STUDIES ON THE ACID AMIDE FRACTION OF THE NITROGEN OF PEAT. I¹

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During the past decade a large amount of work has been done on the distribution of the organic nitrogen in soil organic matter. In this laboratory special attention has been paid to the composition of peat soils. The result of the work has been to show that "in comparison with ordinary proteins, peat contains a larger percentage of acid amide and humin nitrogen and a smaller amount of basic and non-basic nitrogen compounds" (7).

In a general way the total nitrogen in peat is divided as follows:

	<i>per cent of total N</i>
Humin nitrogen.....	2.0-8.0
Ammoniacal nitrogen.....	0.5-0.8
Acid amide nitrogen.....	10.0-20.0
Non-basic amino nitrogen.....	22.0-40.0
Non-basic non-amino nitrogen.....	3.0-10.0
Basic amino nitrogen.....	3.5- 6.0
Basic non-amino nitrogen.....	1.5- 6.5

It will be observed from these figures that the largest two fractions are the acid amide and non-basic amino or mono amino acid fractions. In pursuance of a plan outlined some time ago the former is now receiving special attention in our work.

Before proceeding further with the discussion it might be well to state just what is meant by the acid amide fraction as applied to peat. By this term is meant the nitrogen that is evolved as ammonia upon treatment with acid and subsequent distillation after making alkaline. When dealing with pure protein material the statement is justified that this truly represents the nitrogen present in the form of acid amides. It has been contended, and the authors admit the contention, that in a complex and little known material like peat it is not safe to apply the same criteria to the various subdivisions obtained that are used in the treatment of pure proteins. Hence it must be understood that the term "acid amide fraction" as used in this work refers to a fraction showing the same behavior that acid amides do in proteins, but that

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in peat it may be composed of other sorts of compounds. In other words, strictly speaking it is a term of convenience. It is true that there is no definite idea as to what these compounds might be and from the work of Jodidi (3) the preponderance of such evidence as we have is against their existence, but, nevertheless, in view of the uncertainty of our knowledge of the constitution of peat, it seems advisable to accept the possibility.

THE SIGNIFICANCE OF ACID AMIDES IN SOILS

The value of organic nitrogen compounds as sources of nitrogen for plant nutrition depends largely upon the ease with which they are converted into ammonia. From the readiness with which acid amides can be made to split off ammonia in vitro it would seem to be a logical conclusion that in the soil this fraction would be the one to become ammonified most rapidly, i.e. would be the most "*available*." An attempt was made to connect this fraction with the availability of nitrogen in peat as determined by the alkaline permanganate method but with small success, and the presence of such a large percentage of nitrogen in this form also affords an indication that it is not as easily decomposed as would be anticipated on the above grounds.

Actual experimental data on the behavior of acid amides in soils have been obtained by several investigators, notably Jodidi, Kelley and Lathrop.

Jodidi (3) added weighed amounts of amino acids and acid amides to definite quantities of soil and determined the ammonia produced. He found that the results depended upon the structure of the particular compounds used but that acid amides and amino acids did not differ materially as classes so far as the rapidity of their ammonification in soil was concerned.

Kelley (4) found that the basic nitrogen fraction of the materials investigated was more easily ammonified than either the non-basic or acid amide fractions. As regards the last two groups sometimes one and sometimes the other was decomposed more rapidly.

The most exhaustive work on the subject is that of Lathrop (5, 6), who studied the changes in the various fractions of nitrogen in dried blood when added to soil. He found that during the early stages the amide nitrogen diminished very rapidly. It then increased to a maximum after which it slowly decreased in quantity. The conclusion that Lathrop drew from his work was that through the action of microorganisms the dried blood was destroyed with the synthesis of a new protein which was much more resistant to decomposition than was the original dried blood.

Summarizing the results that have been obtained up to the present it may be said that soil organic matter contains material of a protein nature formed from the decomposition of protein which differs from the latter in having a high content of amide nitrogen compounds. These compounds differ from the acid amides ordinarily found in proteins in that they are very resistant to ammonification by the agents which are usually efficient in causing this. So

pronounced is this characteristic that these compounds may actually be produced in soils which exhibit a very active ammonifying power on the amides of ordinary proteins added to them.

The question which at once presents itself is, Why are the amides of soil organic matter so different from those of proteins in this respect? It has been shown that there is apparently a close relationship between the amide nitrogen contents and the amounts of the dibasic acids glutaminic and aspartic, which are present in proteins. The recent work of Dakin (1) who, in using Foreman's (2) method of separation, found that caseinogen contained a much larger amount of glutaminic acid than had formerly been obtained, will necessitate a revision of the theories on this subject, at least so far as this particular protein is concerned. His discovery of a third dibasic acid, hydroxyglutaminic acid, also tends to complicate the matter. Whether or not these same factors enter into the situation as respects other proteins is as yet unknown and in order to test it out in the case of soil organic matter which contains such an unusually large amount of amide nitrogen we are attempting to determine the relative amounts of the dibasic acids in peat.

That material of the nature of that under investigation contains some glutaminic and aspartic acids is known from the work of Suzuki (8) who isolated both from the hydrolytic products of humus by the esterification method. So far as we know, however, there has never been any attempt made to estimate them quantitatively.

In the course of the extensive work which has been carried on in this laboratory with peat soils, repeated attempts have been made to isolate glutaminic acid as the hydrochloride directly from the hydrolysates. The ease with which it can be obtained from the hydrolysates of many proteins naturally tempted us to try to get it from our material. The following experiments are typical of the methods of procedure employed and the results obtained.

Three hundred grams of oven-dried brown peat were digested with 1500 cc. of concentrated HCl for 16 hours on the hot plate. The product was filtered and the residue boiled with water and filtered through a Büchner funnel, the process being repeated several times. The combined filtrates were then concentrated to a volume of about 100 cc. by the method used in previous work. During this process crystals of calcium sulfate separated out and were removed by filtration. They were probably formed from sulfuric acid carried over from the drying flasks. The resultant solution was saturated with dry hydrochloric acid gas at 0° and allowed to stand some days in the refrigerator. A precipitate of inorganic matter had separated out at the end of this time, which was removed and the filtrate concentrated to a thin sirup. It was again saturated with hydrochloric acid and placed in the refrigerator after being inoculated with crystals of glutaminic acid hydrochloride. A second crop of inorganic crystals resulted. These were removed, the filtrate and washings concentrated, inoculated and placed in the ice box but even on long standing no crystals separated.

It was apparent from this and similar experiments that either there was too little glutaminic acid present to be separated in this way or the conditions for its crystallization were not proper, as a result, perhaps, of the presence of some inhibiting substance. In this connection the following quotation from Foreman (2) is of interest:

The ease with which glutaminic acid hydrochloride separates seems to vary with the protein under treatment and with the amount of glutaminic acid present. Plimmer (1912) states that separation of the hydrochloride "occurs in the case of caseinogen and certain vegetable proteins which contain from 10 to 40 per cent of this amino acid." If the protein contains under 10 per cent, then as a rule, no hydrochloride will separate. Occasionally, however, a separation is obtained; thus Hopkins and Savery (1911) working with Bence-Jones protein obtained 8.05 per cent.

To shed some light on this point 100 gm. of oven-dried peat was mixed with 30 gm. of casein and boiled with 500 cc. of concentrated hydrochloric acid on the hot plate for 48 hours. The mass was filtered through asbestos and washed by repeatedly boiling the residue with water and sucking it dry on a Büchner funnel. The filtrate and washings were combined and concentrated at 50° until a sirupy consistency was reached. This sirup was saturated at 0° with dry hydrochloric acid gas and after inoculation with a few crystals of glutaminic acid hydrochloride was set in the ice box and allowed to stand several days. A precipitate formed which was filtered on asbestos and washed with cold hydrochloric acid. After one recrystallization from hydrochloric acid it was pure white and melted at 197° (uncor.). It weighed 3.97 gm., representing a yield of 13.2 per cent.

It was thus made apparent that with the relative quantities of peat and casein used fairly good returns of glutaminic acid could be expected on the basis of that contained in the casein alone. The indications were that the amount present in the peat must be small. An attempt was next made to determine approximately the limits of precipitation for glutaminic acid from hydrolysates of peat.

Ten grams of casein and 187 gm. of peat were hydrolyzed and worked up in the usual manner, the filtrate and washings being concentrated to a volume of 20 cc. As a control 10 gm. of casein were treated in the same manner. The hydrolysates were saturated with dry hydrochloric acid gas and allowed to stand in the refrigerator for 4 days.

The precipitate from the casein alone weighed 1.45 gm. and melted at 197.5°. A second crop of 0.033 gm. melting at 195° also was obtained. This yield compared well with the results of Osborne and Guest which were the highest obtained up to the time of this work. The conditions seemed to be favorable for the maximum precipitation of the acid.

The precipitate from the peat-casein mixture, however, weighed 8.12 gm. and was largely inorganic, containing the chlorides of iron, calcium and magnesium. It was dissolved in distilled water and boiled with a slight excess of sodium carbonate to precipitate the heavy metals. The precipitate was

filtered off and the filtrate acidified with hydrochloric acid. The greater part of the sodium chloride was precipitated by passing in dry hydrochloric acid gas without cooling the solution. The first crop of sodium chloride thus obtained carried with it no organic matter, but succeeding crops carried an increasing proportion of organic matter. When the glutaminic acid hydrochloride was obtained, however, it still held an appreciable quantity of salt.

The possibility of precipitating the inorganic chlorides first by omitting the inoculation with glutaminic acid hydrochloride was next attempted. Ten grams of casein and 200 gm. of peat were mixed, hydrolyzed and worked up in the usual manner. After concentration the extract was placed in the refrigerator without inoculation. Two crops of inorganic crystals were obtained. The mother liquor was saturated with hydrochloric acid gas, seeded with glutaminic acid hydrochloride and set in the ice box for a week. The precipitate which separated out was filtered, dissolved in distilled water and boiled with an excess of barium hydroxide to drive off ammonia. The excess barium was then removed quantitatively with sulfuric acid, the solution made up to a volume of 100 cc. and the amino nitrogen determined on an aliquot, with the following results: 1 cc. gave 1.72 cc. nitrogen at 23° and 742 mm.

This corresponds to 1.24 gm. of glutaminic acid hydrochloride, a yield slightly lower than that obtained from casein alone.

It was evident that with the quantities present in the last series of experiments considerable difficulty would be experienced in getting satisfactory yields of glutaminic acid by the direct method. Further attempts along this line of attack were discontinued without applying the results of the above work to peat alone because our attention was called to Foreman's method of isolating both glutaminic and aspartic acids which offered greater possibilities than did our own method.

Foreman's method depends upon the insolubility of the calcium salts of the dibasic acids in alcohol and has afforded very valuable results in the hands of its originator. Dakin has also used it to good advantage. As usual, in applying to peat a method which has been developed for use with pure proteins, difficulties were encountered which made deviations from the original procedure necessary. However, we were able to accomplish our purpose and have isolated both glutaminic and aspartic acids from peat. The technic has not yet been perfected to the point where conclusions regarding the quantitative relationships can be drawn, but the results are promising for the early accomplishment of this end.

Foreman found that upon adding an excess of lime to the hydrolysate of caseinogen, filtering it and adding alcohol to the filtrate, a precipitate was formed which consisted of the following fractions:

- (a) Glutaminic and aspartic acids.
- (b) A gummy substance very difficult to crystallize.

(c) A very small quantity of pigmented substance precipitated from the solution by silver sulfate solution.

(d) A substance precipitated by phosphotungstic acid.

The general method of procedure consisted in general in separating the hydrolysate into these fractions.

The first trial was made with a mixture of 10 gm. of casein and 200 gm. of peat, the same quantities used in the second set of preliminary experiments described above. The mixture was hydrolyzed by boiling with a liter of concentrated hydrochloric acid and worked up as in the preceding cases. The sirup resulting from the concentration of the filtrate and washings from the insoluble residue was taken up in about 400 cc. of water and treated with a cooled paste of calcium hydroxide. After shaking thoroughly the precipitate was filtered off and washed free from chlorides by repeated suspension in water and filtering. The combined filtrate and washings were concentrated *in vacuo* until a fairly concentrated but not sirupy solution was obtained. This was transferred to a 2-liter flask and alcohol (about 97 per cent) added in small portions at a time, the mixture being shaken thoroughly after each addition. A black sticky precipitate formed on the bottom and sides of the flask. It was filtered through a Büchner funnel and washed as thoroughly as possible with alcohol. After dissolving it in about 300 cc. of water the calcium was removed by means of oxalic acid. The chlorides and some of the coloring matter were then removed by means of silver sulfate solution. After filtering off the precipitate the excess silver was removed by passing hydrogen sulfide through the solution. The filtrate from the silver sulfide was concentrated *in vacuo* until all of the hydrogen sulfide had been removed. Phosphotungstic acid was then added until precipitation was complete. The solution at this point was still strongly colored. After filtering off the precipitate the excess phosphotungstic acid was removed by means of barium hydroxide and the excess barium in turn quantitatively removed by means of sulfuric acid. The filtrate from the barium sulfate was concentrated as far as possible by distillation *in vacuo* and then transferred to a weighed evaporating dish and dried over phosphorous pentoxide.

In the dry state the residue weighed 11.068 gm. and contained a large quantity of inorganic matter. It was dissolved in water, treated with an excess of barium hydroxide, filtered and the excess barium removed as before. This time the dried residue weighed 9.4907 gm. It was a hard gritty mass and very difficult to triturate with glacial acetic acid which was the next step in the process and required most of a day. The acetic-acid-insoluble residue when dried weighed 4.4055 gm. It was dissolved in water and made up to a volume of 200 cc.

The acetic-acid-soluble part was freed from acetic acid by distillation *in vacuo*. The gummy residue was dissolved in water and made up to a volume of 50 cc.

Then 100 cc. of the solution of the acetic-acid-insoluble part was boiled with an excess of copper carbonate. The filtrate from the excess copper

carbonate was concentrated *in vacuo* and from it on standing overnight was obtained a large crop of fine light blue needles. These were filtered from the mother liquor which was further concentrated but it yielded no more crystals. Two crops of crystals were obtained on recrystallizing the product from hot water. They weighed, respectively, 0.2917 and 0.1055 gm., making a total of 0.3972 gm. from the 100 cc. used, or 0.7944 gm. from the total sample of peat casein mixture. This corresponds to 0.5420 gm. of aspartic acid.

After removing the copper by means of hydrogen sulfide from the filtrate from the copper aspartate and distilling off the excess hydrogen sulfide *in vacuo*, the residue was dried in a desiccator over sulfuric acid. It weighed 1.9211 gm. This residue was boiled with concentrated hydrochloric acid, cooled and saturated with dry hydrochloric acid gas, after filtering off the brown precipitate that formed during the boiling with hydrochloric acid. After inoculating with a few crystals of glutaminic acid hydrochloride the solution was allowed to stand in the refrigerator for 3 days. The perfectly white crystals were filtered on asbestos. They weighed 0.6234 gm. and melted at 197°, being apparently quite pure. A second crop of less pure product was obtained, which after treatment with animal charcoal melted at 196° and weighed 0.0307 gm. The total amount of pure glutaminic acid hydrochloride in the hydrolysate of the peat-casein mixture calculated from these yields was 1.31 gm., corresponding to 1.05 gm. of glutaminic acid. This, of course, does not include that in the mother liquors from the original crystallization and from the recrystallizations. These solutions were transferred to a 100-cc. flask and made up to volume. An analysis for amino nitrogen gave the following results: 1 cc. gave 1.84 cc. nitrogen at 23.5° and 749 mm.

The total amino nitrogen content based on these figures was 0.1017 gm. for the 100 cc., or 0.2034 gm. for the whole hydrolysate. This corresponds to 2.66 gm. of pure glutaminic acid hydrochloride. Undoubtedly there was much glutaminic acid present, but as no attempt was made to remove the ammonia before making the above analysis the figures are not entirely accurate.

The next step was to try out the method on peat alone. As over half of the above quantities of glutaminic and aspartic acids could be accounted for by the presence of the 10 gm. of casein, it was decided to use a sufficient quantity of peat to insure getting measurable amounts of these compounds. Accordingly, 3 kgm. of peat was hydrolyzed as usual with a solution of 9 liters of concentrated hydrochloric acid and 6 liters of water, this giving approximately a constant boiling mixture. The resulting hydrolysate was concentrated *in vacuo* to about a liter, cooled and saturated with dry hydrochloric acid gas. Three crops of inorganic crystals were obtained, the last mother liquor being diluted to about 2 liters and treated with a cooled paste of calcium hydrate. About 125 gm. of CaO were required to give an excess. The large gelatinous precipitate produced was washed free from chlorides and the filtrate and washings concentrated *in vacuo* to about 500 cc. As the

resulting solution was rather viscous 100 cc. of water was added to it and then alcohol in small portions. A black, sticky precipitate was formed and was not completely thrown down by 5 liters of alcohol. However, at this point it was filtered off and the alcohol distilled from the filtrate, which was then further concentrated. Alcohol was then added to it, but precipitation was still incomplete when 5 liters had been added. The precipitate, however, was filtered off at that point, the alcohol distilled from the filtrate and the liquid again concentrated. It was taken up in a small quantity of water and added in small amounts to comparatively large volumes of alcohol, the resulting precipitates being filtered through the same filter.

The precipitates were combined, washed as thoroughly as possible, dissolved in water and freed from calcium by means of oxalic acid. Coloring matter and chlorides were removed with warm silver sulfate solution, the excess silver precipitated with hydrogen sulfide and the filtrate concentrated *in vacuo*.

Phosphotungstic acid was then added until precipitation was complete, a large quantity being required. The excess was removed as usual by means of barium hydrate and the excess of the latter was precipitated quantitatively by sulfuric acid. The final filtrate was concentrated by distillation *in vacuo* and finally dried in a weighed casserole in a vacuum desiccator over sulfuric acid and P_2O_5 . The resulting product was a hard brittle mass weighing 91.8 gm.

Considerable difficulty was experienced in extracting this material with acetic acid, but finally after triturating it in a porcelain casserole with small portions of acid, 41 gm. of a grey powder was obtained.

The extract was concentrated *in vacuo* to a stiff gum and placed in a vacuum desiccator over KOH. Even after standing thus for 5 months it still smelled strongly of acetic acid.

SEPARATION OF GLUTAMINIC AND ASPARTIC ACIDS

The acid-insoluble grey powder contained 41.59 per cent of ash, which consisted apparently of oxides of tungsten, and 8.5 per cent of amino nitrogen figured on the ash-free basis. Two grams of it was dissolved in water, treated with barium hydrate to remove ammonia and the excess barium removed quantitatively with sulfuric acid. After treating with bone-black it was concentrated *in vacuo* and made up to approximately a liter. This solution was treated with an excess of copper carbonate and filtered. The filtrate and washings were concentrated to 500 cc. *in vacuo* and allowed to stand overnight. Light blue needles separated, and were filtered, dried and weighed. The yield was 0.4609 gm.

The filtrate was freed from copper and concentrated *in vacuo* to about 15 cc. It was saturated in the cold with hydrochloric acid, inoculated with glutamic acid hydrochloride and allowed to stand several days in the ice box.

A crop of 0.1248 gm. of a white crystalline compound melting at 197° was obtained. The above work was now repeated with 25 gm. of the acetic-acid-insoluble substance. From this amount of material 5.716 gm. of the copper salt of aspartic acid and 2.00 gm. of glutaminic acid hydrochloride were obtained. These figures are for the purified products and do not take into consideration the residues left in the mother liquors.

0.0556 gm. copper salt was dissolved and made up to 25 cc.; 5 cc. samples gave 1.45 and 1.46 cc. of nitrogen at 22° and 743 mm.; average per cent amino nitrogen found 7.16; theory 7.19.

A portion of this copper salt was converted into the free acid.

0.0593 gm. was dissolved in water and made up to 25 cc.; 5-cc. samples gave 2.31 and 2.28 cc. of nitrogen at 25° and 744 mm.; average per cent amino nitrogen found, 10.51; theory 10.52.

The glutaminic acid hydrochloride melted at 197.5°.

0.0904 gm. was dissolved in water and made up to 10 cc.; 2-cc. samples gave 2.55 and 2.58 cc. of nitrogen at 25° and 736 mm.; average per cent amino nitrogen found 7.62; theory 7.63.

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Estimation of pyrrolidon carboxylic acid

Foreman (6) has suggested a method for estimating the pyrrolidon carboxylic acid present by taking advantage of the fact that on boiling with hydrochloric acid it is converted into glutaminic acid with a corresponding increase in amino nitrogen.

A portion of the gum obtained in the above acetic acid extraction was analyzed for amino nitrogen.

0.0564 gm. gave 4.01 cc. of nitrogen at 23.5° and 742 mm. This corresponds to 3.88 per cent, or 1.971 gm. of amino nitrogen in 50.8 gm. of gum.

A portion of this gum was dissolved in water.

1 cc. of this solution gave 6.19 cc. of nitrogen at 24.5° and 753 mm. corresponding to 3.413 mgm. of amino nitrogen.

A 5-cc. portion was saturated in the cold in a small glass bulb with hydrochloric acid gas, a condenser was attached and the solution boiled for 15 hours. The contents of the bulb were diluted to 25 cc. and analyzed for amino nitrogen.

A 2.5-cc. sample gave 3.42 cc. of nitrogen at 24.5° and 744 mm. This corresponds to 3.724 mgm. per cubic centimeter, or 9.12 per cent of the total amino nitrogen.

The original 50.8 gm. of gum contained 1.971 gm. of amino nitrogen. Hence there would have been for the whole quantity of gum a total increase of 0.1798 gm. corresponding to a content of 1.657 gm. of pyrrolidon carboxylic acid.

This estimation was checked by extracting the pyrrolidon carboxylic acid from the gum with absolute alcohol. A portion of the gum weighing 12.65

gm. was triturated with absolute alcohol. The gum obtained by boiling off the alcohol was dissolved and made up to 50 cc. Of this 4 cc. was diluted to 25 cc.

1 cc. gave 0.72 cc. of nitrogen at 23.5° and 742 mm., corresponding to a total amino nitrogen content of 0.1228 gm. for the 12.65 gm. of the original gum.

Five cc. were hydrolyzed as before and made up to 25 cc.

1 cc. gave 1.30 cc. of nitrogen at 23.5° and 742 mm. This corresponds to a total of 0.1774 gm. of amino nitrogen in the 12.65 gm. of gum, or an increase of 0.0546 gm.

This is equivalent to a content of 2.0214 gm. of pyrrolidon carboxylic acid in the original gum as compared with 1.657 gm. by the former determination.

A 40-cc. portion of the solution of the gum was saturated with dry hydrochloric acid and boiled for 16 hours. After filtering off a flocculent precipitate, treating with animal charcoal and concentrating *in vacuo* it was seeded with glutaminic acid hydrochloride and placed in the refrigerator. After standing a few days a small crop of crystals was obtained, which in melting point and appearance under the microscope appeared to be identical with glutaminic acid hydrochloride. While it is possible that this arose from glutaminic acid dissolved by the acetic acid, it is more likely that it was formed from the pyrrolidon carboxylic acid since treatment of the gum directly without previous hydrolysis failed to yield anything resembling this compound.

DISCUSSION

This preliminary work shows several things of interest. In the first place, it shows that both glutaminic and aspartic acids are obtainable from peat by the methods employed. It also indicates the presence of pyrrolidon carboxylic acid, a fact which, of course, would be expected if glutaminic acid were present.

It is also apparent that while Foreman's method is applicable in a general way, certain details must be changed if it is to give satisfactory results with peat. This statement is true regarding the application to peat of any method devised for pure proteins. The presence of a comparatively high content of inorganic material and of humic organic substances introduces complications which make it necessary to make more or less extensive changes. In the present instance, for example, it becomes advisable to concentrate the original hydrolysate and saturate it with hydrochloric acid gas in order to remove as much of the inorganic matter as possible. But even then subsequent precipitates with the various reagents, such as calcium hydrate, phosphotungstic acid and silver sulfate are abnormally large as compared with those obtained in working up pure protein material. It is correspondingly difficult to wash them clean and at best they probably carry down more or less organic material which is ordinarily soluble.

The separation of the aspartic from the glutaminic acid in the acetic-acid-insoluble residue was not as sharp as was hoped for. The copper aspartate seemed to crystallize out almost completely in the first crop, for a further concentration yielded a very small amount of the light blue needles. When the glutaminic acid had been removed as completely as possible, however, the residue was again worked up for copper aspartate and a considerable quantity obtained. Carrying out the separation in the reverse order yielded no better results, as the glutaminic acid hydrochloride failed to crystallize readily and a humus-like precipitate came down in its place. It is possible that there is present some hydroxyglutaminic acid which complicates matters at this point.

The work is being continued along the lines suggested by this preliminary investigation.

SUMMARY

Attempts to separate glutaminic acid directly from the hydrolysate of peat have failed.

The application of Foreman's method has resulted in the separation of both glutaminic and aspartic acids and the estimation of pyrrolidone carboxylic acid from this material.

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