reduct.	Reduct.
0.25			Ppt.			
0.5				Ppt.	Ppt.	Ppt.

In a second series of experiments the same amounts of the various solutions were used as in Table I but instead of heating the mixture over a free flame, it was heated in actively boiling water for two minutes. The results of these experiments are shown in Table II.

Delicacy of Barfoed's Test.

In a series of experiments to determine the delicacy of the reaction, it was found that when the test is performed with 1 cc. of Barfoed's reagent, 9 cc. of glucose solution and the mixture is heated in boiling water for two minutes, a concentration of 0.08% of glucose gives a very definite reduction and half that concentration gives a faint reduction.

The Barfoed's reagent used in this work was made according to the directions of Barfoed[2] in his original paper. Five cc. of 38% acetic acid were added to 200 cc. of a solution of copper acetate. The copper acetate was made up in the proportion of 1 part of salt to 15 parts of water.

[1] Mathewson, "A Study of Some of the Biochemical Tests," Dissertation, Columbia University, **1912.**

[2] Barfoed, *Z. anal. Chem.*, **12**, 27 (1873).

TABLE II.

Percentage conc. of NaCl.	Percentage concentration of glucose.										
	0.05.	0.1.	0.2.	0.4.	0.6.	0.8.	1.	2.	3.	4.	5.
0.01	V. f. r.										
0.02	V. f. p.	V. f. p.; F. r.	R. +++								
0.04			Ppt.								
0.06				F. p.; Marked r.							
0.08				V. f. r.	V. f. r.						
0.1				Ppt.	Ppt.	V. f. p.; R.	V. f. p.; R.	R. ++	R. ++	R. ++	
0.2								Ppt. +++	Ppt. +++	Ppt. ++	F. p.; F. r.
0.3											
0.4											
0.5											

V. f. r. = very faint reduction; V. f. p. = very faint ppt.

Influence of Sodium Chloride at Room Temperature.—A series of experiments was conducted to show what concentration of sodium chloride will cause a precipitate with Barfoed's reagent when allowed to stand at room temperature. The experiments were conducted as follows: Four cc. of sodium chloride solution were mixed with 1 cc. of Barfoed's reagent and the mixture was allowed to stand at room temperature for twenty-four hours. In these experiments a sodium chloride concentration of 0.32% gave a precipitate. With a concentration of 0.16% sodium chloride, no precipitate was formed.

Effect of Glucose on the Formation of the Greenish White Precipitate.—The next series of experiments was undertaken to determine whether glucose had any effect on the formation of this precipitate at room temperature. The volumes of the various solutions used in these experiments were the same as in Table I and II. The results are shown in Table III.

TABLE III.

Percentage concentration of sodium chloride.	Percentage concentration of glucose.				
	0.25.	0.3.	1.0.	2.5.	5.0.
0.1	Opalescence in 15 hours.				
0.2 to 0.9	Opalescence within two hours in all cases.				
1.0	Marked opalescence within a few minutes.				

Glucose seems to favor the formation of opalescent solutions (colloidal) as indicated by its appearance in a lower sodium chloride concentration than in the experiments without it.

Nature of Greenish White Precipitate.—The greenish white precipitate that is formed, contains copper, sodium, chlorine and the acetic acid

radical. When this compound is allowed to form at room temperature it tends to go into colloidal solution. Boiling hastens the precipitation. The percentage composition of this precipitate has not been determined.

Conclusions.

Under the conditions of these experiments it appears that a very small percentage of sodium chloride interferes with Barfoed's test.

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