

# SOIL SCIENCE

RUTGERS COLLEGE

---

VOL. III

NEW BRUNSWICK, N. J., APRIL, 1917

No. 4

---

## THE ORGANIC MATTER OF THE SOIL: V. A STUDY OF THE NITROGEN DISTRIBUTION IN DIFFERENT SOIL TYPES<sup>1</sup>

By

CLARENCE AUSTIN MORROW, *Professor of Chemistry, Nebraska Wesleyan University*, and ROSS AIKEN GORTNER, *Associate Professor of Agricultural Biochemistry, University of Minnesota*<sup>2</sup>

### INTRODUCTION

The nature of the organic nitrogen of the soil is of more than usual interest to chemists inasmuch as this nitrogen must pass through a specific cycle of processes before it can again become generally available for the higher plants. It is perfectly obvious that these processes may be extremely rapid or very slow, depending upon the manner in which the nitrogen atoms are bound in the organic compound. For example, the nitrogen of an acid amide or an amino acid may be regarded as relatively easily converted into ammonia in contrast with such nitrogen heterocycles as pyridine or quinoline, which are probably very difficultly convertible.

It occurred to the authors that, inasmuch as soils differ widely in ammonification power, etc., it might be well to make a comparative study of the distribution of nitrogenous compounds in different soil types. Such a study was begun in the fall of 1914. Although this is the last paper of the series on the organic matter of the soil to appear, it was the first paper which was planned and to a large extent the first which was completed. Because of this fact certain of the results indicated in the preceding papers of the series could not be applied in this investigation.

---

<sup>1</sup> Received for publication February 26, 1917.

<sup>2</sup> The work reported in this paper was carried out in the Division of Soils, Minnesota Agricultural Experiment Station, mainly during the winter of 1914-15, the authors being respectively Assistant in Soils and Associate Professor of Soil Chemistry. These data are also a part of those recorded in a thesis presented by C. A. Morrow to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

The organic matter of the soil, containing as it does animal and plant residues, living and dead bacteria, protozoa, and fungi, and the reaction products of each upon the others, is a mixture of organic compounds of an almost hopelessly complex nature. This extreme complexity is demonstrated by the variety of organic compounds which have been isolated from soils, mainly through the work of the Bureau of Soils of the United States Department of Agriculture. Approximately 39 compounds have been isolated directly from the soil.<sup>3</sup> Fifteen of these contain nitrogen, i. e., cytosine, xanthine, hypoxanthine, adenine, guanine, choline, creatinine, lecithin, trimethylamine, nucleic acid, tetracarbonimide, a picoline  $\gamma$  carboxylic acid, arginine, histidine and lysine. The latter six of these may be considered as derived from protein materials, but the remaining nine are probably of non-protein origin. However, from other considerations, it seems probable that the protein nitrogen of the soil may exceed all other forms of nitrogen. Potter and Snyder (30) found 20.48 per cent of the alkali-soluble nitrogen of a soil to be of non-protein nature.

#### HISTORICAL

The chemistry of soil nitrogen may to a large extent be considered as being the chemistry of protein undergoing hydrolysis. The isolation of a number of amino acids indicates that proteins are decomposed in the soil in much the same way as in acid hydrolysis or animal digestion. Just how far the cleavages have already gone in the soil previous to acid hydrolysis remains a matter of much work before definite conclusions can be drawn.

Walters (50) has reported the presence of certain decomposition products in the soil, presumably proteoses and peptones, resulting from either a partial hydrolysis of proteins or by the synthetic action of microorganisms. They represent stages of decomposition between that of true proteins and amino acids. Walters concludes "that proteins undergo hydrolytic decomposition in the soil in much the same way as in digestion by enzymes, acids or alkalies, in the laboratory." In an extensive examination of the nitrogen compounds of processed fertilizers, Lathrop (20) has reported the presence of certain protein-like substances similar to those described above.

In his studies on the chemical nature of the organic nitrogen in the soil, Jodidi (17) thought water would be preferable to either acids or alkalies for the purpose of extraction, since it would not be so liable to alter the organic nitrogenous materials. He found that the direct extraction of a soil by boiling with water for 10 hours removed only 2.92 per cent, and for 24 hours the highest amount removed from any soil was 9.96 per cent of the total soil nitrogen. Schmook (36), however, reports 19.10 per cent of the total nitrogen of a Laterite soil of Russia to be water-soluble.

---

<sup>3</sup> This does not include several which have been isolated from soil hydrolyzed with acids, etc.

The literature has been very thoroughly summarized by Potter and Snyder (27) in regard to the determination of ammonia in soils. Both their work and that of Jodidi (16) indicate that the amount of ammonia is small. Kelley and Thompson (19) in a study of some Hawaiian soils reached the conclusion that ammonia and nitrate nitrogen constitute but a small percentage of the total nitrogen, and that the nitrogen is very largely in organic combination.

It is known that only a small part of the soil nitrogen is dissolved by dilute acids, yet it has been shown by Kelley and Thompson (19) that 1 per cent hydrochloric acid dissolves some organic nitrogen, for in every instance the soils contained only about half as much ammonia nitrogen as the total nitrogen extracted by the acid.

In the soil studies of Potter and Snyder (28) they find that the nitrogen extracted by 1 per cent hydrochloric acid varied from about 1.2 to 2.3 per cent of the total nitrogen, except in the case of the peat, where it was only 0.67 per cent. Gortner (9), working with 8 mineral soils, finds a maximum of 4.18 per cent of the total nitrogen soluble in 1 per cent hydrochloric acid with an average of 3.17 per cent. In three peats he finds a maximum of 7.50 per cent with an average of 3.78 per cent, and in 5 samples of unchanged vegetable materials (oat straw, alfalfa hay, oak leaves, sweet fern leaves, and grass from a peat bog) he finds a maximum of 34.58 per cent with an average of 20.10 per cent. These findings would seem to indicate that in the transformation of vegetable materials into the true organic matter of the soil there is a fall in the proportion of the total nitrogen soluble in very dilute acids.

Shorey (37) published results of his investigations which gave the first definite knowledge of the nitrogen distribution in the soil. Working on an Hawaiian soil he applied the method proposed by Osborne and Harris (24) for classifying the decomposition products of proteins resulting from acid hydrolysis. The method is a modification of that proposed by Hausmann (13) and is in short as follows. After hydrolysis the excess of the mineral acid is removed by evaporation, and the nitrogen present as ammonia determined by distilling with an excess of magnesium oxide. After separating the magnesia precipitate from the remaining solution by filtration, the nitrogen was determined in the precipitate by the Kjeldahl method. The di-amino nitrogen in the filtrate was precipitated by phosphotungstic acid and determined by the method of Kjeldahl and the mon-amino nitrogen determined by difference.

He obtained in the acid solution 84.5 per cent of the total nitrogen in the soil, 52.3 per cent of which was found in the magnesia precipitate. This result is in striking contrast to those obtained by Osborne and Harris (24) working on pure proteins, where they found that the nitrogen

contained in the magnesia precipitate does not usually exceed 4 per cent of the total nitrogen and in most cases is very much less. The amount of nitrogen insoluble in the 12 per cent acids used in the digestion may be designated as "humin." The nitrogen in the magnesia precipitate has been designated by most investigators as "humin" nitrogen. The total humin nitrogen in the soil is then represented by the nitrogen in the magnesia precipitate plus that retained by the soil. On recalculation of his data it was found that the insoluble humin *in the soil* after hydrolysis amounted to 15.3 per cent of the total nitrogen, making a total humin nitrogen content of 59.1 per cent. This very high result of total humin nitrogen was undoubtedly due to the soil being hydrolyzed only 7 hours with a relatively low concentration of hydrochloric acid and the insoluble residue boiled the same length of time with sulfuric acid. Complete decomposition of the proteins probably did not take place in the dilute acids used in the short time that they were heated. As a result some proteoses and peptones were precipitated by the magnesium oxide, which would account for the high results.

Shorey (38) concluded that even though we might know much concerning the constitution of the compounds comprising the various groups isolated from protein by this method of analysis, we know nothing concerning their composition when isolated from soil, inasmuch as we are not dealing with a pure protein [*cf* also Gortner (7, 8, 10)].

The work of Suzuki (45) gives us further knowledge of the individual amino compounds formed in the decomposition of soil organic matter. He worked with three samples of humic acid, one obtained from Merck, origin unknown to Suzuki, one prepared from an unmanured soil, and one from a compost heap. After boiling each preparation for 10 hours with strong hydrochloric acid, the undecomposed residue was filtered off, washed, and the residue extracted twice in this manner with strong hydrochloric acid. He determined the amounts dissolved as amide, di-amino, and mon-amino acid nitrogen. From 65 to 75 per cent of the total nitrogen was dissolved by the hydrochloric acid and in the extract 41 to 62 per cent of the nitrogen was not precipitated by phosphotungstic acid. A sample of humic acid was twice extracted with concentrated acid and the residue analyzed. His results calculated on the ash free basis showed the residue to contain 64.11 per cent carbon, 3.35 per cent hydrogen, and 0.80 per cent nitrogen. The residue becomes lower in nitrogen<sup>4</sup>, hydrogen, and ash but richer in carbon as the hydrolysis is continued.

Detmer (5) pointed out that similar results were true in peat beds where the deposits remain undisturbed for years. He found that there is

---

<sup>4</sup> He stated that although the nitrogen content decreases, it is very difficult to remove entirely.

an increasing carbon and nitrogen content of the humus for varying depths. This is shown by the following table:

	Carbon	Hydrogen	Oxygen	Nitrogen
Brown peat, near the surface .....	57.75	5.43	36.02	0.80
Dark peat, 7 feet .....	62.02	5.21	30.67	2.10
Black peat, 14 feet .....	64.07	5.01	26.87	4.05

Suzuki (43, 45) made further studies on a 500-gm. sample of the humic acid obtained from Merck. It was hydrolyzed with concentrated acid and the solution obtained subjected to esterification and fractional distillation according to the method of Fischer (6). He obtained:

Alanine .....	2.39 gm.
Leucine .....	2.16 gm.
Alanine + aminovalerianic acid .....	0.11 gm.
Aminovalerianic acid .....	0.57 gm.
Proline (copper salt of active proline) .....	0.67 gm.
(copper salt of inactive proline) (?) .....	0.50 gm.
Aspartic acid .....	0.06 gm.
Impure aspartic acid (?) .....	3.16 gm.
Glutaminic acid .....	present
Tyrosine .....	trace
Histidine .....	trace
Ammonia .....	1.90 gm.
Copper salts of unknown acids .....	30.30 gm.

As these compounds are typical protein decomposition products, his work proves that the humic acid examined by him was either of a protein nature, a mixture of protein decomposition products, or probably both together with some compounds as yet unknown. Unfortunately, the origin of the acid was unknown to Suzuki, but he states that it was probably prepared from peat.

From a study on Michigan peat soils, Jodidi (16) has concluded that the bulk of the organic nitrogen is made up of acid amides, di-amino acids, and mon-amino acids. He used slightly modified methods. The ammonia was determined as above by distillation with magnesium oxide. The residue from the distillation with magnesia was dissolved in dilute sulfuric acid and the di-amino acids precipitated by phosphotungstic acid. The nitrogen in the precipitate of di-amino acids was determined by the method of Kjeldahl. The filtrate from the di-amino acids containing the mon-amino acids was oxidized by the Kjeldahl method and the nitrogen determined. He secured the mon-amino nitrogen by difference in most cases, stating that it was difficult to get a direct determination of the mon-amino nitrogen by the Kjeldahl method.

He states that "this percentage was usually higher than the one directly found by Kjeldahlizing the filtrate from the phosphotungstic acid

precipitate." In one experiment the percentage of mon-amino nitrogen by direct determination was 62.83 per cent, while by difference the result was 67.22, and in another case the results were 64.25 and 65.06, respectively.

It will be noted that this is a departure from the method used by Shorey (37) in that here the nitrogen is separated into *three* fractions instead of the usual four. The nitrogen in the magnesia precipitate was distributed with the di-amino and mon-amino acid nitrogen.

Van Slyke's (47) nitrous acid method was first applied by Robinson (31) to a study of peat soil, in order to determine the amount of amino nitrogen present. The ammonia nitrogen was removed by previous distillation with magnesium oxide. The only value of Robinson's work seems to be in the fact that his figures for total and amino nitrogen increase to a maximum with increasing time of hydrolysis, in much the same manner that proteins react; thus indicating that the amino groups were not existing free in the peat but in some form of combination which did not react with nitrous acid. For example, after one hour's hydrolysis the total nitrogen of the soil in solution amounted to 29.86 per cent, while the amino nitrogen was 4.62 per cent, or a ratio exceeding 6:1. After 42 hours' hydrolysis the nitrogen of the soil in solution was 51.54 per cent of the total nitrogen and the amino nitrogen was 25.07, or a ratio only slightly exceeding 2:1. This ratio increases again with further hydrolysis so that at the end of 138 hours the ratio is almost 3:1. However, the amount of nitrogen in solution was so small that the experimental error of measuring total and amino nitrogen must have been quite large.

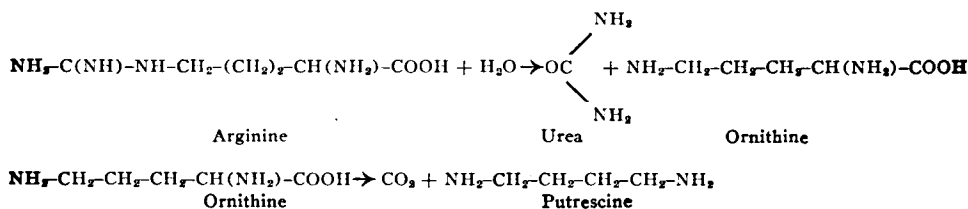
More recently Jodidi (17) made a study of some Iowa soils using a modification of the Osborne and Harris (24) method. The nitrogen removed from the solution by the magnesium oxide was apparently ignored by the author.<sup>5</sup> This contained a part of the so-called "humin" nitrogen. Subtracting the sum of the ammonia<sup>6</sup> and di-amino nitrogen from 100 he found the per cent of the nitrogen in solution as mon-amino nitrogen. It will be readily seen that this conclusion is incorrect. The mon-amino acid nitrogen as determined represents the sum of the humin and mon-amino nitrogen. It is very unfortunate that this mistake should have been made, since this gives us only the actual ammonia and di-amino acid nitrogen for use in comparison with other investigations of the organic soil nitrogen as distributed by acid hydrolysis. Kelley (18), following details as outlined by Jodidi (17), has made the same error and the criticisms above apply with equal force to his data.

<sup>5</sup> Experimental data presented later in this paper will show that this fraction may exceed 9 per cent of the total nitrogen.

<sup>6</sup> He distinguishes the ammonia nitrogen originally present in the soil as such, from that produced by acid hydrolysis.

It is also extremely unfortunate that investigators should in any case rely upon figures obtained "by difference" for any one of their fractions. It is sometimes permissible to use figures obtained in this manner, for example, in case a determination has been lost and lack of time or other consideration prevents a repetition, but constantly to use figures obtained in this manner is undesirable, especially, since by this method we have no means of determining how great the experimental error of the method may have been.

Jodidi (17) called attention to the fact that in the case of protein substances the distillation of the hydrolyzed protein with magnesium oxide gives pure ammonia. This, however, may not hold true for the hydrolyzed portion of soils, since some protein substances through decay yield organic bases. It has been shown by Bocklisch (3) that dimethyl amine is formed through putrefaction of fish, and trimethylamine has been produced by the putrefaction of wheat flour and fish. The bases putrescine and cadaverine result from the decay of organic substances under certain conditions. It is possible for certain di-amino acids to become transformed into di-amines, as for example, arginine can be decomposed into urea and ornithine through bacterial activity. These processes can be expressed by the following equations:



Jodidi found that the ammonia obtained by distilling the evaporated extract of the soil with magnesium oxide, was actually pure ammonia, thereby establishing the absence of any volatile organic bases; but concluded that the phosphotungstic acid precipitate and the filtrate from that precipitate did not represent di-amino and mon-amino acids only.

In order to find out how much of the di-amino and mon-amino nitrogen actually belongs to diamino and mon-amino acids, the solutions were subjected to analysis by the formaldehyde-titration method of Schiff (33, 34, 35) as modified by Sørensen (41), Henriques (14) and Henriques and Sørensen (15).

In a comparison of the amount of di-amino<sup>7</sup> acid nitrogen, calculated as if histidine, arginine, and lysine were present in about equal amounts, he finds that in Plot E, 101.8 per cent; in Plot Q, 84.8 per cent; and in Plot U, 93.9 per cent of the nitrogen in the phosphotungstate fraction was actually present as di-amino acids.

<sup>7</sup> The exact interpretation of his data is difficult to understand.

However, he obtains widely divergent results for mon-amino acid nitrogen, in Plot E, 91.64 per cent; in Plot Q, 52.63 per cent; in Plot U, 40.12 per cent; in Plot H, 88.31 per cent; and in Plot J, 92.11 per cent of the total nitrogen in the "filtrate from the bases" was actually present as mon-amino acid nitrogen, whereas all should have been present in this form if he were dealing with pure proteins only. He, therefore, concludes that the di-amino and mon-amino acids, or in other words *the bases and filtrate from the bases obtained by hydrolyzing soils, contain other products than are formed by hydrolysis of pure protein.*

In a series of fertilized soils studied by Lathrop and Brown (22) they find that almost 98 per cent of the nitrogen in the soil is of organic nature. The ammonia and the nitrate nitrogen constitute the remainder. Employing the same method for the distribution of the soil organic nitrogen as Shorey (37), they boiled 100 gm. of soil with 500 c.c. of hydrochloric acid (sp. gr. 1.115) for 3 hours, and used the filtrate after making to definite volume for the analyses. The figures given for ammonia nitrogen represent the actual amount of nitrogen as ammonia obtained by hydrolysis and do not include the ammonia nitrogen already present in the soil. They find that the plots which have received organic fertilizers give the largest amount of ammonia on hydrolysis, the amount being highest in the plot which has received manure alone and lowest in the check plot.

Of the five soils studied, four contained over 26 per cent of the "humins" nitrogen soluble in acid, while the other showed only about half as much. Since the nitrogen of the soil not soluble in acid may be considered "humins" nitrogen, the total amount in this form in the above four soils was over 53 per cent, while in the other soil, which received dried blood, it amounted to only 43 per cent.

However, the fractions which they determined have actually very little significance in a discussion of protein hydrolysis products, inasmuch as a 3-hour hydrolysis is far too short a time to decompose the protein molecule completely. This explains their high figures for humin nitrogen and low ones for mon-amino acid nitrogen. The figures for di-amino and mon-amino acid nitrogen differ rather widely, but there seems to be no agreement between the form of nitrogen and the plot treatment.

In conclusion they say "these five samples of soil are really the same soil under long continued treatment of different kinds. It is not improbable that work on widely different soils will show even much greater variations than those here noted. The work shows, however, that even in such cases there is a difference in the nitrogenous compounds in the soil, and that different decompositions of the nitrogenous matter have taken place and probably will continue to take place, under the different conditions imposed upon the soils in the field."



A very interesting study has been made by Shmook (36) of the nitrogen distribution in four Russian soils, one of the Podzol type, two of the Chernozem type, and one of the Laterite type, by applying the method of Hausmann (13). The water extract from 100 gm. of the Podzol soil showed a very high content of soluble nitrogenous compounds. This amounted to 0.0452 gm. of nitrogen which constituted 19.10 per cent of the total soil nitrogen. This was distributed as follows: amide nitrogen, 0.0034 gm.; di-amino nitrogen, traces; and mon-amino nitrogen, 0.0408 gm. These results were deducted from the analyses of the hydrolyzed soil.

He finds that the Chernozem and Podzol soils show a similarity in the distribution of amide nitrogen, and that of the amino acid nitrogen, but that the nitrogen distribution in the Laterite soil is entirely different from that of the other types. He concludes that the amount of protein in the soil is not in direct relation to the amount of organic matter, and that the nitrogen insoluble in hydrochloric acid occurs in unknown form and composes only 1.50 to 1.90 per cent of the organic matter of the Chernozem and Podzol soils, but 13.70 per cent of the total organic matter of the Laterite soil after subtraction of the protein nitrogen belonging to this insoluble portion. He suggests these results would indicate that the organic nitrogen existed in the soil in large part as protein material in the Chernozem and Podzol soils, but that a considerable portion was of a non-protein origin in the Laterite soil, since the amount of this insoluble "melanin" in pure proteins amounts to from 0.60 to 1.80 per cent of the total protein nitrogen.<sup>8</sup>

Potter and Snyder (28) made a study of some Iowa soils, using Van Slyke's (47) method of protein analysis. Their soils were of the same type but had received different fertilizer treatment. At the same time they also made a study of a peat soil. The soils were in all cases first extracted with 1 per cent hydrochloric acid "in order to render the humus more soluble." They were then hydrolized by boiling one part of the soil with two parts of 22 per cent hydrochloric acid for 48 hours. They also prepared a 1 per cent sodium hydroxide extract of the acid-leached soils and after precipitation with sulfuric and acetic acids the resulting "humic acid" precipitate was subjected to the above method of analysis.

The authors conclude: (a) that the humin nitrogen as determined by the Van Slyke method in the dilute alkali extract of soils is very high when compared with the amounts in proteins; (b) that no typical class of organic compounds is extracted from the soil by dilute alkali; (c) that

<sup>8</sup> Actually in some cases these results are much lower, and in others are decidedly higher, e. g., Van Slyke (46) finds gelatin contains 0.07 per cent and fibrin 3.17 per cent.

the amounts of amino acid and peptide nitrogen in the soil are found to be very small compared with the amounts of amino acids formed by acid hydrolysis; (d) (and this is the most important for our purpose) that "nothing very significant can be deduced from the variations in the different soils," or in other words, *the organic nitrogen in the same soil type under different fertilizer treatment is essentially the same, and as we shall show later in the experimental part of this paper, the organic nitrogen (as distributed by Van Slyke's method) in different soil types is essentially the same.*

Lathrop (21) recently made a study of protein decomposition in the soil. He added a high-grade nitrogenous fertilizer to the soil and allowed decomposition to proceed at laboratory temperature, and at different periods took samples and subjected them to Van Slyke's method of protein analysis in order to determine how the different fractions were affected by bacteria and other agencies present in the soil.

From his work he concludes that the analysis obtained by the Van Slyke method indicates that there is a formation of protein taking place in the soil in the course of the decomposition of the protein materials, and that apparently the new protein is somewhat resistant to decomposition. He states that "this is indicated in (a) the unequal loss of mon-amino acids and hydrolyzable nitrogen from the soil during the early stages, (b) by an increase in amide nitrogen during the early stages, (c) by an increase in histidine nitrogen during the early stages, (d) by an increase in the arginine nitrogen during the later stages, and (e) by an increase in lysine nitrogen during the later stages." This view that the protein nitrogen in the soil was largely contained in the bodies of bacteria and protozoa had been previously advanced by Shmook (36).

It was stated by Loew and Aso (23) that under favorable conditions of growth protein material is excreted by yeast and bacteria, and that soluble materials can pass through the cytoplasm to the outside on the death of the cell. They also state that the amount of nitrogenous substances partly consisting of peptones excreted by dead cells, is by no means inconsiderable.

#### EXPERIMENTAL

*The problem.* It has been shown in the historical study above that a number of investigators have studied the distribution of the organic nitrogen in the soil by applying either Hausmann's (13) or Van Slyke's (47) method of protein analysis. It has been demonstrated by Potter and Snyder (28) that various plots on a single soil type but under different cultural conditions gave, with Van Slyke's method, essentially the same nitrogen distribution.

The results of Potter and Snyder's work were published some time after the present investigation had been begun, but the problems in the

two instances were not exactly similar. We have made a study of the distribution of the organic nitrogen in *different soil types* in an attempt to see whether the forms in which nitrogen occurs differ from locality to locality, and from soil type to soil type.

*The material.* This study was made using two peats, one muck, seven mineral surface soils and one mineral subsoil. All but one of the soils used are from samples of soils which have been used in the preceding papers of this series. Inasmuch as a complete description of these soils has already been given (9, 11), it will not be repeated.

The soils used were Fargo silt loam, Fargo clay loam, forest-covered loess, prairie-covered loess, Hempstead silt loam, Carrington silt loam (2 samples),<sup>9</sup> Hempstead silt loam subsoil, sphagnum-covered peat, black peat and "muck."

All of the samples were used in an air-dry condition.

*The method.* The method of Van Slyke (47, 48) has been used throughout this investigation because the nitrogen can be separated into a larger number of fractions than when the earlier method of Hausmann (13) is employed. The different fractions, however, are not listed in the same manner as in the Van Slyke method, for since we are not dealing with pure protein material we cannot correctly speak of arginine, histidine, cystine, and lysine nitrogen.

Van Slyke (49) has called attention to the fact that his method was devised for the analysis of *pure protein* material and not for a heterogeneous mixture of nitrogen compounds. This fact is apparently not recognized by certain investigators.

It is obvious that there are other types of organic materials which will interfere with the nitrogen distribution. It seems very probable that in plant materials and in soils there must be many organic nitrogenous compounds which have no relation to the protein molecule, such as purine bases, pyrimidine bases, nitrogenous fats, nitrogenous pigments, as well as other non-protein nitrogenous compounds. Much valuable *comparative* data can be obtained by the application of Van Slyke's method to the analysis of heterogeneous materials; but it is self-evident that no analogy can be drawn between the analysis of pure protein and the analysis of a protein mixed with an unknown amount of foreign nitrogenous compounds. The results obtained from the hydrolysates of soils are of little value in advancing our knowledge of soil proteins or for comparison with analyses of pure proteins, but may be extremely valuable and

---

<sup>9</sup>One sample of Carrington silt loam was from Nerstrand, Rice County, Minnesota, situated on the Kansas glacial drift. This sample was used in the earlier papers of this series. The other sample was from Morristown, Rice County, Minnesota, situated on the Late Wisconsin glacial drift, and had not been used in the preceding studies. Like the sample from Nerstrand, it represents a composite of 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields.

interesting for comparison between themselves and with other analyses of soils carried out under similar conditions. It must be remembered that all data on similar material are strictly comparable when the same method of analysis is followed.

It is possible that many of the non-protein nitrogenous compounds may be split up during the hydrolysis of heterogeneous material. Gortner (7) has shown that uric acid nitrogen is distributed in all four of the major fractions after hydrolysis. The ammonia nitrogen amounted to 15.27 per cent, humin nitrogen 35.98 per cent, basic nitrogen 12.97 per cent, and non-basic nitrogen 35.78 per cent. "The humin nitrogen contained no trace of black color and was probably calcium ureate." Probably all of the purines and pyrimidines would behave in a similar manner.

The general method employed in this investigation will be discussed in detail for two soils, a peat and a mineral soil, inasmuch as the experimental conditions vary in minor details with the two types.

1. *The method in detail for a peat soil.* Duplicate samples were hydrolyzed in the presence of hydrochloric acid for 48 hours. The content of calcium oxide was taken into account, and corrections made so that the hydrochloric acid used was of sufficient concentration to neutralize the lime and at the same time furnish a constant boiling acid. The hydrolysis was carried out in 200-c.c., long-neck, round-bottom Kjeldahl flasks, fitted with Hopkins' condensers made from a test tube which fitted rather loosely into the neck of the flask. The flasks were heated to gentle boiling on the same sand bath over an Argand burner, so that the rate of hydrolysis would be as nearly the same as possible.

After completion of the 48-hour hydrolysis the mixture was evaporated in a Claissen distilling flask under diminished pressure until all the hydrochloric acid possible was driven off. The residue after this distillation was dissolved in 100 to 150 c.c. of water, 100 c.c. of 95 per cent alcohol, and an excess of calcium hydroxide suspended in water was added and the ammonia distilled off into standard acid at a temperature of 40-50° under a pressure of less than 30 mm., distillation being continued for at least a half-hour. The results are listed under "ammonia nitrogen."

The alkaline mixture in the distilling flask was filtered and the precipitate well washed with hot water until free of chlorides. A Kjeldahl determination was made of the filter and its contents, and the results listed under "humin nitrogen."

The filtrate and washings from the humin were acidified with hydrochloric acid and concentrated under diminished pressure to less than 200 c.c., and to this solution was added 18 c.c. of concentrated hydrochloric acid and the whole heated on the water bath until hot. A solution containing 15 gm. of phosphotungstic acid was then added and the

heating on the water bath continued for an hour. The flask was then set aside in a cool place for 48 hours. The precipitate of the bases was then filtered off and washed as directed by Van Slyke (47).

The basic phosphotungstates were suspended in 800 c.c. of water and brought into solution by the cautious addition of a 50 per cent solution of sodium hydroxide, a few drops of phenolphthalein being added to guard against too great an excess of alkali. The phosphotungstic acid was precipitated by the addition of a slight excess of 20 per cent barium chloride, and the barium phosphotungstate was filtered off and washed free of chlorides with hot water.

The filtrate and washings were united, acidified with hydrochloric acid, and concentrated under diminished pressure to a very small volume. After cooling, any residue was filtered off, washed, and the filtrate made to 50 c.c. volume.

The washed precipitate of barium phosphotungstate and filter were subjected to Kjeldahl determination for any nitrogen that might be held by absorption, adsorption, or occlusion, as was also any residue remaining on the filter after the final filtration of the solution containing the basic nitrogen. In all cases some nitrogen was found. This nitrogen is probably derived from the "unadsorbed humin carried down with the basic phosphotungstates" mentioned by Van Slyke (49, p. 284). Inasmuch as this work was done prior to Van Slyke's publication, we added this nitrogen to the total nitrogen content of the bases instead of to the humin.

In no case did we attempt to separate the basic nitrogen into the usual fractions of "arginine," "cystine," "histidine," and "lysine" nitrogen, because we were not dealing with pure protein material. The nitrogen of the arginine determination is listed as "nitrogen set free as ammonia by 50 per cent potassium hydroxide." The solution remaining from this determination was used in the estimation of the total nitrogen of the bases. This was performed according to Van Slyke's directions. The quantity of acid neutralized in this determination was added to that neutralized in the nitrogen set free as ammonia by 50 per cent potassium hydroxide, thus securing the "total basic nitrogen."

The "amino nitrogen of the bases" was determined in Van Slyke's (48) apparatus, using 10-c.c. portions of the solution.

The filtrate from the bases was treated with sodium hydroxide solution until a slight turbid precipitate of lime was formed, and then cleared immediately by the addition of acetic acid. The solution was then concentrated under diminished pressure and on cooling was made to 200 c.c. volume. "Total nitrogen in the filtrate from the bases" was determined on duplicate portions of 25 c.c. each by the method of Kjeldahl. The digestion was continued for 3 hours after the solutions were clear, so

that the phosphotungstic acid would not interfere with the accuracy of the determination. The "amino nitrogen in the filtrate" from the bases was determined on duplicate portions of 10 c.c.

2. *The method in detail for a mineral soil.* Duplicate portions of 250 gm. were hydrolyzed in round-bottom Kjeldahl flasks for 48 hours on different sand baths. Allowance was always made for the lime content of the soil, and the requisite amount of hydrochloric acid added to insure the presence of a constant boiling acid (sp. gr. 1.115), and a volume of approximately 250 c.c. The solutions boiled smoothly and gave no trouble by bumping.

On completion of the hydrolysis the two samples were diluted to 1000 c.c. in measuring flasks and allowed to settle for at least 24 hours. The clear solution was then siphoned off and an aliquot of 500 c.c. analyzed according to the usual method of Van Slyke. In nearly all cases this solution was straw color, as a result of the presence of ferric salts that had been formed during the hydrolysis. No black color, the usual color of a protein hydrolysate, was observed in any instance.

The soil remaining in the measuring flask was washed free from soluble nitrogen with a 1 per cent solution of potassium sulfate, by decantation from tall soil beakers, the solution after settling being siphoned off not oftener than twice a day. The electrolyte was added in order to prevent the clay from forming a colloidal suspension, and at the same time the particular salt chosen would not interfere with the subsequent Kjeldahl determination.

A concrete example of the thoroughness of this washing may well be given. It will be noted that 700 c.c. of the original hydrolysate was siphoned off for the different analyses. This left a total volume of 300 c.c. of residue and solution to be washed by decantation with 1 per cent potassium sulfate. By the methods of calculation given in the following paragraphs it was found that the remaining solution contained 0.1089 gm. of nitrogen. If three-fourths of the wash solution is removed each time, there will remain in the solution at the end of the fourth washing approximately 0.0004 gm. of the original nitrogen. Actual Kjeldahl determinations were made on 250-c.c. portions from the fourth washing in the case of duplicates from the same soil, and the results indicated that 0.0006 gm. of nitrogen still remained in the solution. Since this was within experimental error of the theoretical value, the method of washing by decantation was followed in all the subsequent work with mineral soils, or those which had mineral soils added previous to the analysis.

The residue from the hydrolyzed soil was evaporated to dryness on the steam bath in an evaporating dish, then further dried at about 110° C. After cooling, this dry soil was passed through a 1-mm. sieve and after being thoroughly sampled, duplicate nitrogen determinations were made

on 15-gm. portions and the total nitrogen remaining in the soil calculated. These results are listed as "insoluble humin nitrogen in the soil." The weight of the dry soil divided by the average specific gravity (2.6) represented the actual volume occupied by this soil residue. The total volume of the hydrolysate minus the volume occupied by the insoluble residue gives the actual volume of the soil solution.

Since the analysis was made on 500 c.c. of the soil solution it was necessary to recalculate the total "insoluble humin nitrogen in the soil" in order to determine the amount of this humin nitrogen actually belonging to the aliquot analyzed.

The total nitrogen belonging to the solution analyzed was found by taking the sum of the total nitrogen in the solution and the above insoluble humin nitrogen. Knowing the total nitrogen content of the soil before hydrolysis and the total nitrogen in solution, the per cent of the total nitrogen in solution after hydrolysis can be determined.

The 500-c.c. aliquot was concentrated under reduced pressure until the greater part of the hydrochloric acid had been removed and the ammonia nitrogen determined in the manner outlined under the peat analysis.

The "humin" fraction precipitated by the calcium hydroxide was almost colorless or light yellow because of the iron salts contained in it. This bulky precipitate was always washed by decantation after the method above described, except that distilled water was used, the united washings being concentrated to 200 c.c. or less for the precipitation of the basic nitrogen.

It was found necessary to use 35 gm. of phosphotungstic acid for the precipitation of the bases. The remainder of the analysis was carried out as directed under peats.

3. *The determination of nitrogen.* Nitrogen was determined on the soils and soil extracts in the usual manner, 25 to 35 c.c.  $\text{H}_2\text{SO}_4$ , 10 gm.  $\text{K}_2\text{SO}_4$ , and a crystal of  $\text{CuSO}_4$  being used. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without necessitating a calculation.

#### *The Analytical Data*

1. *Analysis of "fibrin from blood" hydrolyzed in the presence of 100 gm. of ignited subsoil.* This analysis was conducted in order to ascertain, if possible, the effect of soil minerals upon the hydrolysis of a pure protein. Fibrin was selected because it was from a sample already analyzed (10). The subsoil was first ignited to redness in a muffle furnace for an hour, in order to drive off all the organic matter, and subsequently cooled in a desiccator.

Duplicate portions of 5 gm. of fibrin and 100 gm. of ignited subsoil were hydrolyzed in the presence of hydrochloric acid. The analysis was

conducted as described for the mineral soils, excepting that a 600-c.c. aliquot was used for the analysis, this amount of solution being equivalent to 3 gm. of fibrin.

The experimental data showing the grams of nitrogen found and per cent of total nitrogen are given in Table I.

Table II shows a comparison of these analyses with other analyses of the same sample of fibrin hydrolyzed alone, and in the presence of three times its weight of cellulose [data of Gortner (10)].

Differences between these analyses, together with differences between duplicates in each series of analysis, and data showing "maximum" and "average" experimental differences to be expected are given in Table III.

These comparisons will be considered in detail under "Discussion" in the latter part of this paper.

2. *Sphagnum-covered peat.* Duplicate samples were hydrolyzed in the usual manner. It was found in the ammonia determination that all of the ammonia nitrogen could not be driven off in a half-hour when the volume of solution was large and a bulky precipitate of iron and aluminum hydroxides was present. Continued distillation for a further half-hour in this case gave additional ammonia nitrogen amounting to 0.0060 gm. In all subsequent work with both peats and soils the volume was kept smaller by the use of a more concentrated suspension of calcium hydroxide and the distillation was usually continued for one hour.

The calcium hydroxide precipitate containing the "humin" nitrogen was difficult to digest in the subsequent Kjeldahl determination, because of the large amount of organic material present. From 100 to 200 c.c. of sulfuric acid was required for the digestion. After digestion the material was transferred to a 1000-c.c. flask and 250-c.c. portions used for the distillation.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

3. *Sphagnum-covered peat hydrolyzed in the presence of nine times its weight of mineral subsoil.*<sup>10</sup> Duplicate 10-gm. samples were hydrolyzed in the presence of 90 gm. of subsoil with constant boiling hydrochloric acid for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

A comparison between these analyses and those of the peat hydrolyzed alone is made in Table IV, the data of the peat hydrolyzed alone being recalculated from a 15-gm. basis to a 10-gm. basis.

4. *Sphagnum-covered peat hydrolyzed in the presence of metallic tin.* The peat was hydrolyzed in the presence of a reducing agent because

<sup>10</sup> This was the first attempt to determine the nitrogen fractions in the presence of a mineral soil. For certain reasons later analyses have already been reported in this paper. The analyses as reported in this paper are by no means in chronological order, a fact which may explain seeming inconsistencies.



it was thought that possibly the amount of "humin" nitrogen would be reduced, for according to Samuley (32) the formation of this dark-colored product is due to an oxidative process [*cf.* Hlasiwetz and Habermann, and Cohn, cited by Plimmer (26, p. 17)].

It is perhaps significant that the "humin" nitrogen was reduced 3.88 per cent by the presence of a reducing solution. It is not known whether there was sufficient tin present to maintain a reducing solution throughout the hydrolysis, inasmuch as the ferric iron in the peat would have an oxidizing action on the stannous salt. The sample was known to contain iron but the amount was not determined.

Duplicate 15-gm. samples were hydrolyzed with 100 c.c. of hydrochloric acid (sp. gr. 1.115) for 48 hours in the presence of 5 and 10 gm. of tin, respectively. The tin was first partially dissolved in the acid before the samples of peat were added.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

5. *Calcareous black peat.* Duplicate samples of 15 gm. were hydrolyzed for 48 hours in the presence of 100 c.c. of constant boiling hydrochloric acid, and the analysis conducted as described under the method for a peat soil.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

6. *Acid "muck" soil.* One 25-gm. sample was hydrolyzed in the presence of 125 c.c. of concentrated hydrochloric acid for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

7. *Fargo clay loam.* Duplicate portions of 250 gm. were hydrolyzed for 48 hours.

The "humin" precipitate required 100 c.c. of sulfuric acid for the digestion. After digestion the material was transferred to a 500-c.c. flask and 250-c.c. portions used for the distillation. This method was followed subsequently with the "humin" nitrogen determination of all the mineral soils.

The addition of 15 gm. of phosphotungstic acid to the filtrate from "humin" did not entirely precipitate the bases. Five-gm. portions were added from time to time until a total of 50 gm. had been used. After standing the usual length of time the precipitate of the bases was filtered off, but even then the wash water caused the formation of a small additional precipitate in the filtrate. After warming on the steam bath this final solution was perfectly clear, and on standing over night, only a trace of precipitate was formed; so the precipitation was considered complete. It appears probable that a portion of this precipitate is due to the formation of inorganic phosphotungstates which consume a very large

amount of the phosphotungstic acid, for if all of this precipitate had consisted of basic nitrogen compounds the amount of nitrogen recovered should have been greater than the amount which was actually found. In all the subsequent work 35 gm. of phosphotungstic acid was used for the precipitation of the bases in the hydrolysates from mineral soils. The phosphotungstate precipitate dissolved very slowly in the sodium hydroxide, as did all other phosphotungstic acid precipitates of the mineral soils studied.

During the concentration of the filtrate from the bases so much precipitate separated, that this was filtered off and the solution made up to 300 c.c. volume. The salt remaining was dissolved in water and also made up to a volume of 300 c.c. Aliquot portions were taken from each solution and combined for the different determinations.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

8. *Fargo silt loam.* Two 125-gm. portions were hydrolyzed for 48 hours with 500 c.c. of hydrochloric acid (sp. gr. 1.18).

The resulting hydrolysates from the two flasks were combined and diluted to 2 liters. After settling, a 1-liter portion was siphoned off and analyzed by the usual method. Two 100-c.c. portions were used for the determination of total nitrogen in solution.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

9. *Carrington silt loam.* Sample I represents soil from Nerstrand, Minnesota, situated on the Kansan drift, and Sample II from Morristown, Minnesota, situated on the Late Wisconsin. A single hydrolysis of 250 gm. was made in each instance.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

10. *Hempstead silt loam.* Duplicate 250-gm. samples were hydrolyzed for 48 hours.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

11. *Prairie-covered loess.* In Sample I, 250 gm. were hydrolyzed in the presence of 250 c.c. of constant boiling hydrochloric acid for 48 hours. The hydrolysate on cooling was diluted to 1000 c.c. and a 500-c.c. portion was siphoned off and used for the analysis. In Sample II, two 125-gm. samples were hydrolyzed in the same manner outlined under Fargo silt loam (500 c.c. of constant boiling hydrochloric acid to 125 gm. of soil). The dilution and aliquot used for analysis were also the same.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

12. *Forest-covered loess.* In Sample I, 300 gm. of soil were hydrolyzed and diluted in the same manner as Sample I of prairie-covered loess. In Sample II, 300 gm. of soil were hydrolyzed under the same conditions as Sample II of prairie-covered loess.

The volume of acid used in the hydrolysis had but little effect on the proportion of the different fractions. The only observed difference was in the insoluble humin nitrogen retained by the soil residue, and this was slightly larger in Sample II, which was hydrolyzed in the presence of the greatest excess of acid. In connection with this it must also be noted that there was a somewhat larger quantity of nitrogen in solution in Sample II than in Sample I. Much the same results were found with the prairie-covered loess. All increases or decreases in the various fractions due to the greater excess of acid may well be considered to be within the experimental error.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

13. *Analysis of a 1 per cent hydrochloric acid extract of sphagnum-covered peat.* Acid extraction was made of the two peats in direct contact with the 1 per cent hydrochloric acid. For the extraction 125-gm. portions were placed in 2.5-liter acid bottles and 2 liters of 1 per cent acid added, a total of 500 gm. of peat being used. The bottles were shaken at intervals during 5 days and then the contents filtered through two thicknesses of cheese-cloth and squeezed in the hands. The resulting solution was then filtered through two thicknesses of filter paper on a Büchner funnel. This extract was light-straw colored.

It has been shown by a number of investigators, *e. g.*, Jodidi (16), Kelley and Thompson (19), and Gortner (9), that considerable amounts of nitrogen are dissolved from certain soils by this preliminary treatment. The acid solution thus obtained should contain the ammonia, acid amides, amines, amino acids, and all other organic nitrogenous compounds soluble in water or very dilute acid. The 1 per cent hydrochloric acid extracted 8.57 per cent of the total nitrogen from the peat.

Duplicate nitrogen determinations were made on 250-c.c. portions of the acid extract and from these results the total nitrogen in the bulk solution determined. The 5500 c.c. of solution containing 0.6468 gm. of nitrogen were used for analysis. This solution was concentrated under reduced pressure to about 200 c.c. and then hydrolyzed for 48 hours, after 75 c.c. of concentrated hydrochloric acid were first added. During evaporation under reduced pressure considerable hydrolysis took place, for the solution turned dark brown in color.

The analysis of this extract from the sphagnum-covered peat shows that over 65 per cent of the nitrogen is in the form of ammonia. Potter and Snyder (28) have shown that a very small amount of the nitrogen

in the 1 per cent hydrochloric acid extract of soils exists in the soil as ammonia nitrogen. It seemed probable that if an extract of the peat contained so much ammonia nitrogen after hydrolysis, the air-dry peat must contain an appreciable amount in the ordinary condition. The ammonia nitrogen was determined directly on a 5-gm. sample of the air-dry material. An excess of calcium hydroxide solution was added and the mixture distilled under reduced pressure for 45 minutes. It was found that 5.40 per cent of the total nitrogen of the soil existed in the form of ammonia nitrogen.

The precipitate containing the "humins" nitrogen was washed by decantation until practically all the dissolved nitrogen was removed. After digestion the material was diluted to 500 c.c. and 250-c.c. portions used for distillation. Before concentration the filtrate from the "humins" precipitate was a reddish color, and when finally concentrated a cherry red.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

14. *Analysis of that portion of sphagnum-covered peat soluble in 4 per cent sodium hydroxide and precipitated by hydrochloric acid.* The organic material soluble in 4 per cent sodium hydroxide was next extracted from new portions of the peat. Twelve 5-gm. portions were leached with 1 per cent of hydrochloric acid to the absence of calcium and the excess of acid removed by washing with distilled water, until the filtrate indicated only a faint trace of free acid when tested with Squibb's litmus paper. After leaching and washing, each 5-gm. portion was washed into tall glass-stoppered cylinders of 500 c.c. capacity with 4 per cent sodium hydroxide, and filled to the mark. These were thoroughly shaken and placed on their sides, thus allowing the peat to settle on the sides of the cylinder and exposing a very large surface to the action of the hydroxide. The shaking was repeated at intervals for 9 days. The cylinders were then thoroughly shaken, placed in a vertical position and allowed to settle for 4 days before the supernatant liquid was siphoned off and filtered.

These filtered solutions were neutralized with hydrochloric acid (solution tested faintly acid) when a brown flocculent precipitate separated. This was allowed to settle for several hours and the colorless solution siphoned off. The brownish-black precipitates were filtered and after draining over night were thoroughly mixed with a large volume of water and again filtered and drained. The resulting precipitates were hydrolyzed with 200 c.c. of hydrochloric acid for 48 hours. This amount of concentrated acid was added and the flask brought to boiling until hydrogen chloride fumes were evolved, showing the presence of constant boiling acid.

The entire hydrolysate was used for the ammonia determination. After this determination the "humin" precipitate was thoroughly ground in a mortar to insure complete disintegration, although this seemed hardly necessary, as the solid was already in a fairly fine state of division. This precipitate was washed in the usual manner by decantation, the filtrate concentrated by evaporation and made to 250 c.c. volume. Duplicate portions of 25 c.c. were used for the determination of total nitrogen in the solution. The remaining 200-c.c. portion was used for precipitation of the bases and subsequent analysis.

The high content of carbonaceous organic matter made the "humin" precipitate very difficult to digest. The sulfuric acid required was 130 c.c. and the digestion extended over 10 days before complete decoloration was effected. Of course, precautions were taken to prevent the absorption of ammonia from outside sources. The material was diluted to 500 c.c. and 250-c.c. portions used in the distillation.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

15. *Analysis of that portion of sphagnum-covered peat soluble in 4 per cent sodium hydroxide and not precipitated by hydrochloric acid.* The filtrates remaining from the brownish-black precipitate formed by acidifying the sodium hydroxide extracts of the soil with hydrochloric acid (*cf.* Section 14) were concentrated in the usual manner to about 700 c.c., when a heavy precipitate of sodium chloride separated. On standing over night there also separated a heavy flocculent brown precipitate. This may have been due to the salting out effect of the sodium chloride on some of the organic substances in the solution. The solution was saturated with hydrogen chloride in the cold and the mixture then divided into two portions and hydrolyzed for 48 hours. After hydrolysis the portions were united and filtered through glass wool and the precipitate washed with concentrated hydrochloric acid. The filtrate was allowed to stand in a tall soil beaker when more salt separated. This was due to the increased concentration of the hydrochloric acid. The salt that separated was freed from the mother liquid by packing in a centrifuge and washing a number of times with acid. The salt, washed as free of the solution as possible, was dried on the steam bath. It was nearly white in color. The glass wool was dried and ground with the salt. After being sampled, 15-gm. portions were used for Kjeldahl determinations. The results were listed as "nitrogen retained by the salt."

The combined filtrates were concentrated and analyzed.

The average nitrogen distribution in per cent of total nitrogen is given in Table VI.

16. *Summary Tables.* Certain of the preceding analyses have been summarized in Tables V and VI.

In Table V are shown the amounts and percentages of soil dissolved by the acid during hydrolysis as well as the amount of nitrogen and percentage of the total nitrogen dissolved.

Table VI summarizes the average nitrogen distribution of all the soils analyzed. Because of the large amount of space required, the tables showing the analyses in detail, both as regards grams of nitrogen in the different fractions and the concordance of duplicate determinations, have been omitted, and only the summary table is given. The detailed tables will ultimately be available in a printed thesis from the Graduate School of the University of Minnesota.

TABLE I  
NITROGEN DISTRIBUTION IN THREE GRAMS OF MERCK'S "FIBRIN FROM BLOOD" HYDROLYZED IN THE PRESENCE OF 100 GRAMS OF IGNITED SUBSOIL

	Gm. Nitrogen		Per Cent Total Nitrogen		
	I	II	I	II	Av.
Total N .....	0.4577	0.4591	.....	.....	.....
Ammonia N .....	0.0457	0.0455	9.98	9.91	9.95
Insoluble humin N in soil .....	0.0125	0.0130	2.73	2.83	2.78
N precipitated by $\text{Ca}(\text{OH})_2$ .....	0.0220	0.0221	4.81	4.81	4.81
Basic N <sup>1</sup> .....	0.1219	0.0995	26.63	21.68	24.15
Arginine N .....	0.0598	0.0440	13.07	9.58	11.32
Histidine N .....	None	None	.....	.....	None
Lysine N .....	0.0597	0.0531	13.04	11.57	12.30
Cystine N .....	.....	.....	.....	.....	<sup>2</sup> 0.51
Amino N in bases .....	0.0775	0.0746	16.93	16.25	16.59
N in filtrate from bases .....	0.2725	0.2909	59.54	63.36	61.45
Amino N in filtrate from bases .....	0.2671	0.2702	58.36	58.85	58.61
Non-Amino N in filtrate from bases...	0.0054	0.0207	1.18	4.51	2.84
Total N regained .....	0.4746	0.4710	103.69	102.59	103.14

<sup>1</sup> The barium phosphotungstate retained 0.0022 gm. of nitrogen in Sample I and 0.0020 gm. in Sample II.

<sup>2</sup> From data of Gortner (10).

## DISCUSSION

*Changes in nitrogen distribution in a protein when hydrolyzed in the presence of a mineral soil.* From a study of Table II it is seen that the histidine nitrogen of fibrin hydrolyzed alone is 4.36 per cent and when hydrolyzed in the presence of cellulose it is 4.86 per cent of the total nitrogen. However, when fibrin is hydrolyzed in the presence of ignited subsoil we see the histidine nitrogen is entirely lacking<sup>11</sup> and that the nitrogen precipitated by calcium hydroxide amounts to 4.81 per cent. This corresponds very closely to the amount of histidine found in the other two cases. In other points the three analyses agree within experimental error.

<sup>11</sup> It is of interest to note that Brewster (4) found a similar behavior in the hydrolysis of vegetable materials. On hydrolyzing whole Kafr grain he obtained no histidine fraction, while the pure protein of Kafr grain showed a histidine content of 1.64 per cent.

It appeared possible that the histidine nitrogen might have been converted into the nitrogen fraction precipitated by calcium hydroxide. It is well known that histidine can be precipitated by silver salts in slightly alkaline solutions; and it was thought possible that the histidine might be precipitated by some of the mineral constituents of the soil and thus be found with the calcium hydroxide precipitate.

TABLE II  
COMPARATIVE ANALYSES OF THREE GRAMS<sup>1</sup> OF MERCK'S "FIBRIN FROM BLOOD"  
HYDROLYZED ALONE AND IN THE PRESENCE OF CARBOHYDRATE  
AND OF IGNITED SUBSOIL

	3 gm. of Fibrin hydrolyzed alone [data of Gortner (10)]	3 gm. of Fibrin hydrolyzed in the presence of 9 gm. of cellulose [data of Gortner (10)]	3 gm. of Fibrin hydrolyzed in the presence of 100 gm. of ignited subsoil (average data of Table I)
	Per cent total nitrogen	Per cent total nitrogen	Per cent total nitrogen
Ammonia N .....	10.15	9.85	9.95
Humin N .....	2.83	7.72	7.59
Arginine N .....	10.91	8.56	11.32
Histidine N .....	4.36	4.86	None
Lysine N .....	12.05	11.04	12.30
Cystine N .....	0.51	0.71	0.51
Amino N in filtrate from bases.....	55.43	52.02	58.61
Non-amino N in filtrate from bases..	2.51	3.91	2.84
Total N regained .....	98.75	98.67	103.14

<sup>1</sup> The 3-gm. portion contained approximately 0.4550 gm. of nitrogen.

With this idea in mind the behavior of histidine in the presence of an ignited subsoil was tested, only three fractions being determined. A 0.5000-gm. sample of histidine di-hydrochloride<sup>12</sup> and 50 gm. of ignited subsoil were boiled in the presence of 100 c.c. of hydrochloric acid (sp. gr. 1.18) for 48 hours. The solution was diluted to 500 c.c. in a graduated flask and two 200-c.c. portions siphoned off and analyzed. The solution was deep straw color. The determinations for ammonia nitrogen gave negative results. The precipitate formed by calcium hydroxide was washed by decantation until no soluble nitrogen could be detected in the wash water and the precipitate Kjeldahled. This precipitate was bulky because of the presence of large amounts of ferric and aluminum hydroxides. The average nitrogen content of this fraction was only 0.0006 gm.

The filtrate from the calcium hydroxide precipitate was concentrated to a small volume and the entire solution used for the nitrogen determination. Sample I contained 0.0371 gm. in the filtrate, and Sample II, 0.0365 gm., making an average of 0.0368 gm.

<sup>12</sup> The histidine di-hydrochloride was prepared from dried blood as outlined by Abderhalden (1). Total nitrogen found was 18.42 per cent; calculated, 18.42 per cent.

The residual soil was practically colorless, and a determination indicated that it was nitrogen free. The volume occupied by the soil residue was 17.3 c.c. By calculation it was found that the total nitrogen regained in the original solution was 0.0903 gm., theoretical 0.0921 gm., or a recovery of 98.01 per cent.

Thus, practically all of the histidine was recovered in the filtrate from the calcium hydroxide precipitate, indicating that the above hypothesis was incorrect.

TABLE III  
DIFFERENCE BETWEEN DUPLICATE ANALYSES (DUE TO EXPERIMENTAL ERRORS), THE DIFFERENCES APPARENTLY DUE TO THE ADDITION OF CARBOHYDRATE AND OF IGNITED SUBSOIL, AS WELL AS VAN SLYKE'S "MAXIMUM" AND "AVERAGE" DIFFERENCES TO BE EXPECTED

	Per cent difference between duplicates of fibrin alone [data of Gortner (10)]	Per cent difference between duplicates of fibrin and 3 times its weight of carbohydrate [data of Gortner (10)]	Per cent difference between duplicates of fibrin and 100 gm. of ignited subsoil	Average % difference between fibrin hydrolyzed alone and with 3 times its weight of carbohydrate [data of Gortner (10)]	Average % difference between fibrin hydrolyzed alone and in the presence of 100 gm. of ignited subsoil	Van Slyke's (46) experimental differences	
						"Maximum" per cent	"Average" per cent
Ammonia N .....	0.01	0.48	0.07	-0.30	-0.20	0.37	0.12
Humin N .....	0.11	0.25	0.10	+4.89	+4.76	0.39	0.20
Arginine N .....	0.73	0.86	3.49	-2.35	+0.41	1.27	0.73
Histidine N .....	0.07	0.66	None	+0.50	-4.36	2.14	0.79
Lysine N .....	0.08	0.11	1.47	-1.01	+0.25	<sup>1</sup> (0.93)	0.61
Cystine N .....	0.10	0.00	....	+0.20	....	0.11	0.05
Amino N in filtrate from bases.....	2.12	2.15	0.49	-3.41	+3.18	1.60	0.63
Non-Amino N in filtrate from bases...	0.27	0.40	3.23	+1.40	+0.33	<sup>1</sup> (0.60)	0.68
						1.20	

<sup>1</sup> The figure in parentheses represents the second greatest difference between duplicates observed by Van Slyke (46).

Table III shows the differences between the duplicate determinations of the analysis of fibrin alone and in the presence of carbohydrate and of subsoil, and the differences apparently due to the addition of 100 gm. of ignited subsoil to the 3 gm. of fibrin. Van Slyke's (46) "maximum" and "average" differences to be expected between duplicate determinations are also given in the table for reference.

The table shows that the differences between the analyses of fibrin hydrolyzed alone and in the presence of ignited subsoil were, in most cases, within the maximum allowed by Van Slyke for experimental



error. The only differences which were *certainly* greater than experimental error were those of humin nitrogen, histidine nitrogen, and amino nitrogen in the filtrate from the bases. It was observed that practically the same error occurred with amino nitrogen in the filtrate from the bases when hydrolysis was carried out in the presence of carbohydrate.

From this analysis one can only draw the conclusion that even if *the organic matter of the soil consisted entirely of pure protein, one would not obtain the same nitrogen distribution by the Van Slyke analysis in the presence of soil that one would obtain in the absence of the soil*, or in other words, the presence of ignited mineral subsoil interferes with the Van Slyke analysis in much the same manner as carbohydrates [Gortner (10)].

TABLE IV

COMPARATIVE ANALYSES OF SPHAGNUM-COVERED PEAT HYDROLYZED ALONE AND IN THE PRESENCE OF NINE TIMES ITS WEIGHT OF A MINERAL SUBSOIL

	Grams Nitrogen			Apparent distribution of N in subsoil in per cent of total nitrogen
	Peat	Peat + Subsoil <sup>1</sup>	Increase (+) or Decrease (—)	
Total N .....	0.2000	0.2441	+0.0441	.....
Ammonia N .....	0.0466	0.0623	+0.0157	35.60
Humin N .....	0.0527	0.0658	+0.0131	29.71 <sup>~</sup>
Basic N .....	0.0195	0.0268	+0.0073	16.55
N set free as NH <sub>3</sub> by 50% KOH...	0.0060	0.0100	+0.0040	9.07
N not set free by 50% KOH.....	0.0135	0.0168	+0.0033	7.48
Amino N of bases .....	0.0105	0.0178	+0.0073	16.55
Non-Amino N of bases .....	0.0090	0.0090	.....	.....
N in filtrate from bases .....	0.0860	0.0951	+0.0091	20.64
Amino N in filtrate from bases.....	0.0776	0.0825	+0.0049	11.11
Non-Amino N in filtrate from bases	0.0084	0.0126	+0.0042	9.52
Total N regained .....	0.2048	0.2500	+0.0452	102.50

<sup>1</sup> Ninety gm. of subsoil contained 0.0441 gm. of soil nitrogen.

*The humin nitrogen, its origin and significance.* In such a discussion one must first consider the source of humin nitrogen in pure proteins.

Osborne and Jones (25) suggest that perhaps tryptophane and histidine are responsible for the humin formation, basing their postulation on the fact that zein, which contains no tryptophane and but little histidine, gives only small amounts of humin on hydrolysis.

Gortner and Blish (12) hydrolyzed zein in the presence of both tryptophane and of histidine and found that a large part of the tryptophane was converted into humin nitrogen, whereas none of the histidine was converted into humin but was all recoverable in the bases. It has been shown above that histidine is practically all recovered in the filtrate from the humin when it is hydrolyzed in the presence of an ignited mineral

subsoil. Histidine, therefore, may be eliminated as a factor in the formation of humin nitrogen in the soil. Gortner and Blish conclude that "in all probability the humin nitrogen of *protein* hydrolyses has its origin in the tryptophane nucleus."

Gortner (10) has shown that the humin nitrogen is increased by the addition of carbohydrate material to protein, and suggests that this increase may be due to both physical and chemical causes.<sup>13</sup> He presents evidence to show that the action of carbohydrate is probably due to the furfural produced from the carbohydrate and shows that increasing quantities of furfural cause the humin nitrogen to increase steadily.

TABLE V  
PERCENTAGES OF SOIL AND OF SOIL NITROGEN DISSOLVED BY HYDROLYZING  
THE DIFFERENT SOIL TYPES

Soil Type	Sample	Grams soil taken (dry basis)	Grams soil dissolved	Per cent soil dissolved	Grams nitrogen in soil	Grams nitrogen dissolved <sup>1</sup>	Per cent of dissolved nitrogen
Fargo clay loam.....	I	240.2	43.2	17.99	0.6005	0.4252	70.99
	II	240.2	43.2	.....	.....	.....	.....
Fargo silt loam.....	I	222.8	54.8	24.60	1.8336	1.4237	77.65
Carrington silt loam.	I	235.5	36.5	16.61	0.8738	0.6191	70.91
Hempstead silt loam.	I	242.3	32.3	13.33	0.6201	0.4395	70.87
	II	242.3	33.3	13.74	.....	0.4508	72.69
Prairie-covered loess.	I	230.3	42.3	18.37	0.6933	0.5260	75.91
	II	230.3	45.3	19.64	.....	0.4991	72.02
Forest-covered loess.	I	294.4	29.4	9.98	0.3768	0.2735	72.19
	II	294.4	32.4	11.11	.....	0.2511	66.65

<sup>1</sup> The figures in this column were obtained by subtracting the "insoluble humin nitrogen" remaining in the residual soil from the nitrogen figures obtained by multiplying the original weight of soil taken (dry basis) by the nitrogen content of the soil. These figures may or may not agree with the figures obtained in Kjeldahling a portion of the solution, as a result of experimental errors, and perhaps to errors introduced in using a uniform factor (2.6) for specific gravity. The figures in this column are free from any error of this sort.

Shmook (36) states that during the hydrolysis of his soils there separated on the walls of the condenser a substance violet blue in color, and that this appears during the hydrolysis of pure protein and is recognized as Liebermann's reaction for protein substances. The above conclusion in regard to the hydrolysis of a pure protein is incorrect, since no color appears on the neck of the flask or condenser in such an analysis. When

<sup>13</sup> Practically the same increase in humin nitrogen occurred when fibrin was hydrolyzed in the presence of a mineral subsoil. The humin in this case was not due to the presence of carbohydrate, since the soil had lost all of its organic matter by ignition.

furfural is heated alone with hydrochloric acid a characteristic colored substance is deposited on the condenser. It has been shown [Gortner (10)] that at the same time a polymerization (?) of furfural to humin takes place very rapidly. Our soils on hydrolysis gave a deposit on the condenser similar to that described by Shmook. The reaction indicates the presence of furfural, which is in turn formed from the carbohydrates in the soil.

The humin nitrogen of protein origin actually present in the hydrolyzed soil may easily be a very small part of the nitrogen found. It is evident that there must be many nitrogenous organic compounds present in the soil which have no relation to protein material, such as purine and pyrimidine bases, nitrogenous fats, and nitrogenous pigments, besides a number of other non-protein substances. It is certain that the humin nitrogen will be greatly changed by the presence of many of these compounds. The calcium hydroxide here drags down all the organic nitrogenous compounds which are soluble in dilute acids, but insoluble in hot water and dilute calcium hydroxide, together with the calcium salts of nitrogenous organic acids, the calcium salts of the purine and pyrimidine bases in addition to the humin formed from the protein material, and other organic compounds that are adsorbed, absorbed, occluded, or combined with the iron and aluminum hydroxides present.

From Table VI we find that from 3.26 to 9.21 per cent of the total nitrogen is precipitated by calcium hydroxide. This does not represent true humin nitrogen, since the calcium hydroxide precipitate does not contain any black colored substances formed by hydrolysis. The solution from which it is precipitated is colored only by ferric compounds; therefore, *the organic material in this precipitate must consist of colorless organic compounds adsorbed by or combined with the lime*. This portion of the nitrogen consists almost certainly of non-protein material. In all pure proteins the nitrogen retained in the calcium hydroxide precipitate is supposed to consist entirely of deeply colored substances. This study of the distribution of organic nitrogen in the soil has led to this new fraction, not previously reported. Certain of the analyses were carried out before the possible importance of this fraction was realized, but in most of the analyses this fraction is reported as "nitrogen retained by calcium hydroxide." Investigations as to the chemical nature of this fraction are highly desirable.

The *true humin nitrogen* of protein origin remains in the residual soil after hydrolysis. The amount of nitrogen in this fraction varies from 22.93 per cent to 28.27 per cent of the total nitrogen for the mineral soils studied. This represents more nearly the true humin nitrogen, in that the black coloring matter formed by acid hydrolysis remains in this fraction, but in addition we should also find here all organic nitrogenous

TABLE VI  
AVERAGE NITROGEN DISTRIBUTION IN PER CENT OF THE TOTAL NITROGEN

	Sphagnum peat	Sphagnum peat + subsoil	Sphagnum peat + tin	1% HCl extract of sphagnum peat	Sphagnum peat soluble in 4% NaOH not pptd. by HCl	Sphagnum peat soluble in 4% NaOH and pptd. by HCl	Calcareous black peat	"Muck"	Fargo clay loam	Fargo silt loam	Carrington silt loam Sample I	Carrington silt loam Sample II	Hempstead silt loam	Prairie-covered loess	Forest-covered loess
Ammonia N .....	23.27	25.52	20.18	65.40	26.58	16.22	19.26	19.49	24.00	26.56	28.55	28.07	29.44	30.53	28.69
Insoluble humin N in soil .....	*	*	*	*	2.73	*	*	*	28.27	22.93	25.84	24.59	27.56	24.35	26.92
"Humin" N pptd. by Ca(OH) <sub>2</sub> .....	*	*	*	*	8.97	*	*	*	9.21	13.26	5.93	6.63	4.80	5.19	4.84
Total "humin" N .....	26.38	26.96	22.48	10.16	11.70	33.22	26.07	27.61	37.48	26.19	31.77	31.22	32.36	29.54	31.76
Total basic N .....	9.73	10.98	12.28	6.01	8.67	10.80	10.60	13.55	9.58	12.11	11.71	14.74	11.39	12.68	13.98
Basic N set free as NH <sub>3</sub> by 50% KOH .....	2.98	4.09	3.31	2.80	1.87	2.45	3.27	3.10	3.23	3.20	3.36	3.17	2.28	2.92	3.69
Basic N not set free as NH <sub>3</sub> by 50% KOH .....	6.75	6.89	8.97	3.21	6.80	8.35	7.33	10.45	6.35	8.91	8.35	11.57	9.10	9.76	10.29
Amino N of bases .....	5.26	7.29	6.86	4.11	4.28	6.08	6.35	9.13	6.44	7.52	8.04	7.99	7.44	7.48	7.57
Non-amino N of bases .....	4.47	3.69	5.41	1.90	4.39	4.72	4.25	4.42	3.14	4.59	3.67	6.75	3.95	5.20	6.41
N in filtrate from bases .....	43.00	38.96	46.40	20.64	53.69	41.08	42.40	38.81	32.71	35.14	25.47	28.25	28.10	28.80	27.21
Amino N in filtrate from bases .....	40.45	33.82	43.40	17.11	46.07	33.74	39.46	33.82	30.77	32.64	**	25.85	24.56	26.24	25.17
Non-amino N in filtrate from bases .....	2.55	5.14	2.99	3.53	7.62	7.34	2.94	4.99	1.95	2.50	.....	2.40	3.54	2.56	2.04
Total N regained .....	102.28	102.42	101.34	102.21	100.64	101.32	98.33	99.46	103.77	100.00	97.50	102.28	101.29	101.56	101.64

\* Not determined separately.

\*\* Solution lost at this point.

1 By difference.

\* Calculated.

compounds insoluble in a fairly strong solution of hydrochloric acid, all of the nitrogen adsorbed by the carbohydrate humins, etc. Potter and Snyder (28) express surprise at the large proportion of nitrogen in this fraction, but when one considers the heterogeneous nature of the soil organic matter it is perhaps more surprising to find that over 60 per cent of the nitrogenous compounds are soluble in strong hydrochloric acid. Further study is necessary before the full significance and origin of this humin nitrogen can be thoroughly understood.

*The effect of the quantity of acid used for the hydrolysis on the amount of nitrogen dissolved and the nitrogen distribution in soils.* Throughout this investigation acid at least as strong as constant boiling hydrochloric acid was used for the hydrolysis, inasmuch as that is the strength ordinarily employed in the hydrolysis of proteins.

In the case of two soils, however, one of the duplicates was hydrolyzed in the presence of 1000 c.c. of concentrated acid to 250 gm. of soil, the other being hydrolyzed in the presence of 500 c.c. of constant boiling acid to 250 gm. of soil, in order to see if any noticeable differences would be observed between the resulting analyses. The two soils thus hydrolyzed were the prairie-covered loess and forest-covered loess.

The results show little difference between the duplicates. Table V shows that the larger volume of the stronger acid dissolved a greater per cent of the soil, because of the fact that more of the mineral constituents were soluble in acid of this concentration. At the same time, however, the amount of nitrogen extracted was less. It is perfectly obvious that sufficient acid was used in all experiments to secure uniform and maximum hydrolysis.

*The percentage of soil nitrogen extracted by acid hydrolysis.* Shorey (37) working with a single Hawaiian soil extracted 84.68 per cent of the total soil nitrogen by acid hydrolysis. Jodidi (17) working with 11 Iowa soils found from his studies a minimum of 68.90 per cent, a maximum of 83.94 per cent, and an average of 75.77 per cent; Lathrop and Brown (22) in 5 Pennsylvania soils found a minimum of 70.60 per cent, a maximum of 73.71 per cent, and an average of 71.78 per cent; Shmook (36), working with 4 Russian soils, found a minimum of 60.60 per cent in the Laterite soil, a maximum of 87.67 per cent in the Podzol soil, and an average of 68.33 per cent; Kelley (18), working with 9 soils of the Laterite class common to the Hawaiian Islands, found a minimum of 67.51 per cent, a maximum of 91.80 per cent, and an average of 82.17 per cent; and Potter and Snyder (28), in 7 Iowa soils, found a minimum of 68.68 per cent, a maximum of 76.47 per cent, and an average of 74.41 per cent.

The grand average of all of these 37 soils from widely different origin gives 75.91 per cent of the soil nitrogen in solution in the hydro-

chloric acid extract. In these studies there was found a minimum of 66.63 per cent, a maximum of 77.65 per cent, and an average of 72.19 per cent extracted by the acid.

These results indicate that the nitrogen of practically all soils, in so far as investigated, dissolves to about the same extent during acid hydrolysis.

*A consideration of nitrogen distribution in different extracts from the sphagnum-covered peat.* Nitrogen distribution was determined on extracts of a sphagnum-covered peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide and *not* precipitated by acidification, and (c) 4 per cent sodium hydroxide and precipitated by acidification with hydrochloric acid. Of the three extracts only the second approximates the distribution of nitrogen in a pure protein. The figures for the ammonia nitrogen are abnormally high in the hydrochloric acid extract.

The humin nitrogen is high in all the extracts, but is excessive in fraction (c). It is clear that carbohydrates from the soil must be present in all three fractions used, and must have some share in bringing the humin nitrogen up to such high figures. The nucleic acids [Shorey (39, 40)] would be found in the hydrochloric acid precipitate from the sodium hydroxide solution, and the purine and pyrimidine components of these nucleic acids, as well as the lecithins [Aso (2), Stoklasa (42)] and nitrogenous fats and nitrogenous acids would be precipitated with the true humin by the calcium hydroxide.

The basic nitrogen figures are not widely divergent, although there may be some significant differences.

The differences between the nitrogen in the filtrate from the bases is perhaps the most significant of all. An amino nitrogen of only 17.11 per cent in the filtrate from the bases such as is found in the hydrochloric acid extract, is far lower than has ever been obtained in an analysis of a pure protein and indicates that the nitrogen of this extract is essentially non-protein.

Unfortunately it was impossible to complete the corresponding analyses on the calcareous black peat, but the fractions obtained indicated a distribution similar to that of the sphagnum-covered peat.

#### *General Conclusions in Regard to the Distribution of Soil Nitrogen in Different Soil Types*

From a study of Table VI a great similarity is observed between the different analyses of different soil types. Practically the same deduction was made by Potter and Snyder (28) in their study of a single soil type under different fertilizer treatment.

We find that the nitrogen distribution in a soil is very uniform, whether in the same soil type under different fertilizer treatment, or in

different soil types. This is to be expected, for if we were to take at random 50 Van Slyke analyses of proteins and compare the average analysis with that of another 50 analyses, we should expect to find results agreeing closely with each other. This expectation should also hold true for the hydrolysate of soils, since in each soil are to be found many of the nitrogenous compounds contained in the plant and animal products that find their way to the soil together with their decomposition products. Since there is such a great variety of different nitrogenous substances in the soil, it stands to reason that the nitrogen distribution in soils is an *average* distribution, and as such should not be expected to vary widely from soil to soil.

#### SUMMARY

This paper deals with a study of the nitrogen distribution, Van Slyke's method being used, in different soil types. Tables have been presented showing such distribution for the following materials:

- a. Fibrin hydrolyzed in the presence of an ignited mineral subsoil, (together with data of fibrin hydrolyzed alone and in the presence of carbohydrates).
- b. A calcareous black peat.
- c. An acid, sphagnum-covered peat, hydrolyzed alone, in the presence of a mineral subsoil, and in the presence of stannous chloride.
- d. An acid "muck" soil.
- e. Seven samples of mineral surface soil representing the following soil types: Fargo clay loam, Fargo silt loam, Carrington silt loam (two samples from different glacial drifts), Hempstead silt loam, prairie-covered loess, and forest-covered loess.
- f. Extracts of a sphagnum-covered peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide but precipitated by acid, and (c) 4 per cent sodium hydroxide and *not* precipitated by hydrochloric acid.

The following conclusions are evident:

1. The figures for the ammonia nitrogen in a protein analysis are not appreciably changed when the hydrolysis is carried out in the presence of an ignited mineral soil equal to twenty times the weight of the protein material.
2. The "humin" nitrogen was greatly increased by hydrolysis in the presence of ignited mineral soil. The histidine fraction entirely disappeared.
3. Attention is called to the fact that the analysis of a pure protein in the presence of an ignited mineral soil does not give reliable results for the different fractions. Therefore, the figures obtained for the nitrogen distribution in soils are of value only when used for purposes of comparison. Such data should not be compared with analyses of pure proteins.

4. Since practically all mineral soils give furfural on treatment with acid it is very likely that a very considerable amount of the total humin nitrogen found is due to the presence of carbohydrates in the soil, which give rise to furfural during hydrolysis. This may combine with certain of the nitrogenous compounds and cause an increase in the humin nitrogen, as well as adsorb or occlude nitrogenous compounds in the "humin" formed from furfural by polymerization.

5. This investigation of the distribution of organic nitrogen in the soil has indicated a new fraction which should be recorded separately. This is the fraction of nitrogen removed from a colorless solution by calcium, iron, and aluminium hydroxides on the addition of calcium hydroxide. The nitrogen retained in this fraction must consist almost entirely of material of non-protein origin, since the organic substances in this precipitate have been shown to be *colorless* organic compounds adsorbed by or combined with the metallic hydroxides. This fraction has been reported as nitrogen precipitated by calcium hydroxide.

6. The *true* humin nitrogen remains in the residual soil after hydrolysis, but in addition non-humin nitrogenous compounds are also retained in this fraction.

7. The strength and volume of the hydrochloric acid used in hydrolysis has little effect on the nitrogen distribution of the hydrolysate, provided acid as strong as constant boiling acid is used, in the proportion of at least two parts of acid to one of soil.

8. Results gained from a study of different soils indicate that the organic nitrogen dissolves during hydrolysis, to almost the same extent regardless of the origin and nature of the soil.

9. Some very interesting figures are found in the comparison of the different extracts from sphagnum-covered peat. The portion soluble in sodium hydroxide and *not* precipitated by hydrochloric acid, gives a nitrogen distribution approximating very closely that of a normal plant protein. The nitrogen dissolving in the preliminary hydrochloric acid leaching shows a nitrogen distribution which is *certainly* not due exclusively to protein materials, *e. g.*, an ammonia nitrogen percentage of 65.40 and amino nitrogen in filtrate from bases of 17.11 per cent.

10. The most significant fact brought out by this study is that the organic nitrogen distribution in *different soil types* is very uniform. This is to be expected, since it has been pointed out that the nitrogen distribution in soils is an *average* distribution of all the plant and animal nitrogenous products that find their way into the soil.

#### LITERATURE CITED

(1) ABDERHALDEN, EMIL.

1910. Handbuch der Biochemischen Arbeitsmethoden. Bd. II, 1101 p.  
Urban and Schwarzenberg. Berlin and Wien.



- (2) Aso, K.  
1904. On organic compounds of phosphoric acid in the soil. *In* Bul. Col. Agr., Tokyo Imp. Univ., v. 6, p. 277.
- (3) BOCKLISCH, O.  
1885. Ueber Fäulnisbasen (Ptomaine) aus Fischen. *In* Ber. Deut. Chem. Gesell., Bd. 18, p. 87-89.
- (4) BREWSTER, J. F.  
1917. Nitrogen distribution in various cereals and other feeding stuffs. Program of Work. U. S. Dept. Agr. 1917, p. 280.
- (5) DETMER, W.  
1871. Die natürlichen Humuskörper des Bodens und ihre landwirtschaftliche Bedeutung, Dissertation, Leipzig. Also *in* Landw. Vers. Stat., Bd. 14, p. 248. *Abs. in* Jahresber. Agr. Chem., 1870-72, p. 68-72.
- (6) FISCHER, EMIL.  
1901. Ueber die Hydrolyse des Caseins durch Salzsäure. *In* Ztschr. Physiol. Chem., Bd. 33, p. 151-176.
- (7) GORTNER, R. A.  
1913. Studies on the chemistry of embryonic growth : I. Certain changes in the nitrogen ratios in developing trout eggs. *In* Jour. Amer. Chem. Soc., v. 35, p. 632-645.
- (8) GORTNER, R. A.  
1914. Studies on the chemistry of embryonic growth II. Comparative analyses of the eggs and newly hatched larvæ of the giant salamander (*Cryptobranchus Allegheniensis*). *In* Jour. Amer. Chem. Soc., v. 36, p. 1556-1566.
- (9) GORTNER, R. A.  
1916. The organic matter of the soil: I. Some data on humus, humus carbon, and humus nitrogen. *In* Soil Sci., v. 2, p. 395-441. 2 pl.
- (10) GORTNER, R. A.  
1916. The origin of the humin formed by the acid hydrolysis of proteins. II. Hydrolysis in the presence of carbohydrates and of aldehydes. *In* Jour. Biol. Chem., v. 26, p. 177-204.
- (11) GORTNER, R. A.  
1917. The organic matter of the soil: III. On the production of humus from manures. *In* Soil Sci., v. 3, p. 1-8.
- (12) GORTNER, R. A., and BLISH, M. J.  
1915. On the origin of the humin formed by the acid hydrolysis of proteins. *In* Jour. Amer. Chem. Soc., v. 37, p. 1630-1636.
- (13) HAUSMANN, W.  
1899. Ueber die Vertheilung des Stickstoffs im Eiweissmolekül. *In* Ztschr. Physiol. Chem., Bd. 27, p. 95-108.
- (14) HENRIQUES, V.  
1909. Über quantitative Bestimmung der Aminosäuren im Harne. *In* Ztschr. Physiol. Chem., Bd. 60, p. 1-9.
- (15) HENRIQUES, V., and SÖRENSEN, S. P. L.  
1910. Über die quantitative Bestimmung der Aminosäuren Polypeptide und der Hippursäure im Harne durch Formoltitration, II. *In* Ztschr. Physiol. Chem., Bd. 64, p. 121-143.
- (16) JOBIDI, S. L.  
1909. Organic nitrogenous compounds in peat soils. Mich. Agr. Exp. Sta. Tech. Bul. 4, 28 p.

- (17) JODIDI, S. L.  
1911. The chemical nature of the organic nitrogen in the soil. Iowa Agr. Exp. Sta. Research Bul. 1, 46 p. 1 fig.
- (18) KELLEY, W. P.  
1914. The organic nitrogen of Hawaiian soils. I. The products of acid hydrolysis. *In* Jour. Amer. Chem. Soc., v. 36, p. 429-434.
- (19) KELLEY, W. P., and THOMPSON, ALICE R.  
1914. The organic nitrogen of Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 33, 22 p.
- (20) LATHROP, E. C.  
1914. The nitrogen of processed fertilizers. U. S. Dept. Agr. Bul. 158, 24 p.
- (21) LATHROP, E. C.  
1916. Protein decomposition in soils. *In* Soil Sci., v. 1, p. 509-532.
- (22) LATHROP, E. C., and BROWN, B. E.  
1911. Studies in organic soil nitrogen. *In* Jour. Indus. Engin. Chem., v. 3, p. 657-660.
- (23) LOEW, O., and ASO, K.  
1906-8. On changes of availability of nitrogen in soils. I. *In* Bul. Col. Agr., Tokyo Imp. Univ., v. 7, p. 443-448.
- (24) OSBORNE, T. B., and HARRIS, I. F.  
1903. Nitrogen and protein bodies. *In* Jour. Amer. Chem. Soc., v. 25, p. 323-353.
- (25) OSBORNE, T. B., and JONES, D. B.  
1910. A consideration of the sources of loss in analyzing the products of protein hydrolysis. *In* Amer. Jour. Physiol., v. 26, p. 305-328.
- (26) PLIMMER, R. H. A.  
1912. The Chemical Constitution of the Proteins, Part I, 2d ed. 188 p. Longmans, Green and Co., New York.
- (27) POTTER, R. S., and SNYDER, R. S.  
1914. The determination of ammonia in soils. Iowa Agr. Exp. Sta. Research Bul. 17, 19 p.
- (28) POTTER, R. S., and SNYDER, R. S.  
1915. Amino acid nitrogen of soil and the chemical groups of amino acids in the hydrolyzed soils and their humic acids. *In* Jour. Amer. Chem. Soc., v. 37, p. 2219-2227.
- (29) POTTER, R. S., and SNYDER, R. S.  
1915. The amino acid nitrogen of soils. *In* Jour. Indus. Engin. Chem., v. 7, p. 1049-1053.
- (30) POTTER, R. S., and SNYDER, R. S.  
1916. Soluble non-protein nitrogen in soil. *In* Jour. Agr. Research, v. 6, p. 61-64.
- (31) ROBINSON, C. S.  
1911. Organic nitrogenous compounds in peat soils. II. Mich. Agr. Exp. Sta. Tech. Bul. 7, 22 p.
- (32) SAMUELY, FRANZ.  
1902. Über die aus Eiweiss hervorgehenden Melanine. *In* Beitr. Chem. Physiol. u. Path., Bd. 2, p. 355-388.
- (33) SCHIFF, HUGO  
1900. Über Methylenasparagine. *In* Annalen, Bd. 310, p. 25-44.
- (34) SCHIFF, HUGO  
1901. Trennung von Amin- und Säurefunktion in Lösungen von Eiweisskörpern. *In* Annalen, Bd. 319, p. 287-303.

- (35) SCHIFF, HUGO  
1902. Trennung von Amin-und Säurefunktion mittels Formaldehyd. III. *In Annalen*, Bd. 325, p. 348-354.
- (36) SHMOOK, ALEXANDER  
1914. "Some data pertaining to the forms of nitrogen in soils." (In Russian.) *In Zhur. Oputu. Agron.* (Russ. Jour Expt. Landw.), v. 15, p. 139-153.
- (37) SHOREY, E. C.  
1905. Report on agricultural investigations in Hawaii (report of the chemist), U. S. Dept. Agr. Off. Exp. Sta. Bul. 170, p. 25-38.
- (38) SHOREY, E. C.  
1906. Organic nitrogen in Hawaiian soils. *In Hawaii Agr. Exp. Sta. Ann. Rpt.* 1906, p. 37-59.
- (39) SHOREY, E. C.  
1911. Nucleic acids in soils. *In Biochem. Bul.*, v. 1, p. 104.
- (40) SHOREY, E. C.  
1912. Nucleic acids in soils. *In Science*, v. 35, p. 390.
- (41) SÖRENSEN, S. P. L.  
1908. Enzymstudien. *In Biochem. Ztschr.*, Bd. 7, p. 45-101.
- (42) STOKLASA, J.  
1911. Biochemischer Kreislauf des Phosphat-Ions im Boden. *In Centbl. Bakt.* (etc.), Abt. 2, Bd. 29, p. 385.
- (43) SUZUKI, S.  
1906-8. On the formation of humus. *In Bul. Col. Agr., Tokyo Imp. Univ.*, v. 7, p. 95-99.
- (44) SUZUKI, S.  
1906-8. Studies on humus formation. II. *In Bul. Col. Agr. Tokyo Imp. Univ.*, v. 7, p. 419-423.
- (45) SUZUKI, S.  
1906-8. Studies on humus formation. III. *In Bul. Col. Agr., Tokyo Imp. Univ.*, v. 7, p. 513-529.
- (46) VAN SLYKE, D. D.  
1910. Eine Methode sur quantitativen Bestimmung der aliphatischen Amino-gruppen; einige Anwendungen derselben in der Chemie der Proteine, des Harns und der Enzyme. *In Ber. Deut. Chem. Gesell.*, Bd. 43, p. 3170-3181.
- (47) VAN SLYKE, D. D.  
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *In Jour. Biol. Chem.*, v. 10, p. 15-55.
- (48) VAN SLYKE, D. D.  
1912. The quantitative determination of aliphatic amino groups. II. *In Jour. Biol. Chem.*, v. 12, p. 275-284.
- (49) VAN SLYKE, D. D.  
1915. Improvements in the method for analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *In Jour. Biol. Chem.*, v. 22, p. 281-285.
- (50) WALTERS, E. H.  
1915. The presence of proteoses and peptones in soils. *In Jour. Indus. Engin Chem.*, v. 7, p. 860-863.