2. The adsorption is not a function of the granule surface per unit weight.

3. The amount of the adsorption is much greater for sodium hydroxide than for either hydrochloric acid or sodium chloride.

4. In the case of starch-hydrochloric acid, the ordinary adsorption rule is followed for solutions up to about 0.4 *N*, except in the case of maize starch.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE SOIL, SECTION IOWA AGRICULTURAL EXPERIMENT STATION.]

THE CHEMICAL NATURE OF THE ORGANIC NITROGEN IN THE SOIL.

BY S. L. JODIDI.

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The data given in this paper, as well as in a previous one,[1] show, in agreement with numerous investigations by others, that the amount of ammonia in soils is quite small, ranging from a few thousands to a few hundreds of one per cent. The proportion of nitrites and nitrates, too, is in the great majority of cases insignificant, being but a few thousands of one per cent. and less, which is true of the soils herein examined. Hence, practically all of the nitrogen occurring in soils is of an organic nature. This emphatically points to the importance of researches with a view to finding out the nature of the organic nitrogenous compounds present in the soil. Such knowledge would enable us, in the first place, to better understand the important biological processes of ammonification, nitrification and denitrification; to comprehend why the nitrogen in one soil is more useful or available than in another; to see whether there are among the nitrogenous compounds such that are detrimental to plant life; and last but not least, it will enable us to utilize the soil nitrogen to better advantage than is now the case.

While some nitrogenous bodies like urea, for instance, readily furnish ammonia through fermentation, others do not. While such compounds like asparagine, *e. g.*, represent plant food, tyrosine is considered as being hurtful to plants, whereas leucine and similar compounds, in the presence of nitrites, can give rise to the process of denitrification, so much so that under favorable conditions leucine is thus quantitatively converted into leucinic acid; other compounds with different chemical structure cannot. Important as is the knowledge of the organic matter in the soil, our pres-

[1] THIS JOURNAL, 31, 396; also *Technical Bull.* 4 (1909), Michigan Agricultural Experiment Station.

ent scope of information is extremely limited, to say the least. This is very well illustrated by the fact that out of 3794 pages in which Beilstein[1] treats of organic chemistry, but two pages are devoted to humus and humin substances.

In a previous article[2] we have demonstrated that the organic nitrogen in Michigan peat soils is made up of acid amides, monoamino acids and diamino acids. What is true of peat soils must not necessarily hold good for other soil types, for a number of reasons. While peat soils are exceedingly rich in humus and nitrogen, other soil types usually contain a small amount of both of them; whereas the humification process in peat soils, because saturated and oftentimes covered with water, is essentially one of putrefaction taking place in the absence of air, the humification in other soils is one of eremacausis inasmuch as it occurs chiefly in the presence of air.

Further, it will be well to recollect that the method applied for the separation of the organic nitrogenous bodies in peat soils was essentially the Hausmann-Osborne[3] method used in protein chemistry. However, the very assumption that organic nitrogenous compounds occurring in soils can be separated by the same method as applied for protein decomposition products is of a somewhat hypothetical nature and needs direct verification.

The nature of the organic nitrogen in soils being of considerable general interest as well as of paramount importance to agriculture, it may be worth while briefly to show what considerations have led the writer to the above assumption which, it may be said right here, has proved to be correct. Starting from the idea that soil organic matter or humus is the result of decomposed plants, all of which contain a large if not predominant portion of their nitrogen in the shape of proteins, it was but natural to assume the existence in the soil of some disintegration products of proteins. Now, proteins can be split into their constituent parts either by chemical means (*e. g.*, mineral acids or alkalies), or through the activity of certain micro-organisms, or through the agency of enzymes. Of these means the first mentioned is out of the question, inasmuch as even peat soils—the most acid soils known—usually show but a slight acidity largely due to the presence of weak organic acids and acid salts. On the other hand, decomposition of proteins in the soil can and undoubtedly does take place through bacterial activity or the agency of enzymes widely distributed in the vegetable kingdom. Furthermore, disintegration in

[1] See Beilstein's *Handbuch d. org. Chemie*, third edition, first volume, pages 1108–1109.

[2] See "Organic Nitrogenous Compounds in Peat Soils," THIS JOURNAL, **31**, 396.

[3] *Z. physiol. Chem.*, **27**, 95 (1899); **29**, 136 (1900); also THIS JOURNAL, **25**, 323 (1903).

the soil of protein-holding materials into simpler molecules can be accomplished also in a purely chemical way. A. E. Taylor,[1] *e. g.*, has shown that leucine can be recovered from a steril suspension of casein in pure water; he was also able to recover arginine from a solution of protamine sulfate in pure water, after the lapse of about one year. These facts assume considerable significance in processes taking place for a considerable length of time, such as in the soil.

Theoretically, then, the formation in the soil of smaller molecules (amino acids, etc.) out of the various protein compounds contained in decaying vegetable materials seems to be altogether possible and even probable. Nevertheless such deductions as these must be verified by direct experiments as presented in this article. While details will be found in the experimental part, a very brief outline of the questions involved may be given here. The hydrochloric acid extract of the soil was evaporated practically to dryness and distilled with cream of magnesia. The distillate contained, in the form of ammonia, all the nitrogen corresponding to the amides present in the soil. The residue from distillation with magnesia was extracted with water, and the extract, after acidulating with sulfuric acid, treated with PTA.[2] The precipitate contained the diamino acids, and the filtrate from that precipitate represented the monoamino acids.

Now, in the case of proteins proper the distillation of the acid-treated protein with cream of magnesia gives pure ammonia. This, however, may not necessarily hold true for soils recalling that some proteins, through decay, yield organic bases. Thus, methylamine,[3] dimethylamine,[3] and trimethylamine[3] are formed through putrefaction of fish and various protein-holding substances. Likewise decay of organic materials furnishes, under certain conditions, putrescine and cadaverine. Putrescine can also be formed from arginine[4] and ornithine,[5] through bacterial activity, just as cadaverine can result from lysine.[5] Hence, it is not out of the question that the distillation of the acid-treated soil with magnesia may yield, in addition to ammonia, also organic bases. If the distillate contains ammonia only, then it must, when saturated with hydrochloric acid and evaporated to dryness, yield a salt which heated in a test tube will not melt nor be charred, but will sublime. The same

[1] A. E. Taylor, "On Fermentation," Univ. Calif., Publ. Pathol., 1, 223 (1907).

[2] The abbreviation "PTA" will stand for the words: phosphotungstic acid.

[3] *Ber.*, 18, 1922 (1885); 18, 86 (1885); also *Jahresbericht über die Fortschritte der Chemie*, 1858, 231, etc.

[4] *Z. physiol. Chem.*, 43, 338 (1904–5).

[5] *Ibid.*, 29, 334 (1900).

salt when treated with chloroplatinic acid must yield a compound, the analysis of which is bound to lead to the formula $(NH_4)_2PtCl_6$. This latter salt when decomposed with hydrogen sulfide must give pure ammonium chloride. All of these operations were actually made with positive result, thus showing the presence of ammonia only.

Further, if the PTA precipitate actually contains diamino acids we logically must expect them to show all the precipitation reactions which diamino acids generally display. Moreover, inasmuch as protein hydrolysis leads to α-amino acids of the general type $R.CH.NH_2.COOH$ all of which, with the exception of glycocoll, contain an asymmetric carbon atom, both the diamino acids and the monoamino acids if they be such compounds must, in accordance with the fruitful theory of van't Hoff[1] and Le Bel, be optically active. Furthermore, both must show the presence in their molecules of amino groups as well as of the carboxyl group. The presence of two amino groups in the diamino acids must cause them to show a distinctly alkaline reaction, while the monoamino acids in which one amino group is neutralized by one carboxyl group must show a neutral or slightly acid reaction. And if the NH_2 groups of the amino acids are fixed, *e. g.*, converted into the $-N=CH_2$ group by treating with formaldehyde,[2] then the COOH group must come into play by causing acid reaction, etc. All of the above-mentioned experiments and reactions having as their object the demonstration of the presence of acid amides and amino acids in the soil extracts have actually been carried out with positive results and are given in the experimental part of this paper.

Experimental.

In order to find out the nature of soil humus formed from various organic materials under field conditions the Experiment Station field located on the Wisconsin drift was divided into a number of plots of $^1/_{10}$ or $^1/_{20}$ of an acre each. The various plots were treated with materials like manure, hay, straw, etc., which on cultivated fields actually represent the principal sources for humus formation. For the purpose of rendering the sources of nitrogen in each plot easily reviewable it was thought best to put down the data in question in the form of a table (see Table I).

The percentage of total nitrogen found in the soil samples of the various plots (E, J, Q and U) was as follows: E, 0.278; J, 0.219; Q, 0.263; U, 0.262.

How much of this nitrogen was of organic nature could be answered only after the determination of the inorganic nitrogen has been made.

[1] van't Hoff, *Lagerung der Atome im Raume*. Braunschweig, 1894; also *Ber.*, 18 2277; 20, Ref. 448; 21, 265, etc.

[2] S. P. L. Sörensen, *Biochem. Z.*, 7, 48 (1907).

TABLE I.—THE SOURCES OF NITROGEN IN THE VARIOUS PLOTS.

Plot.	Treatment (per acre).	Crop.	V.	Remarks.
E	In 1906, a heavy application of manure	In 1906	Corn	The soil samples, the investigation of which is given in this paper, were collected in 1909, at the end of the summer, prior to the treatment of 1909 (which treatment for this reason has been omitted in the table). Each sample is a composite one of 12 or 24 borings taken from each plot at equal distances and equal depth, namely, from the surface down to seven inches depth. The samples collected were dried in the air, passed through a 20-mesh sieve, thoroughly mixed and bottled.
		In 1907	Oats	
	In 1907, one ton of timothy	In 1908	None	
	In 1908, one ton of timothy	In 1909	None	
J	In 1906, a heavy application of manure	In 1906	Corn	
		In 1907	Oats	
	In 1907, two tons of clover hay	In 1908	None	
	In 1908, two tons of clover hay	In 1909	None	
Q	In 1906, a heavy application of manure	In 1906	Corn	
		In 1907	Cow-peas	
	In 1907, no treatment	In 1908	Corn	
	In 1908, no treatment	In 1909	Oats	
U	In 1906, a heavy application of manure	In 1906	Corn	
		In 1907	Oats and clover	
	In 1907, no treatment			
	In 1908, no treatment	In 1908	Corn	
		In 1909	Corn	

The estimation of ammonia was carried out after Schlösing's[1] method slightly modified. The results will be found in Table II.

TABLE II.—PERCENTAGE OF AMMONIACAL NITROGEN IN THE VARIOUS PLOTS.

		Ammoniacal nitrogen found.				Ammoniacal nitrogen found.		
Plot.	Air-dry soil used. Grams.	Grams.	Per cent. of oven-dried soil.	Per cent. of total nitrogen in soil	Moist soil used. Grams.	Grams.	Per cent. of oven-dried soil.	Per cent. of total nitrogen in soil.
E	100	0.00261	0.0027	0.99	100	0.00182	0.0022	0.78
J	100	0.00317	0.0033	1.50	100	0.00197	0.0023	1.06
Q	100	0.00317	0.0033	1.25	100	0.00119	0.0014	0.51
U	50	0.00159	0.0033	1.26	100	0.00204	0.0023	0.89

Examining the data of Table II we notice that the figures of the air-dried samples do not agree with those of the moist samples despite the fact that they were simultaneously taken from the soil and prepared for analysis in exactly the same manner. The reason is that between the ammonia estimations of the air-dried samples which were made first and those of the moist samples there was a lapse of from three to five weeks. From the analytical data it is to be concluded that a part of the ammoniacal nitrogen in the moist samples must have changed. It was but natural to suppose, as we did, that the ammonia has been transformed into nitrates in which case the percentage of nitrates in the moist soil samples must be correspondingly higher than in the air-dried samples.

[1] *Technical Bull.* 4 (1909), Michigan Exp. Sta., page 11.

This supposition has been fully confirmed by the experiment, as can be seen from the data given in Table III.

TABLE III.—PERCENTAGE OF NITRIC NITROGEN IN THE VARIOUS PLOTS.

		Nitric nitrogen found.				Nitric nitrogen found.		
Plot.	Air-dry soil used. Grams.	Grams.	Per cent. of oven-dried soil.	Per cent. of total nitrogen in soil.	Moist soil used. Grams.	Grams.	Per cent. of oven-dried soil.	Per cent. of total nitrogen in soil.
E	214.02	0.00227	0.0011	0.40	344.59	0.01729	0.0060	2.16
J	264.15	0.00508	0.0020	0.91	418.61	0.02134	0.0060	2.75
Q	246.29	0.00031	0.0001	0.05	335.23	0.00637	0.0022	0.82
U	203.86	0.00026	0.0001	0.05	390.83	0.01312	0.0038	1.46

In looking over Table III we can readily see that the percentage of nitric nitrogen in the moist samples was found to be higher than in the air-dried samples, both calculated to the dry basis. However, by comparing Tables II and III we find that the decrease of ammonia in the moist soils was more than covered by the increase of nitrates in them, the reason being that between the nitrate estimations in the air-dried soils and those in the moist soils several more weeks have elapsed. Besides, it should be borne in mind that the soil samples contained, in addition to ammonia and nitrates, also organic nitrogen which in the moist soil constantly undergoes changes and makes the transformation in question more complicated. Whereas the increase of nitrates in the moist samples in a general way points to conditions in the plots being fairly favorable for the nitrification process, it should be remembered that it is the air-dried samples, the ammonia and nitrate content of which represents the amounts actually present in the soil.

Having ascertained that the sum of ammoniacal and nitric nitrogen is quite small, being but a few thousandths of one per cent. calculated to the oven-dried soils or not quite two per cent. of the total soil nitrogen, the next thing to do was to find a proper solvent for the organic nitrogen in the soils. Water, if it could dissolve out enough nitrogen, would be preferable to either acids or alkalies since it is likely to least change the organic nitrogenous bodies contained in the soils. It is for this reason that water was tried first with the results given in Table IV.

TABLE IV.—EXTRACTION OF SOIL NITROGEN WITH WATER.

				Nitrogen extracted.		
Plot.	Air-dry soil. Grams.	Distilled water. cc.	Hours digested.	Gram.	Per cent. of oven-dried soil.	Per cent. of total soil nitrogen.
E	50	750	10	0.00387	0.008	2.92
J	50	900	21	0.00835	0.017	7.88
Q	50	750	16	0.00768	0.016	6.06
U	40	800	16	0.00751	0.019	7.44

It will be observed that boiling for ten hours was not sufficient to get any appreciable amount of nitrogen in solution. And even boiling

during twenty-one hours did not dissolve out any considerable quantity, 7.88 being the highest percentage of nitrogen obtained. It was, therefore, decided to use acid for the extraction of soil nitrogen. Preference was given to hydrochloric acid because any excess of it can easily be removed by evaporation. A few preliminary digestions were made to ascertain the optimum conditions for the extraction of nitrogen from the soil samples. The digestions have demonstrated that boiling the soils with concentrated hydrochloric acid for from ten to fifteen hours or with 20 per cent. acid for from twenty to thirty hours extracted the maximum percentage of nitrogen, 83.94 being the highest percentage obtained.

The extraction of the nitrogen from the various soils, as well as its separation into the different organic groups, was accomplished as follows: Four or five or even six round-bottom flasks, each of which contained a mixture of 50 grams air-dry soil and 750 or 900 cc. hydrochloric acid, were heated to the boiling point and kept boiling under reflux condensers for from fifteen to twenty-four hours, depending on the concentration of the acid used. On cooling, the flask contents were made up with water to a definite volume, thoroughly shaken and filtered through dry filters. All of the filtrate or soil extract thus obtained, unless otherwise stated, was used for the experiments in connection with each individual plot.

A portion[1] of the extract, mostly 1000 cc., was evaporated on the water bath practically to dryness and the residue distilled in a 750 or 1000 cc. Kjeldahl flask with cream of magnesia from which every trace of ammonia was previously expelled by long boiling. The distilling substance was absorbed in 0.1 N H_2SO_4. Subtracting from the total ammonia found by titration the amount of ammoniacal nitrogen originally contained in the soil we find the proportion of the amido-nitrogen.

The residue in the Kjeldahl flask was twice extracted with boiling, ammonia-free water being filtered and washed with hot water after each extraction. Filtrates and washings were evaporated on the water bath to 100 cc. and, on cooling to 20°, treated with five grams of sulfuric acid and thirty cc. of a solution containing twenty grams of PTA and five grams of sulfuric acid per 100 cc. After twenty-fours hours the phosphotungstates were filtered out and washed with about 200 cc. of a solution containing 2.5 grams of PTA and 5 grams of sulfuric acid per 100 cc., the washing having been carried out by rinsing the precipitate from the filter into a beaker and returning to the filter three consecutive times. The ammonia resulting from Kjeldahlization of the precipitate represented the nitrogen of the diamino acids present in the soil.

[1] With very few exceptions, all of the estimations given in this paper were made in duplicates and their averages only recorded in the tables.

Subtracting the sum of ammoniacal, amido, and diamino nitrogen from 100, we find the percentage of monoamino nitrogen. For the sake of convenience the data were arranged in tabular form (see Table V).

TABLE V.—AMOUNT OF NITROGEN IN THE VARIOUS COMPOUNDS.

Plot.		Gram.	Per cent. of oven-dried soil.	Per cent. of total soil nitrogen.	Per cent. of nitrogen in solution.
E	Total nitrogen in solution (obtained by boiling with hydrochloric acid)....	0.10380	0.226	81.20	100.00
	Ammoniacal nitrogen.....	0.00127	0.0027	0.99	1.22
	Nitrogen of acid amides.....	0.02643	0.0575	20.67	25.46
	Nitrogen of diamino acids.....	0.01306	0.0284	10.21	12.58
	Nitrogen of monoamino acids (difference from 100).....	0.06304	0.1371	49.32	60.74
J	Total nitrogen in solution (obtained by boiling with hydrochloric acid).....	0.12795	0.1669	76.23	100.00
	Ammoniacal nitrogen.....	0.00252	0.0033	1.50	1.97
	Nitrogen of acid amides.....	0.03435	0.0448	20.46	26.85
	Nitrogen of diamino acids.....	0.01663	0.0217	9.91	13.00
	Nitrogen of monoamino acids (difference from 100).....	0.07445	0.0971	44.36	58.19
Q	Total nitrogen in solution (obtained by boiling with hydrochloric acid).....	0.1161	0.1983	75.41	100.00
	Ammoniacal nitrogen.....	0.00192	0.0033	1.25	1.65
	Nitrogen of acid amides.....	0.03835	0.0655	24.91	33.03
	Nitrogen of diamino acids.....	0.01432	0.0245	9.30	12.33
	Nitrogen of monoamino acids (difference from 100).....	0.06151	0.1051	39.95	52.98
U	Total nitrogen in solution (obtained by boiling with hydrochloric acid).....	0.09312	0.1864	71.12	100.00
	Ammoniacal nitrogen.....	0.00165	0.0033	1.26	1.77
	Nitrogen of acid amides.....	0.02858	0.0572	21.83	30.69
	Nitrogen of diamino acids.....	0.01137	0.0228	8.68	12.21
	Nitrogen of monoamino acids (difference from 100).....	0.05152	0.1031	39.35	55.33

Having separated the soil nitrogen into the various groups, the next thing to do was to demonstrate that the groups obtained in the manner described actually represented the acid amides, diamino acids and monoamino acids.

Acid Amides.

Inasmuch as acid amides, by boiling with mineral acids, split off their nitrogen as ammonia, we should naturally expect that the evaporated hydrochloric acid extract of a soil containing amides when distilled with magnesia would give pure ammonia, provided the soil does not contain, in addition to amides, any volatil organic bases. To establish the nature of the distillate the following experiments were carried out:

A definit amount of the soil extract, usually from 1000 to 1500 cc.,

was evaporated on the water bath to dryness, the residue distilled in a Kjeldahl flask with cream of magnesia, the distillate being absorbed in hydrochloric acid. It was next evaporated to dryness, the remainder taken up with some water whereupon chloroplatinic acid was added and the whole concentrated to syrupy consistency. On cooling, the substance was treated with 80 per cent. alcohol and set aside for a few hours. The chloroplatinate was filtered out, washed with alcohol and dried at 100°. After examining a few crystals under the microscope, a portion of the chloroplatinate was analyzed as to its platinum content. Another portion of it was dissolved in hot water and decomposed with hydrogen sulfide. The filtrate from platinum sulfide was (after decolorizing with animal charcoal, if necessary) evaporated to dryness, and the behavior of the residue observed on heating in the test tube over a burner.

Diamino Acids.

The residues which remained in the Kjeldahl flasks, after the amido nitrogen was expelled with magnesia in the form of ammonia, were thoroughly extracted with ammonia-free hot water, whereupon the extracts were concentrated to 100 cc. and separated by means of PTA into diamino acids (precipitate) and monoamino acids (filtrate) in the manner already described. In order to set free the diamino acids, the precipitate was treated with barium hydroxide and the excess of the latter removed with carbon dioxide when the filtrate from barium carbonate was evaporated to a few cubic centimeters. A small part of the liquid was used for various tests, especially for precipitation reactions, the bulk of it being used for polariscopic examination as well as for estimation of the diamino acids by the formaldehyde titrimetric method.

Monoamino Acids.

The filtrate from the PTA precipitate, supposed to contain monoamino acids, was freed from PTA by means of barium hydroxide, the excess of which was removed with carbon dioxide. The filtrate from barium carbonate was evaporated until crystallization began. On cooling, the crystals were separated by suction from the mother liquor, which on concentration yielded two more crystallizations. All of the crystals proved to consist principally of barium chloride mixed with some potassium and sodium chloride. The mother liquors were separated by suction from the crystals which were treated and washed with alcohol for the removal of organic matter adhering to them. Filtrates and washings were evaporated to a few cc. in order to completely remove the alcohol. The remainder was dissolved in some water, decolored with animal charcoal, filtered, concentrated to a small volume and used for the observation of the rotatory power. It was also analyzed by the formaldehyde titrimetric method. The essential data will be found in Table VI.

TABLE VI.

	Acid amides.			Diamino acids.		Monoamino acids.	
Plot.	Behavior in the test tube.	Microscopic examination of the platinochloride.	Analysis of the platino-chloride. % Pt.	Reactions	Rotatory power (in the 2 dm. tube).	Reaction with formaldehyde.	Rotatory power (in the 2 dm tube).
E	The chloroplatinate, it will be recalled, was prepared by the addition of chloroplatinic acid to the distillate which was obtained by distilling the evaporated acid extract of the soil with magnesia.	Examination of the platinochloride crystals, especially under the microscope, showed beautiful yellow, heavy, hard, translucent	43.94	The diamino acids (PTA precipitate) extracted from the plots (E, J, Q and U) showed the following reaction: 1. The aqueous solution was strongly alkaline.	E. The rotation of the diamino acid solution, with 0.013 gr. nitrogen, on acidulating with hydrochloric acid, was $\alpha = +0.1$ (Ventzke).	Addition of neutralized formaldehyde to the monoamino acids extracted from each of the soils examined, immediately brought about acid reaction pointing to the presence of carboxyl and amino groups.	E. The rotation of the aqueous solution of the monoamino acids (obtained from about 100 g. soil) was $\alpha = +0.35°$ (Ventzke). On acidulating with hydrochloric acid the same solution showed $\alpha^1 = +1.54°$.
J	The chloroplatinate thus obtained was dissolved in hot water, treated with hydrogen sulfide, the	octahedrons or prisms. This is	44.19	2. PTA gave a heavy, white precipitate.			J. Not examined.
Q	filtrate from platinum sulfide evaporated to dryness and the residue introduced into a test tube. On heating over the Bunsen burner the	true of all the plots, with the exception of J, the platino-	43.98	3. Mercuric chloride gave a gray flocculent precipitate, especially in the presence of barium hydroxide.	J. Not examined.		Q. The aqueous solution of the monoamino acids, under conditions similar to Plot E, showed the rotation $\alpha = -0.30°$. On acidulating with hydrochloric acid, it showed $\alpha = +0.45°$.
U	substance did not melt, but at once commenced to sublime, evolving white fumes which condensed on the cooler parts of the test tube. The sublimate could be driven forwards as heat was applied. It showed all the reactions of pure ammonium chloride. The above observations were made with all the plots.	chloride from which was not examined under the microscope.	44.09	4. Silver nitrate gave a grayish white precipitate, soluble in excess of ammonia. 5. Phosphomolybdic acid gave a yellow precipitate. 6. On addition of neutralized formaldehyde to the alkaline solution it soon became acid, indicating the presence of carboxyl and amino groups.	Q. The rotatory power of the diamino acid solution, under conditions similar to Plot E, was $\alpha^1 = +0.17°$. U. The rotation of the diamino acid solution, under conditions similar to Plot E, was $\alpha = +0.14°$.		U. The acidulated solution of the monoamino acids, under conditions similar to Plot E, showed the rotation $\alpha = +0.64°$.

[1] The amount of nitrogen contained in the tube could easily have been determined by Kjeldahlizing its content. It was, however, saved for the quantitative determination of the diamino (monoamino) acids by the formaldehyde-titrimetrical method. In addition, it should be borne in mind that estimation of the specific rotatory power was not feasible because of the probable presence of several diamino (monoamino) acids. Here, as in the other cases, it was simply intended to establish the fact as to whether or not the substances in question possess rotatory power.

Examining Table VI we find that the behavior in the test tube of the evaporated filtrate from platinum sulfide, as well as the microscopic picture of the platinochloride, leaves no doubt but that the substance in question represented pure ammonium chloride. This was also confirmed by the analysis of the chloroplatinates. In all cases the percentage of platinum found was a trifle above 43.91 per cent. of platinum required by the formula $(NH_4)_2PtCl_6$. The presence of an organic base would have diminished the percentage of platinum in the double salt.

The optical activity of the substances examined as well as the presence in their molecules of carboxyl- and amino-groups, as shown by the formaldehyde reaction, establish beyond doubt that they actually are amino acids and diamino acids, the latter being also verified by their precipitation reactions and the alkalinity of their aqueous solutions.

The Formaldehyde Titration Method.

In a series of remarkable articles Hugo Schiff[1] was the first to point to the importance of the reaction taking place between formaldehyde and amino acids. He showed that by means of this reaction the amino function can be separated from the acid function, since the formaldehyde neutralizes the influence of the NH_2 group by converting the atom complex $= C\begin{matrix}COOH\\NH_2\end{matrix}$ into $= C\begin{matrix}COOH\\N:CH_2\end{matrix}$. Thus, alanine, *e. g.*, is converted into methylene-alanine according to the equation

$$CH_3.CH\begin{matrix}NH_2\\COOH\end{matrix} + HCOH = CH_3.CH\begin{matrix}N:CH_2\\COOH\end{matrix} + H_2O.$$

As a consequence of this reaction amino acids, the aqueous solutions of which are practically neutral, show acid reaction immediately upon the addition of neutralized formaldehyde to their solution. This seemed to indicate that it might, perhaps, be possible to work out a method for the quantitative determination of amino acids. In following up this thought, Schiff studied protein solutions as well as a number of amino acids, like glycocoll, alanine, leucine etc., and succeeded in finding out some of the conditions which are necessary for the separation of the base function from the acid function. Thus, this author has laid the first foundation of the titrimetrical method for the quantitative estimation of amino acids, polypeptides and similar compounds. However, because he has not fully taken into consideration the influence of all reagents present in the above reaction, Schiff has not quite succeeded in working out the exact conditions of rendering the method a quantitative one. It is to Sörensen[2] and his coworkers that we owe the complete elabora-

[1] *Ann.*, 310, 25 (1900); 319, 59, 287 (1901); 325, 348 (1902).

[2] *Biochem. Z.*, 7, 48, 407 (1907); also *Z. physiol. Chem.*, 60, 1 (1909); 64, 120 (1910).

tion of this method. S. P. L. Sörensen has emphatically pointed out that the reaction $\begin{matrix}CH_3\\|\\CHNH_2\\|\\COOH\end{matrix} + HCOH + KOH \rightleftarrows \begin{matrix}CH_3\\|\\CHN:CH_2\\|\\COOK\end{matrix} + H_2O$ is reversible, and that the equilibrium of the system depends upon the quantity of each of the chemicals contained in it. While, for instance, an increase of formaldehyde or of potassium hydroxide (*i. e.*, of hydroxyl ions) will shift the station of equilibrium in the direction from left to right, an increase of the quantity of water is bound to shift the station of equilibrium in the opposite direction. From the above it is evident that, if the reaction is to take place in the direction from left to right as completely as possible, a considerable excess of formaldehyde is to be used, whereas the amount of water must be limited to a certain minimum. For this very reason it makes quite a difference whether the titration is performed with *N* or with 0.1 *N* solutions. The experiments by Sörensen have shown that titration with 0.2 *N* solutions gives the most satisfactory results, and in order to eliminate incidental influences of the individual reagents applied, Sörensen recommends to run simultaneously with the formaldehyde[1] titration of the substance also a check analysis with water, taking pains that the end volumes in both should be approximately equal. He further pointed out that if the titration of amino acids and check analyses is effected to pink color only (first phase), phenolphthalein being used as indicator, then the amount of standard alkali used is smaller than theory requires. Through further titration to distinctly red color (second phase) the analytical data are coming nearer to the theory. And by continuing the formaldehyde titration to strongly red color (third phase), the results obtained very closely approach and in some cases equal the theory. In other words, by titrating to a high enough hydroxyl ion content the results obtained by formaldehyde titration are as accurate as those furnished by most of the common titrimetrical methods.

All of the formaldehyde titrations given in this article were performed in accordance with the principles laid down by Sörensen. The reagents applied were: a one-half per cent. phenolphthalein solution; a formaldehyde mixture[2] made up of 50 cc. of 30 to 40 per cent. formaldehyde and 1 cc. of the above phenolphthalein solution to which mixture 0.2 *N* barium hydroxide was added until a weak pink color was reached.

For each individual formaldehyde titration we used 10, 20 or 30 cc. of the solution to be examined simultaneously with 10, 20 or 30 cc. of

[1] Sörensen uses the shorter expressions: "Formol-titration, formol-titrate" instead of the phrases: "Formaldehyde-titration, etc."

[2] *Biochem. Z.*, **7**, 64 (1907).

boiled distilled water as a check solution. To the solution to be estimated 10 cc. of formaldehyde and enough of 0.2 *N* barium hydroxide was added to obtain a red color. Then a couple cc. more of 0.2 *N* barium hydroxide were allowed to run in for the precipitation of carbonates and phosphates present in the soil. Then it was titrated back with 0.2 *N* hydrochloric acid to weak pink color. The check solution was treated exactly in the same way, *i. e.*, 10 cc. of the formaldehyde mixture were added to it and then so much of 0.2 *N* barium hydroxide as to get on titrating back to pink color with 0.2 *N* hydrochloric acid about the same volume as in the amino acid solution. The addition of one drop of 0.2 *N* barium hydroxide made the check solution turn distinctly red (second phase).

To the solution under examination 0.2 *N* barium hydroxide is added by drops until the red color of the check solution is reached.

Through the addition of two more drops of 0.2 *N* barium hydroxide to the check solution it turns strongly red (third phase). Now to the solution to be examined 0.2 *N* barium hydroxide is added dropwise until just this color has been reached.

Inasmuch as the formaldehyde titration method is quite a new one the desire was natural to find out by personal experience as to whether and to what degree the results obtained by that method are accurate. For this purpose we have prepared from casein, according to the directions given by Hlasiwetz[1] and Habermann, several amino acids among which the formaldehyde titrations of leucine and aspartic acid may be given here.

Leucine.—The preparation repeatedly recrystallized and dried at 100–105° C. yielded 10.67 per cent. N, whereas theory requires 10.70 per cent.

10 milligram-molecules, *i. e.*, 1.3117 grams, were dissolved in 50 cc. 0.2 *N* barium hydroxide and made up with water to 100 cc. Thus, a 0.1 *N* solution was obtained to every 2 cc., of which 1 cc. of 0.2 *N* barium hydroxide was added in advance. The formaldehyde titration of the solution, with phenolphthalein as indicator, yielded the following results: 20 cc., 10 cc. and 30 cc. of the solution of leucine required, after allowing for the blank, 9.7 cc., 4.95 cc. and 14.9 cc. of the 0.2 *N* barium hydroxide corresponding to 97.0, 99.0 and 99.3 per cent. of the theory.

Aspartic Acid.—The aspartic acid, which we have obtained as one of the decomposition products of casein, was carefully recrystallized and then dried at 100–105°. Its nitrogen content was found to be 10.64 per cent. Theory requires 10.55 per cent.

15 milligram-equivalents = 15/2 mg.-molecules (0.9983 g.) were dissolved in 75 cc. 0.2 *N* barium hydroxide and made up with water to 150 cc., so that a 0.1 *N* solution was obtained to every 2 cc., of which 1 cc. of 0.2 *N* barium hydroxide was added in advance. The formaldehyde titrations effected to strong red color (third phase), with phenolphthalein as indicator, as before, gave the following results: 10 cc., 20 cc., and 30 cc. of the aspartic acid solution required after allowing for its blanks, 4.95 cc.,

[1] *Ann.*, 169, 150 (1873).

10.0 cc. and 15.05 cc. of 0.2 *N* barium hydroxide, corresponding to 99, 100, and 100.3 per cent. of the thing.

We shall now give the results of the formol titrations with the diamino acids and monoamino acids which were obtained from the plots E, J, Q and U. Before doing so, however, it will be well to recall that while the monoamino acids contain in their molecule one carboxyl group and one amino group, the case is different with the diamino acids, as far as the number of amino groups is concerned. Whereas such diamino acids as lysine, diaminoacetic acid and diaminotrihydroxydodecanic acid contain for one carboxyl group two amino groups, histidine and arginine have, in addition to one amino-group, the imidazole ring and the guanidine group respectively. In other words, for one carboxyl group the monoamino acids have in their molecule one nitrogen atom, while lysine, diamino acetic acid and diaminotrihydroxydodecanic acid have two nitrogen atoms, and histidine and arginine three and four respectively. Inasmuch as the existence among the protein products of diaminoacetic acid recognized as such by Drechsel[1] is now very doubtful[2] and the diaminotrihydroxydodecanic[3] acid has been found so far among the decomposition products of casein only, it is probably safe to assume that the diamino acids most likely to occur in soils are histidine, arginine and lysine; and under the assumption that they are contained in soils in about equal proportions, we shall have to calculate three nitrogen atoms for each

TABLE VII.

Plot.	Diamino acids.	Monoamino acids.
E	50 cc. liquid containing 0.024 g. nitrogen extracted from the soil of this plot were subjected to the formaldehydetitration with the following result: (1)15 cc. substance required 0.85 cc. 0.2 *N* $Ba(OH)_2$. (2) 10 cc. substance required 0.60 cc. 0.2 *N* $Ba(OH)_2$. Average: 50 cc. substance required 2.91 cc. 0.2 *N* barium hydroxide which are equivalent to 0.03259 g. nitrogen (135.8 per cent.) if arginine alone were present; or to 0.02444 g. nitrogen (101.8 per cent.) if histidine only were present. The latter figure will also hold good if, in addition to any amount of histidine, the arginine and the lysine were present in about equal proportions. In other words, in both cases all of the nitrogen classified in Table V as that of diamino acids actually represents diamino nitrogen.	135 cc. liquid containing 0.10541 g. nitrogen were subjected to formaldehyde titration with this result: (1) 20 cc. substance required 5.0 cc. 0.2 *N* $Ba(OH)_2$. (2) 30 cc. substance required 7.7 cc. (3) 10 cc. substance required 2.6 cc. 0.2 *N* $Ba(OH)_2$. Average: 135 cc. substance required 34.50 cc. 0.2 *N* barium hydroxide equivalent to 0.09660 g. nitrogen = 91.64 per cent. This means that 91.64 per cent. of the nitrogen classified in Table V as that of monoamino acids actually represents monoamino nitrogen, the remaining 8.36 per cent. belonging to classes other than amino acids.

[1] *Ber. k. sächs. Ges. Wiss.*, **44**, 115 (1892).

[2] *Ber.*, **35**, 1378 (1902).

[3] E. Fischer and Abderhalden, *Z. physiol. Chem.*, **42**, 540 (1904).

TABLE VII (*Continued*).

Plot.	Diamino acids	Monoamino acids
J	The liquid containing the diamino nitrogen was not subjected to formaldehyde titration (having been lost by accident).	40 cc. liquid containing 0.0546 g. monoamino nitrogen were given the formaldehyde titration and required 17.96 cc. 0.2 *N* barium hydroxide equivalent to 0.05029 g. nitrogen equal to 92.11 per cent. In other words, 92.11 per cent. of what is classified in Table V as nitrogen of monoamino acids actually represent monoamino nitrogen.
Q	60 cc. liquid with 0.0305 g. nitrogen were given the formaldehyde titration with this result: (1) 10 cc. substance required 0.5 cc. 0.2 *N* $Ba(OH)_2$. (2) 20 cc. substance required 1.05 cc. 0.2 *N* $Ba(OH)_2$. Average: 60 cc. substance required 3.08 cc. 0.2 *N* barium hydroxide equivalent to 0.03450 g. nitrogen (113.1 per cent.) if arginine only were present; or to 0.02587 g. nitrogen (85.8 per cent.) if in addition to any amount of histidine, the other two, *i. e.*, arginine and lysine, were present in equal proportions.	60 cc. liquid with 0.1310 g. nitrogen were subjected to formaldehyde titration with the following result: (1) 10 cc. substance required 4.14 cc. 0.2 *N* $Ba(OH)_2$. (2) 20 cc. substance required 8.13 cc. 0.2 *N* $Ba(OH)_2$. Average: 60 cc. substance required 24.62 cc. 0.2 *N* barium hydroxide equivalent to 0.06894 g. nitrogen (52.63 per cent.). In other words, 52.63 per cent. of what is classified in Table V as such of monoamino acids actually represent monoamino nitrogen, the remaining 47.37 per cent. consisting of compounds not belonging to the amino acid class.
U	As the result of formaldehyde titration it was found that 65 cc. substance with 0.0203 g. nitrogen are equivalent to 2.27 cc. 0.2 *N* $Ba(OH)_2$ corresponding to 0.02542 g. nitrogen (125.22 per cent.) if arginine only were present; or to 0.01907 g. nitrogen (93.9 per cent.) if in addition to any amount of histidine, the arginine and lysine were present in equal proportions.	65 cc. liquid containing 0.08724 g. nitrogen were subjected to formaldehyde titration with the following result: (1) 10 cc. substance required 1.96 cc. 0.2 *N* $Ba(OH)_2$. (2) 20 cc. substance required 3.77 cc. 0.2 *N* $Ba(OH)_2$. Average: 65 cc. substance required 12.50 cc. 0.2 *N* barium hydroxide which are equal to 0.03500 g. nitrogen (40.12 per cent.). This means that of the nitrogen classified in Table V as such of monoamino acids, 40.12 per cent. actually belong to this class.

carboxyl group found by the formaldehyde titration. The conditions are quite simple with regard to the monoamino acids containing as they do one carboxyl and one amino group, hence by formaldehyde titration with 0.2 *N* barium hydroxide we have to calculate 2.8 milligrams of nitrogen for each cc. of 0.2 *N* barium hydroxide used.

The formaldehyde titration of the diamino acids and monoamino acids extracted from the various soils was carried out exactly in the same manner as that of leucine and aspartic acid, and is presented in Table VII.

Summary.

The methods described in this publication enable one to extract the bulk of the nitrogen present in the soil, and to separate this nitrogen into the various groups.

All of the organic nitrogen found, by the mode of separation herein described as amido nitrogen, is actually made up of acid amides.

Practically all of the organic nitrogen found as diamino nitrogen actually consists of diamino acids.

Concerning the monoamino nitrogen calculated by difference from 100, it was found that from 40.12 to 92.11 per cent. of what is given in Table V as nitrogen of monoamino acids actually belong to this class of compounds.

The amount of total nitrogen extracted from the soils of the various plots examined, by boiling with hydrochloric acid, was on the average 75.99 per cent. (from 71.12 to 81.20 per cent.), the balance (from 28.88 to 18.80 per cent.) having remained undissolved.

The acid-soluble nitrogen is made up as follows:

Ammoniacal nitrogen, from 1.22 to 1.97 per cent. (from 0.99 to 1.50 per cent. of the total soil nitrogen);

Nitric[1] nitrogen, from 0.07 to 1.19 per cent. (from 0.05 to 0.91 per cent. of the total soil nitrogen);

Nitrogen of acid amides, from 25.46 to 33.03 per cent.;

Nitrogen of diamino acids from 12.21 to 13.00 per cent.;

Nitrogen of monoamino acids from 22.20 to 55.66 per cent.

The rest of the organic nitrogen consists of compounds other than acid amides and amino acids.

By boiling with water, only a small proportion of nitrogen, namely from 2.92 to 7.88 per cent. of the total soil nitrogen, could be extracted from the various soils investigated. This, taken together with the fact that the bulk of the soil nitrogen is made up of acid amides and amino acids all of which are fairly soluble in water, makes it very likely that these compounds are present in the soil not in a free state, but in some kind of combination.

There is a marked difference between the organic nitrogen formed in the soil from comparatively fresh organic materials on the one hand and organic nitrogen formed from comparatively old organic materials on the other. While in the first case (Plots E and J), with the exception of some 8 per cent., the organic nitrogen consists of acid amides, diamino acids and monoamino acids, it contains in the second case (Plots Q and U) a considerable percentage (from 47 to 60 per cent.) of compounds belonging to classes other than acid amides and amino acids.

AMES, IOWA.

[1] The nitric nitrogen was estimated in water extracts.