CCCXXIII. THE DETERMINATION OF GLUTAMINE IN THE PRESENCE OF ASPARAGINE.[1]

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Although glutamine has been known for many years to be a constituent of certain plant tissues [Schulze and Bosshard, 1883], no method whereby this substance can be separately distinguished from asparagine and determined with accuracy has appeared until recently. Glutamine has indeed frequently been isolated but its properties are such that isolation has only qualitative significance. Chibnall and Westall [1932] noted that the amide group of glutamine is extensively hydrolysed by heating at 100° for 3 hours at p_H 8, whereas asparagine is scarcely affected by this treatment. Accordingly they suggested that the glutamine content of a plant tissue extract might be estimated with an error of about 10% if the increase in ammonia, on hydrolysis of the extract at p_H 8, were multiplied by a factor (1·4) derived from the results of experiments on the pure substance. They further observed diminution of the amino-N, during such hydrolysis of glutamine, at a rate parallel with the production of ammonia; this fall in the amino-N of a plant tissue extract, under the conditions of hydrolysis mentioned, provides valuable additional evidence of the presence of glutamine and distinguishes the latter from other substances, especially urea and allantoin, which likewise give rise to ammonia on mild hydrolysis.

Recent experience with Chibnall and Westall's method in the Connecticut Agricultural Experiment Station laboratory indicated that the accuracy could be appreciably improved by certain modifications in the technique, and in particular, in the reaction chosen for the hydrolysis. Chibnall and Westall found that glutamine is apparently very little hydrolysed at p_H 6 and 7; the observations at New Haven, on the contrary, showed it to be completely hydrolysed at these reactions. A joint study in the two laboratories of the factors involved revealed the presence of a systematic error in the method used by Chibnall and Westall to determine the ammonia produced by hydrolysis at these particular reactions. They had employed phosphate buffers at p_H 6 and 7 and had subsequently determined ammonia by distillation *in vacuo* with excess of magnesium oxide. Under these conditions insoluble magnesium ammonium phosphate may form, which is not completely decomposed during distillation at 40°. We have since found that Kostychev [1931] has drawn attention to the possibility of this source of error in the determination of ammonia.

The extreme scarcity of glutamine at the time of Chibnall and Westall's experiments prevented the thorough investigation of the stability of glutamine

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at various reactions which would have revealed this difficulty. Recently however an improved method of isolation [Vickery *et al.*, 1935] has permitted re-investigation of the stability of glutamine in both the New Haven and the South Kensington laboratories with a view to improving the method of its determination in plant tissues. The results are embodied in the present paper.

The stability of glutamine.

The fundamental operation upon which all determinations of amides depend is the estimation of ammonia. Some of the difficulties that beset this determination have been discussed by Pucher *et al.* [1935]. Their procedure (modified from Boussingault [1850]) was employed for the following determinations.

Effect of p_H. A specimen of glutamine containing 18·94% N was employed. Of this 0·1056 g. was dissolved in water and diluted to 200 ml.; 5 ml. therefore contained 0·25 mg. of amide-N. The solution was unchanged in amide-N after 5 days at 0°. Aliquots of 5 ml. were mixed with 10 ml. of an appropriate buffer solution in 25 × 200 mm. test-tubes which were closed by rubber stoppers carrying a short length of fine-bore capillary tubing. At reactions of p_H 7 or higher a small bulb trap charged with 3 ml. of 0·1 *N* HCl was attached to the capillary to provide against loss of ammonia. The test-tubes were placed in a boiling water-bath for 2 or 3 hours and were then kept in cold water until analysed (not over 3 hours). The buffer solutions used were mixtures of 0·05 *M* succinic acid and 0·05 *M* borax for the range p_H 3 to 5, and 0·1 *M* potassium dihydrogen phosphate and 0·05 *M* borax for the range p_H 6 to 9 [Kolthoff, 1926]. The individual mixtures were checked by the quinhydrone electrode at room temperature (22 to 24°), and were all within 0·05 unit of the required p_H. After being heated with glutamine as described, the reactions did not change more than $\pm 0{\cdot}1$ p_H unit.

The data in Table I show that glutamine is least unstable in the region of its isoelectric point, but, outside this range, is rapidly and completely hydrolysed. The data for p_H 3, 4 and 5 confirm the earlier figures of Chibnall and Westall.

Table I. *Hydrolysis of glutamine at different reactions in buffered solutions at 100°.*

Figures are % of the amide-N liberated as ammonia.

p_H ...	3·0	4·0	5·0	5·3	5·5	5·7	6·0	7·0	8·0	9·0
2 hours' hydrolysis	89·8	77·0	69·2	73·1	76·3	79·0	98·6*			
3 hours' hydrolysis	91·0	85·3	81·6	81·1	81·2	89·4	98·3†	99·0	98·4	99·6

* The average of 11 determinations ranging from 94 to 103.
† The average of 6 determinations ranging from 96 to 101.

Effect of temperature. 5 ml. samples of standard glutamine solution were heated in a water-bath at different temperatures with 10 ml. of buffer for 2 hours; the tubes were cooled and Nessler's solution was added to the contents, which

Table II. *Hydrolysis of glutamine at* p_H *6·5 in 2 hours at various temperatures.*

Figures are % of the amide-N liberated as ammonia.

Temperature ° C.	Hydrolysis %
44 ± 2	3·2 to 3·8
63 ± 2	17·0 to 19·0
80 ± 2	53·0 to 56·0
100	98 to 100

were then diluted to 50 ml. and read at the spectrophotometer. Suitable controls with standard ammonia solution showed that this procedure was permissible. The results in Table II show that it is necessary to heat glutamine to 100° in order to bring about complete hydrolysis in 2 hours.

Effect of time. The data given in the fifth column of Table III show the time course of the hydrolysis of glutamine at p_H 6·5. Within the accuracy of the method as applied to the measurement of 0·25 mg. of NH_3-N, hydrolysis is evidently complete in 2 hours.

The products of hydrolysis of glutamine.

Table III shows the rate at which amino-N disappears when a solution of glutamine is heated to 100° at p_H 6·5. 5 ml. samples of standard glutamine solution containing 0·5 mg. of amide-N were heated with 10 ml. of buffer for the stated times. The ammonia was then distilled off in the usual way, the residue was acidified with 2 ml. of glacial acetic acid and diluted to 50 ml., and of this solution 5 ml. were used to determine amino-N in the Van Slyke manometric apparatus [Peters and Van Slyke, 1932]. The values for 15 and 30 min. are each the average of 6 closely agreeing determinations, that for 60 min. the average of 4, and the rest are the averages of duplicates.

Table III. *The rate of hydrolysis of glutamine at* p_H *6·5 and 100°, as measured by the change in the α-amino-N as well as by the production of ammonia; 1·0 mg. of glutamine-N present in each case.*

Time of heating min.	Apparent amino-N mg.	Disappearance of α-amino-N %	Ammonia-N mg.	Increase of ammonia-N as % of total amide-N %	Ratio decrease in amino-N/ increase in ammonia-N
0	0·901	—	0	0	—
15	0·592	38·4	0·145	29·0	2·13
30	0·421	58·2	0·235	47·0	2·04
60	0·151	86·0	0·399	79·8	1·88
90	0·051	94·6	0·470	94·0	1·81
120	0·014	98·4	0·492	98·4	1·80

The rate of ammonia production was ascertained in a separate series of experiments in which the hydrolysates were treated directly with Nessler's solution.

Glutamine in freshly prepared solution yields 90 % of its total N as nitrogen gas in the Van Slyke manometric apparatus in 4 min. at 22·5°. This observation confirms that of Chibnall and Westall who found 92 % after 10 min. shaking in the ordinary Van Slyke amino-N apparatus and, assuming that all of the α-amino-group reacts in this time, indicates that approximately 80 % of the amide group also is decomposed. The figures in column 3 of Table III are calculated on the assumption that the amide-N that was not hydrolysed reacted to the extent of 80 % with nitrous acid. A correction of this magnitude was therefore applied to the apparent amino-N figures in order to ascertain the proportion of the α-amino-N that had disappeared from the system; the figures agree reasonably well with the proportion of the amide-N that was hydrolysed (column 5). It is obvious that the hydrolysis of glutamine is accompanied by a parallel loss of α-amino-N.

A number of observations indicate, however, that the behaviour of glutamine in neutral aqueous solution is by no means simple. For example, a solution which has been stored at 0° for several days may yield only 84 % or less of its

N in the Van Slyke apparatus although in this period no appreciable quantity of ammonia is liberated. Furthermore, partly hydrolysed solutions of glutamine invariably contain less α-amino-N than they should when this is calculated according to our best information regarding the behaviour of glutamine amide-N in the Van Slyke apparatus. Perhaps when the true structure of glutamine has been elucidated by modern physico-chemical methods the anomalous behaviour of this substance in solution may become clear.

The ultimate product of the amide hydrolysis of glutamine is evidently a substance which does not contain amino-N. The most obvious explanation is that hydrolysis is accompanied by lactam formation to give pyrrolidonecarboxylic acid.

In order to make certain that this is the case, 1·0 g. of glutamine (0·190 g. N) was boiled with 100 ml. of water for 4 hours; the solution then contained 0·0845 g. of ammonia-N and 0·0117 g. of apparent amino-N, indicating hydrolysis of 88·9 % of the amide groups and disappearance of 96·4 % of the α-amino-groups. The solution was concentrated *in vacuo* to 10 ml. and a drop of silver nitrate solution was added; this precipitated a trace of brown flocculent material which was filtered off and discarded. The filtrate was then evaporated in a vacuum desiccator to 8 ml. and 1·2 g. (1 equivalent) of silver nitrate dissolved in 2 ml. of water were added. Crystallisation of the silver salt began at once, and the white needles were filtered off and washed with alcohol after the solution had been chilled for a few hours. The yield was 1·281 g. or 82·3 % of the theoretical, calculated from the amino-N data, or 89·1 % calculated from the data for amide hydrolysis. The crystals contained 45·4 % Ag and 5·92 % N (calc. 45·7 % and 5·94 % respectively). Clearly therefore the chief products of the hydrolysis of glutamine in substantially neutral solution are pyrrolidonecarboxylic acid and ammonia.

The stability of asparagine.

Inasmuch as asparagine is frequently present in plant extracts together with glutamine, the stability of the former amide was also studied. The conditions adopted were identical with those employed with glutamine, save that the standard solution of asparagine contained 1 mg. of amide-N in the 5 ml. aliquot added to the buffer solution. The data in Table IV show that asparagine, like glutamine, is most stable in the region of its isoelectric point, but that the proportion hydrolysed at p_H 7 and above in 3 hours is quite appreciable. It is obviously inadvisable to conduct glutamine determinations in the presence of asparagine if the reaction of hydrolysis is above p_H 7; the result of the hydrolysis at p_H 6·5 for 2 hours shows that interference from asparagine with a glutamine determination at this reaction is ordinarily negligible.

Table IV. *Hydrolysis of asparagine at different reactions in buffered solutions at 100°.*

Figures are % of the amide-N liberated as ammonia.

p_H ...	4	5	6	6·5	7	8	8·5	9
3 hours' hydrolysis	2·4	1·8	2·1	2·6*	6·6	14·0	16·3	18·5

* Hydrolysis for 2 hours.

In order to hydrolyse the amide group of asparagine completely it is necessary to employ dilute acid. Vickery and Pucher [1931] recommended 2 *N* H_2SO_4, and a hydrolysis time of 6 hours. Subsequent experience has shown these conditions to be unnecessarily severe. When asparagine is heated with *N* H_2SO_4

86·7 % of the amide-N is hydrolysed in 1 hour, 94·9 % in 2 hours and 100·2 % in 3 hours. Evidently therefore a 3-hour period of hydrolysis at 100° with N H_2SO_4 is adequate.

The determination of glutamine in solution.

The method of determining glutamine that has been developed from the foregoing data depends on the observation that its amide group is completely hydrolysed in 2 hours at 100° within the range p_H 6 to 7, whereas the amide group of asparagine is very slightly affected under these conditions. Owing to differences of equipment, the practical details of the method differ slightly in the two laboratories. In the New Haven laboratory the procedure is as follows.

A suitable aliquot of the solution (not over 5 ml.) is pipetted into a 25 × 200 mm. test-tube together with 10 ml. of buffer solution at p_H 6·5. The tube is closed with a rubber stopper carrying 20 cm. of 1 mm. bore heavy wall glass tubing, the lower surface of the stopper and the orifice of the tube being previously moistened with a few drops of water. The tube is placed in a constant-level boiling water-bath for exactly 2 hours and is then removed and cooled in cold water, a few drops of water being at the same time allowed to be drawn down through the capillary in order to wash back any ammonia that may have volatilised. The contents of the tube are washed into the ammonia distillation apparatus [Pucher *et al.*, 1935] with 20 ml. of water, and the ammonia is distilled *in vacuo* at 40° after addition of 3 ml. of a reagent prepared by dissolving 5 % of borax in 0·5 N NaOH. The distillate is diluted, treated with 5 ml. of Nessler's [Koch and McMeekin, 1924] solution, made up to a volume of 50 ml. and the extinction coefficient determined by means of a Zeiss-Pulfrich spectrophotometer. The quantity of ammonia in the distillate is obtained from the calibration curve of the instrument and is corrected for the apparatus blank.

For the determination of glutamine in plant tissue extracts a suitable aliquot, together with water to make 5 ml., is placed in the test-tube together with 10 ml. of a phosphate-borate buffer of such reaction and molar strength as to give a final reaction, after the 2-hour hydrolysis, close to p_H 6·5. In many cases a 0·1 M buffer of p_H 7·0 is satisfactory, but occasionally buffers of two or four times this concentration may be required. Trials are necessary in each new case. The hydrolysis and determination of the ammonia are conducted as described, and the final figure is corrected for the free ammonia of the tissue.

The procedure employed in the South Kensington laboratory is similar, but the ammonia in the distillate is normally determined by titration with standard acid in the usual way.

The determination of asparagine in solution.

The method is based on the data for the stability of asparagine already given. An aliquot of the solution together with sufficient water to make a total volume of 5 ml. is mixed with 1 ml. of 6 N H_2SO_4, and heated in a test-tube as described for 3 hours at 100°. The solution is then washed into the ammonia distillation flask with 20 ml. of water; 5 ml. of 1 N NaOH are added followed by 5 ml. of the alkaline borate mixture and the ammonia is distilled, determined and corrected for the blank in the usual way.

This procedure, when applied to a plant tissue extract, gives the total amide-N together with the free ammonia-N of the solution. Corrections for the glutamine amide-N and the free ammonia-N are accordingly subtracted, and the difference is regarded as asparagine amide-N. It is evident that any error

arising from the presence of interfering substances such as urea and allantoin is calculated as asparagine; further discussion of this point is reserved, however, for a subsequent paper.

The determination of glutamine and asparagine in mixtures.

In order to calculate the glutamine and asparagine amide-N content of a mixture of these two substances, it is necessary only to determine the total amide-N after hydrolysis with *N* acid and the glutamine amide-N after hydrolysis at p_H 6·5. Data from a series of such determinations shown in Table V indicate that satisfactory results are obtained.

Table V. *Analysis of mixtures of glutamine and asparagine.*

Figures are mg. of amide-N.

Asparagine		Glutamine		Total amide	
Taken	Recovered	Taken	Recovered	Taken	Recovered
0·40	0·383	0·10	0·117	0·500	0·488
0·40	0·384	0·10	0·116	0·500	0·508
0·40	0·385	0·10	0·115	0·500	0·506
0·40	0·389	0·10	0·111		
0·40	0·385	0·10	0·115		
	Av. 0·385		Av. 0·115		Av. 0·500
0·40	0·399	0·25	0·243	0·650	0·644
0·40	0·390	0·25	0·252	0·650	0·640
0·40	0·393	0·25	0·249		
	Av. 0·394		Av. 0·248		Av. 0·642

The preparation of plant tissue for amide determinations.

For the determination of the glutamine content of plant tissues it is essential to prepare a representative sample in a form suitable for analysis without danger of partial hydrolysis of the glutamine. Four methods hold out some promise of success; first, extraction with cold water after cytolysis of the fresh tissue with ether according to the method of Chibnall [1923]; a convenient technique has been described by Pucher *et al.* [1935], but it has been found that in order to extract amides completely the residue from the hydraulic press must be thoroughly ground in a plate-type grinding mill before being washed as they describe for the extraction of ammonia; furthermore it is probably desirable in most cases to heat the extract rapidly to 80° to coagulate any protein, and to cool, make to volume and filter before aliquots are taken for analysis. A second method is that of grinding with sand in a mortar, the resultant pulp being diluted with water, rapidly heated to 80° to coagulate protein, cooled, filtered and the residue extensively washed. This method is particularly advantageous for small samples of succulent tissue and is customarily employed in the South Kensington laboratory. A third method consists of freezing the tissue, properly protected against loss of water, best with carbon dioxide snow; subsequently the material is rapidly thawed, the juice is expressed and the tissue is washed substantially as in the first method mentioned. This procedure is likewise applicable in general only to small quantities of tissue. Lastly, there is the method of drying at some suitable controlled temperature in an oven equipped with means to circulate air over the tissue, a method that can be applied to any quantity of material up to the capacity of the drying oven.

The choice among these methods depends on a number of factors. Among the more important is the question of size of sample necessary to ensure that it shall be adequately representative. A few g. only of a small-leaved species may be sufficient; with large-leaved species such as tobacco, much larger samples are essential. Equipment is likewise very important; laboratories that do not possess a hydraulic press are necessarily restricted in their choice. In addition there is the question of preservation of material until the analytical operations can be undertaken.

Information available at present indicates that only the first two methods, which imply prompt analysis of the fresh tissue extract, are entirely safe. The method of drying possesses so many advantages however and is in fact necessary in so many practical cases, that we have devoted much study to it.

The equipment with which the following tests were carried out consists of a commercial cabinet drier with 12 trays 16×30 in. arranged in two banks. Air is circulated at the rate of approximately 217 ft. per min. over the trays by a motor-driven fan, and heat is supplied from a gas burner protected by baffles in the lower part of the cabinet. The gas supply to the burner is controlled by a thermostat which may be adjusted to any temperature between 50 and 120° and which controls within $\pm 2°$.

Tomato plants were dissected into stem and leaf portions, which were separately cut up with shears into small pieces. Samples were withdrawn from each portion for extraction by the ether-cytolysis method and for drying. Determinations of ammonia-N, total amide-N, glutamine amide-N and amino-N were carried out immediately upon the fresh tissue extracts, and similar analyses were performed on aqueous extracts of the dry tissue. These extracts were prepared by heating 3–5 g. at 80° with 80 ml. of water for 10 min. with constant stirring; the beaker was then rapidly chilled, the contents transferred to a 100 ml. flask, made to volume and centrifuged, and aliquots of the clear fluid, filtered if necessary through a plug of glass wool, were taken for analysis. That this method of extraction is adequate was shown by parallel determinations of free ammonia in samples of dry tissue and in the extracts. In 10 experiments the average ratio between extract ammonia-N and dry tissue ammonia-N was 1·01, and the maximum variation in only two cases exceeded 5%. Such close agreement of the free ammonia-N values indicates that hydrolysis of glutamine during the preparation of the dry tissue extracts was negligible.

Table VI shows the results of analyses of the leaf and stem tissues from three separate batches of plants, dried at the specified temperatures, compared with data obtained on extracts prepared from the fresh tissue. One batch was used for each experiment, collections being made at weekly intervals. The plants were grown in crocks in a greenhouse and had been heavily fertilised with ammonium sulphate in order to stimulate the synthesis of glutamine [Vickery *et al.*, 1934].

It is clear that the agreement between the values found in the leaf tissue is satisfactory; even when dried at 90° the value in the dry tissue is less than 4% lower than in the extracted tissue. The data for the stem tissue are somewhat less satisfactory, the glutamine values in the dried tissue being somewhat higher than in the extracts. This was not due to failure to extract completely, since glutamine determinations on the extracted residues showed that less than 2% of the glutamine originally present remained unextracted. In general it may be concluded that the differences between the glutamine values in extracts and in dried tissue are within the analytical variations to be expected of the method, and it is clear that, if the drying be carried out in the vicinity of 80° under proper conditions, satisfactory determinations of glutamine may be secured.

Table VI. *Comparison of the composition of tomato leaves and stems as determined from analyses of fresh tissue extracts and of tissue dried at various temperatures.*

Three batches of plants collected at weekly intervals, one for each temperature.
Figures not otherwise designated are g. in 1000 g. of fresh tissue.

	Leaves						Stems					
Temperature (° C.) ...	70		80		90		70		80		90	
	Extd.	Dried	Extd.	Dried	Extd.	Dried	Extd.	Dried	Extd.	Dried	Extd.	Dried
Free ammonia-N	0·210	0·237	0·134	0·164	0·228	0·232	0·248	0·260	0·168	0·218	0·192	0·199
Asparagine amide-N	0·075	0·084	0·105	0·120	0·109	0·105	0·151	0·148	0·120	0·108	0·319	0·293
Glutamine amide-N	0·353	0·347	0·366	0·356	0·394	0·380	0·651	0·692	0·664	0·714	0·934	0·846
Amino-N	0·984	1·14	0·911	0·936	1·10	1·06	1·65	1·44	1·51	1·29	2·24	1·77
Amino-N after hydrolysis at p_H 6·5	0·351	0·460	0·299	0·335	0·396	0·590	0·328	0·340	0·318	0·342	0·528	0·483
Ratio: decrease amino-N/ glutamine amide-N	1·79	1·95	1·67	1·69	1·78	1·55	2·03	1·59	1·80	1·33	1·71	1·53
Soluble N	1·91	2·54	1·79	2·25	2·09	2·55	2·68	2·94	2·47	2·66	3·20	3·28
Insoluble N	4·32	4·12	4·33	4·08	4·05	4·09	1·10	1·04	1·28	1·20	1·15	1·02
Time of drying (hours)	1		1		1		2		1·5		1·25	
Ratio: glutamine amide-N of dried/glutamine amide-N of extracted sample	0·982		0·972		0·964		1·06		1·07		0·906	

An experiment was conducted in which a drying temperature of 60° was used. The data were not satisfactory for several reasons, the chief being that a sample of tissue exceptionally low in glutamine was employed. Although the dried sample showed little increase in free ammonia or decrease in glutamine over the extracted sample, there was a very marked increase in soluble N and in amino-N, indicating that during the slow drying at 60° (2·25 hours) considerable autolysis of protein occurred; a drying temperature in excess of 60° is therefore to be desired. It may be inferred that the time during which the tissue is exposed to the temperature of the drying oven is of great importance; the conditions should be so adjusted that this shall be as short as possible.

The determination of glutamine in plant tissues.

Apart from the problem of preparing tissue for analysis in such a manner as to avoid significant hydrolysis of glutamine, a serious difficulty arises from the possible presence of other substances that may evolve ammonia during the analytical operations essential to determination of the amide. Chibnall and Westall have discussed the stability of urea and allantoin in this connection; both give rise to appreciable amounts of ammonia under the conditions of the glutamine hydrolysis, and our more recent studies of these substances show that interference, particularly from urea, may become serious if more than traces are present. Fortunately, however, it is relatively easy to demonstrate the absence of these substances. Inasmuch as the decomposition both of urea and of allantoin is incomplete in 2 hours at p_H 6·5 and 100°, it suffices to conduct hydrolyses under these conditions for 2- and 4-hour periods; if there is no increase in ammonia in the longer period, the absence of urea and allantoin may be inferred.

The data in Table VII are presented chiefly to illustrate the reproducibility of glutamine determinations carried out according to the directions in a preceding section. The tissues had been dried at 80° as already described, and the analyses were conducted on extracts of the dried tissue in order to eliminate interference from proteins. The data for the tomato plant illustrate the extraordinary

Table VII. *Glutamine content of various dried plant tissues.*

		NH_3-N of dry tissue %	NH_3-N after p_H 6·5 hydrolysis %	Glutamine amide-N %	Glutamine %
Tobacco leaf E		0·018	0·042	—	—
		0·018	0·043	—	—
		0·016	0·045	—	—
		0·017	0·042	—	—
	Av.	0·0172	0·043	0·026	0·135
Tobacco stem E		0·012	0·053	—	—
		0·013	0·052	—	—
		0·014	0·055	—	—
		0·011	0·053	—	—
	Av.	0·0125	0·053	0·041	0·213
Tomato leaf, ammonia culture		0·160	0·299	0·239	—
		0·163	0·301	0·238	1·24
Tomato leaf, nitrate culture		0·044	0·061	0·017	—
		0·043	0·063	0·020	0·096
Tomato stem, ammonia culture		0·148	0·712	0·564	—
		0·147	0·705	0·558	2·92
Tomato stem, nitrate culture		0·026	0·082	0·056	—
		0·026	0·080	0·054	0·28
Beet root, ammonia culture		0·007	0·370	0·363	—
		0·007	0·374	0·367	1·90

enrichment in glutamine which occurs when this species is grown in culture solutions that provide ammonia as the sole source of N; attention has been directed to this elsewhere [Vickery *et al.*, 1934].

The behaviour of the apparent amino-N during the hydrolysis of glutamine.

Chibnall and Westall pointed out that the decrease in the apparent amino-N of the solution when the amide group of glutamine is hydrolysed in nearly neutral solution may serve as qualitative evidence of the presence of this substance in a plant extract. This behaviour cannot be employed for quantitative calculation because of the possibility that unstable peptides may be present which may undergo hydrolysis with the production of actual amino-N. Furthermore, glutamic and hydroxyglutamic acids likewise lose amino-N, owing to ring formation, on being heated in neutral solution and, if present, would introduce an additional source of error. Nevertheless the criterion is frequently one of real value in forming a judgment as to the qualitative composition of plant extracts.

The ratio of the decrease in apparent amino-N to the increase in ammonia-N which occurs when a solution of pure glutamine is hydrolysed at p_H 6·5 varies from 2·1 to 1·8 as the hydrolysis progresses (see last column, Table III); the final value is that to be expected from a plant extract that contains glutamine, provided that no other substances are present which produce ammonia or which change in amino-N content under the same conditions.

Data illustrating the magnitude of the ratio as observed in tissues high in glutamine are shown in Table VI. Further data are given in Table VIII. It is clear that the results are unequivocal only when quite appreciable amounts of glutamine are present.

Table VIII. *The changes in amino-N of various plant extracts on hydrolysis at p_H 6·5 for 2 hours at 100°.*

Figures not otherwise designated are mg. N per g. dry tissue.

1	Before hydrolysis 2	After hydrolysis 3	Decrease 4	Glutamine amide-N 5	Ratio column 4/ column 5 6
Tomato stems, high ammonia culture	12·15	4·30	7·85	5·65	1·39
Tomato roots, high ammonia culture	3·97	2·43	0·54	1·05	0·51
Tomato leaves, low ammonia culture	4·27	3·49	0·78	0·61	1·27
Tomato stems, low ammonia culture	5·55	3·02	2·53	2·38	1·06
Tomato roots, low ammonia culture	3·60	2·69	0·91	0·73	1·24
Tomato leaves, nitrate culture	3·00	2·82	0·18	0·19	0·94
Tomato stems, nitrate culture	2·40	2·17	0·23	0·55	0·42
Tomato roots, nitrate culture	1·58	1·57	0·01	0·22	0·00

The present method of determining glutamine must be applied with discrimination and only after careful consideration of the possible sources of error. In the case of tissues known from other data to store relatively large proportions of this substance there is little doubt of the trustworthiness of the results, for the error introduced by the partial decomposition of such traces of urea, allantoin or other unstable substances as may be present is probably negligible. The method is therefore extremely valuable as a means of investigating the metabolism of the amides in known glutamine-storing plants. But the general application of the method to the determination of an additional factor in the customary examination of the N fractions of plant tissues, especially when the quantity involved is relatively small, must be deprecated in the present very incomplete state of our qualitative knowledge of the composition of plants.

Summary.

The procedure advocated by Chibnall and Westall for the determination of glutamine in plant tissues has been revised in the light of more recent information on the stability of this substance. The amide group has been found to be completely hydrolysed when glutamine is heated at 100° for 2 hours at p_H 6·5; accordingly the quantity of glutamine amide-N in an extract of plant tissue can be determined by estimation of the increase in ammonia-N that occurs on hydrolysis under these conditions; interference from asparagine is negligible.

Attention is drawn to the possibility of interference from urea and allantoin, and a method is suggested whereby such interference may be detected.

The decrease in amino-N of an extract, on hydrolysis under the conditions mentioned, is shown to be a valuable qualitative criterion of the presence of glutamine, as has previously been indicated by Chibnall and Westall.

The chief application of the method as at present developed is to the study of plant material known from other considerations to elaborate glutamine.

Data on the stability of glutamine with respect to hydrogen ion activity and to temperature are included, together with a demonstration that the chief products of the hydrolysis of glutamine at essentially neutral reaction are ammonia and pyrrolidonecarboxylic acid.

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