XLVII. THE PROTEINS OF GRASSES.

I. PRELIMINARY COMMUNICATION.

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INTRODUCTION.

A METHOD for the preparation of leaf-proteins was described by one of us some years ago [Chibnall, 1923]. It has since been applied to the leaves of many different types of plants, but not always with success, for proteins in a state of purity were obtained in good yield only from certain herbaceous plants having a leaf cell sap which was alkaline with respect to the isoelectric point of the protein. From other leaves especially of ligneous plants and even of herbaceous plants in which the conditions, such as the p_H of the leaf sap *etc.*, were favourable, the yields of protein were disappointingly small, and the products were impure. At that time no explanation of this difference in behaviour could be put forward [Chibnall and Grover, 1926].

During the past two years we have attempted to use this method to prepare proteins from various grasses. To obtain any appreciable yield of protein it has been necessary to modify it in certain essential details, but even so it appears that the difficulties inherent in preparations of this type have not yet been completely overcome, because we find that a procedure which will give a good yield of protein from a sample of cocksfoot cut in spring will give no protein at all from a similar sample cut from the same plot two or three months later. We feel however that a definite advance in the technique of leaf-protein preparation has already been achieved, and the present paper describes in some detail the modifications of the original method which we now recommend and gives also a preliminary account of our analyses of the proteins of cocksfoot which have so far been prepared.

EXPERIMENTAL.

The preparation of proteins from cocksfoot (Dactylis glomerata).

It will be convenient to describe briefly the original ether method of Chibnall [1923], as experience in its application has enabled us to obtain a clearer conception of the nature of the separation which is obtained between

the protein and the simpler water-soluble nitrogenous products present in the leaf cells.

The leaves are first cytolysed with ether. On cytolysis the cell protoplasts shrink and become freely permeable to the aqueous contents of the vacuoles. The cellulose cell wall is permeable to water, consequently the cells lose their turgidity and become flaccid. The leaves are next enveloped in filter-cloth and pressed in the Buchner press. The cells become flattened, but the cellulose wall is not broken, so that the aqueous liquid which is forced through it will contain only those water-soluble constituents of the cell whose molecules are sufficiently small to allow them to diffuse freely through the pores of the cellulose wall. The fatty materials, proteins, *etc.*, of the protoplasm, as well as any complex polysaccharides, proteins or proteoses present in the original vacuole fluid are retained inside the cell. The cell wall, in fact, is used as a very convenient ultrafilter. The press extract thus obtained is a clear brown fluid, while the residue inside the flattened cell is a deep green gel. On removal from the press the flattened leaves readily imbibe water and swell up to their original volume. By alternate pressing and soaking in water the simpler water-soluble constituents of the cells can be readily washed away. The leaf residues are next ground in a meat chopper with water. The leaf cells are now torn open, and the deep green gel of cytoplasmic material is dispersed into colloidal solution. The cell wall material is removed by squeezing through silk gauze and a green liquid is thus obtained which is heavily charged with colloidal protein and fatty material.

It is possible to separate this protein into two fractions by filtration through paper pulp. In former papers the fraction retained on the filter-pad, which cannot be readily separated from the fatty material, has been referred to as the "combined" protein, while that which passes freely through the filter has been referred to as the "soluble" protein. If the green colloidal solution is flocculated by heat the product will contain both the above-mentioned protein fractions. It will be referred to in the present and future papers as the "whole" protein of the leaf. Until more is known about the chemical and physical properties of the leaf-proteins such arbitrary distinctions are unfortunately necessary.

When applied to various samples of cocksfoot and ryegrass the above-mentioned ether method was unsatisfactory. The green colloidal solution obtained by grinding yielded very little colloidal protein, and on filtration through paper pulp it gave no "soluble" protein at all. It was clear that on cytolysis the leaf-proteins had undergone some form of denaturation similar to that which had already been observed in the case of *Cochlearia amorcea* [Chibnall and Grover, 1926]. Experiments were therefore made to find out in which way the procedure could be modified so as to prevent this apparent denaturation.

In previous researches the leaf material after cytolysis had been enveloped in filter-cloth and pressed between two flat iron plates in the Buchner press; in the present research it has been found more convenient to use a steel cylinder,

11 ins. high and of 6 ins. internal diameter, the pressure being applied by a well-fitting plunger. The "effective pressure" on the leaf material could in this way be made very much greater than when the former procedure was employed.

The cocksfoot used in the present research was obtained from the Agricultural Research Station of the Imperial Chemical Industries Ltd., at Jealott's Hill, Bracknell, Berks. About three weeks before a sample was required the plot was cut low with a scythe and heavily dressed with nitrogenous fertiliser. When the new growth stood 8 to 10 ins. high the upper 6 ins. of the blades of cocksfoot were removed with a scythe and used for the protein experiments.

Exp. 1. A sample of cocksfoot (Batch O), cut at 7 a.m. on September 17th, 1930. The fresh weight was 10 kg., the dry weight 13.5 % and the total N 3.5 % of the dry weight. As it was possible that the apparent denaturation of the protein which occurred in earlier grass experiments had been brought about by the ether it was decided to use ether-water as the cytolysing agent in the present case. The grass was accordingly immersed in ether-water for 20 minutes and then pressed for 10 minutes at 200–400 kg./cm.² Three washings with water were given, and the green colloidal solution obtained after grinding the leaf residues was divided into two equal portions of 22.5 litres. One portion was filtered through paper pulp and the "soluble" protein flocculated at the isoelectric point by adding the requisite amount of mineral acid. It was then coagulated by heating on a water-bath to 95°, filtered, extracted twice with boiling water, again filtered, and then pressed to a hard cake in the Buchner press. The press-cake was powdered, extracted overnight with ether in a Soxhlet apparatus, and then extracted three times successively, first with boiling 95 % alcohol and finally with boiling absolute alcohol. The "soluble" protein, dried at 110°, weighed 25 g. and it contained 13.7 % of N and 2.7 % of ash. The N, ash-free, was 14.0 %. The other portion of the green colloidal solution was heated to 95° to coagulate the protein, which was then washed in the manner described above. The "whole" protein, dried at 110°, weighed 55.6 g., and it contained 11.4 % of N, and 7.7 % of ash. The N, ash-free, was 12.4 %.

Table I. *Comparison of the yields of "soluble" and "whole" proteins obtained from cocksfoot and spinach [Chibnall, 1924].*

	Percentage of total leaf-N		Percentage of total protein-N	
	Cocksfoot	Spinach	Cocksfoot	Spinach
"Whole" protein-N	28.8	30.9	34.3	42.6
"Soluble" protein-N	13.6	14.1	16.2	19.4
Water-soluble non-protein-N	15.9	27.6	—	—
Unextracted-N	55.3	40.5	65.7	57.4

The yields of the proteins are given in Table I, together with those from spinach obtained in an earlier experiment in which ether was used as the cytolysing agent [Chibnall, 1924]. The blades of cocksfoot are thin and fibrous,

and undergo much less disintegration in the meat chopper than fleshy leaves such as spinach. For this reason the yields of the grass proteins were considered satisfactory, even though about 65 % of the total protein remained in the cellular residues, presumably in cells that had not been torn open during the grinding.

Exp. 2. The pressing and washing of large amounts of leaf material are laborious, and the next experiment was planned to investigate in what way the yield of "soluble" protein was influenced by the magnitude of the pressure and the time during which it was applied.

2 kg. of cocksfoot (Batch S), cut at 7 a.m. on October 17th, 1930, were cytolysed about 3 hours later with ether-water and pressed for 20 minutes at the maximum pressure (500 kg./cm.²). The hard press-cake was allowed to imbibe water for 10 minutes and again pressed at the maximum pressure for 20 minutes. The washing and pressing were repeated 4 times in all. On grinding the final press-cake with water very little material passed into colloidal solution, and the yield of "soluble" protein was negligible.

About 4 hours later another 2 kg. sample of the same batch of grass was treated in the same way, except that only one washing was given. The yield of "soluble" protein was 6 % of the total leaf-N. The preparation contained 11.7 % of N and 4.4 % of ash. The N, ash-free, was 12.3 %. Both the yield and purity of the protein were poor, but the experiment demonstrated that the pressing should not be too severe, and that the time of pressing and washing should not be unduly prolonged.

Attempts to increase the purity of the preparation by redissolving an aliquot of the freshly flocculated "soluble" protein and filtering the solution a second time through paper pulp were unsuccessful.

Exp. 3. Further supplies of pure strain grass were not available until late in the following spring. Batch E was cut at 7 a.m. on June 15th, 1931. The fresh weight was 12 kg., the dry weight 13.0 % and the N 3.2 % of the dry weight. The treatment applied was that which the earlier experiments suggested would be the most favourable for good yields of "soluble" protein.

A sample of grass weighing 2 kg. was cytolysed in ether-water for 10 minutes, and then pressed for 4 minutes at the maximum pressure. The residue was allowed to imbibe water for 4 minutes, and pressed as before. The washing was twice repeated. The final press-cake was ground with water and the "soluble" protein obtained in the usual way. The cellular residue was again minced with water to obtain a second green colloidal extract. Successive 2 kg. samples of grass were treated in a similar way at such time intervals that there was always sufficient green colloidal solution to permit of continuous filtration through the large paper pulp pad. The time required to carry through the treatment of each sample of grass to the filtration stage was only 55 minutes. The "soluble" protein from the 12 kg. of grass weighed 36.5 g., and it contained 12.6 % of N and 1.4 % of ash. The N, ash-free, was 12.8 %. The yield was 9.1 % of the total leaf-N.

It was noticed during this experiment that the amount of colloidal material in the green extracts increased with each successive sample of the grass, and that whereas the first gave a "soluble" protein filtrate which was coloured only a faint brown and contained very little protein, the fourth, fifth and sixth gave filtrates which were deeply coloured and contained a very large amount of protein. This observation suggested that some change affecting the "aggregation" of the colloidal leaf-proteins had taken place within a few hours of cutting, so that the sample treated after 3 hours gave much less "soluble" protein than that treated after 6-8 hours. Such a result was entirely unexpected, as it was contrary to that of previous researches in which ether had been used as the cytolysing agent [Chibnall and Nolan, 1924]. To obtain confirmatory evidence the following experiment was made.

Exp. 4. Batch F was cut from the same plot as Batch E at 7 a.m. on June 27th, 1931. The dry weight of the grass had increased to 16 %, due, presumably, to active photosynthesis during this period. Three samples of 2 kg. each were treated at $3\frac{1}{2}$, 7 and 10 hours respectively after cutting. The "soluble" proteins contained (ash-free) 12.4 %, 12.6 % and 12.6 % of N and the yields were 2.4 %, 7.4 % and 4.7 % of the total leaf-N respectively. This experiment showed quite clearly that the optimum working time for the best yield of protein was about 8 hours after cutting.

No further supplies of pure strain grass were available until September, 1931. With the experience gained in the above-mentioned experiments it was then confidently hoped that samples of about 500 g. of both "whole" and "soluble" proteins of cocksfoot could be prepared for amino-acid analysis. The expectation was not realised as the yield of protein from every sample treated was negligible. At the present time we are quite unable to explain this curious difference in behaviour. Many of the samples taken were from the identical 2-year old grass beds which had yielded abundant protein in the spring of 1931 and in September and October of 1930. Others were from 1-year old beds which had not before been used for protein experiments. To obtain samples of grass rich in protein all the beds had been heavily fertilised with either calcium nitrate or ammonium sulphate, and as the summer of 1931 was abnormally cloudy it is possible that some derangement of the normal carbon-nitrogen balance, or of the distribution of inorganic nutrients in the leaf, had affected the "aggregation" of the leaf-proteins in such a way that after cytolysis they were not readily dispersed into colloidal solution by grinding with water.

Analysis of the "soluble" proteins.

Purity of the various preparations. The ash and N analyses are collected in Table II. The N content is not constant throughout, and in most cases is disappointingly low, showing that the preparations contained 15-25 % of N-free material. A similar impurity had been encountered in a previous research [Chibnall and Grover, 1926] and, as in the present case, the amount present decreased with increase in the yield of "soluble" protein, suggesting that the

N-free material was not necessarily a prosthetic group in the protein molecule, but was some constituent of the leaf cell which had similar solubility properties to the "soluble" proteins.

Table II. *Showing details of "soluble" proteins prepared from cocksfoot.*

Preparation	N (%)	Ash (%)	Ash-free N (%)	Yield (% of total leaf-N)
O	13.6	2.7	14.0	13.6
S	11.7	4.4	12.3	6.0
E	12.6	1.4	12.8	9.1
F ₁	12.2	1.8	12.4	2.7
F ₂	12.4	1.7	12.6	7.4
F ₃	12.4	1.3	12.6	4.7
G	12.5	5.1	13.2	2.4

The nature of this impurity has been investigated in some detail. All the protein preparations gave only 1% of furfuraldehyde on distillation with hydrochloric acid, showing that the impurity was not a pentosan or polyuronide such as pectin, both of which might well have been adsorbed on the protein when it was first flocculated from solution by acid. As all the preparations gave a strong Molisch test for carbohydrate the amount of reducing sugar yielded on mild acid hydrolysis was next investigated.

Estimation of reducing sugars after acid hydrolysis. The protein (1 g.) was hydrolysed with either 4 % or 10 % sulphuric acid for various periods. The hydrolysate was filtered, neutralised to Congo red with sodium carbonate and an aliquot used for determining reducing sugar. In some of the preliminary experiments Hanes's [1929] modification of the Hagedorn and Jensen method was employed. The results varied between 7 % and 10 % of the protein, and suggested that the impurity was a polysaccharide. It was then found that crystalline edestin when submitted to similar treatment gave 4.1 % of "reducing sugar," showing that the ferricyanide must be reacting with the hydrolysis products of the protein, and that therefore the method was inapplicable to the determination of reducing sugars in crude protein preparations. The method of MacLean [1919] gave lower, and what appeared to be more reliable results. The value for edestin was 1.4 %, and was clearly not due to any

Table III. *Showing the amount of reducing sugar obtained from the "soluble" proteins by acid hydrolysis.*

Preparation	N (ash-free) (%)	Strength of acid (%)	Number of hours hydrolysis			
			1 Reducing sugar (%)	2 Reducing sugar (%)	4 Reducing sugar (%)	16 Reducing sugar (%)
O	14.0	4	—	—	—	3.0
O	14.0	10	—	—	—	3.1
S	12.3	4	—	—	—	4.6
E	12.6	4	2.9	3.3	2.9	3.2
E	12.6	10	4.2	4.4	3.7	3.0
Edestin	18.5	10	—	1.4	—	1.4

reducing sugar, because a yellowish-white turbidity instead of a precipitate of red cuprous oxide was formed during the boiling with the copper reagent. In the case of the "soluble" leaf-proteins the reduction proceeded normally, and the values for reducing sugar given after various periods of hydrolysis are recorded in Table III. Assuming that this reducing sugar was derived from a polysaccharide the amount of the latter was clearly insufficient to account for all the N-free material present in the protein preparations unless on acid hydrolysis non-reducing substances were produced as well.

Estimation of polysaccharides by alkaline hydrolysis. To determine the optimum conditions of hydrolysis the following experiments were made. "Soluble" protein E (0.5 g.) was hydrolysed with 5 % or 10 % barium hydroxide ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$) (5 cc.) at 100° for various periods. The mixture was then filtered and the residue washed with hot water. The filtrate and washings were made just acid to Congo red with sulphuric acid, the precipitated barium sulphate was filtered off, and the clear liquid made up to 50 cc. Reducing sugars were determined by the method of MacLean both before and after hydrolysis with 0.7 % hydrochloric acid for 2 hours. The results are given in Table IV. Before the acid hydrolysis no appreciable amount of cuprous oxide

Table IV. *Estimation of polysaccharides in "soluble" protein E by alkaline hydrolysis.*

Time (hours)	After treatment with 5 % barium hydroxide for 2 hours at 100°		After treatment with 10 % barium hydroxide for 2 hours at 100°	
	Before hydrolysis. Sugar (%)	After hydrolysis. Increase in sugar (%)	Before hydrolysis. Sugar (%)	After hydrolysis. Increase in sugar (%)
0.5	—	—	0.56	2.02
1	0.25	2.07	0.23	2.34
2	0.25	2.33	0.36	2.08
4	0.31	2.41	—	—
7	—	—	0.85	1.62

was formed during the reduction stage, although subsequently there was a small titration value for "reducing sugar." After acid hydrolysis the reduction proceeded normally. It will be seen that the maximum yield of polysaccharide was obtained after hydrolysis with 5 % barium hydroxide for 2 hours. Several other "soluble" proteins from leaves, and also edestin, were then hydrolysed with 5 % barium hydroxide for 2 hours, and reducing sugars determined both before and after acid hydrolysis. The results are given in Table V. The edestin and the protein from spinach, both of which are free from carbohydrate (as shown by the Molisch test), gave a yellow turbidity instead of a precipitate of cuprous oxide during the reduction, and the titration value obtained must be due to the interaction of certain amino-acids with the MacLean reagents.

In the case of the proteins from *Zea Mays* and cocksfoot the reduction proceeded normally, suggesting that a small amount of polysaccharide had been set free during the alkaline hydrolysis. It was not found possible to estimate

the total polysaccharide in the leaf-proteins by the colorimetric method of Tillmans and Philippi [1929] because they had been coagulated and therefore were insoluble in dilute alkali.

Table V. *Estimation of polysaccharides in certain leaf-proteins and in edestin (after hydrolysis at 100° with 5 % barium hydroxide for 2 hours).*

Protein	N (ash-free) (%)	Increase in reducing sugar after hydrolysis with acid (%)
Grass "soluble" protein S	12.3	2.2
Grass "soluble" protein E	12.8	2.1
Grass "soluble" protein O	14.0	1.6
<i>Zea Mays</i> * "soluble" protein	14.4	2.0
Spinach† "soluble" protein	16.2	1.0
Edestin	18.4	0.8

* Original sample prepared by Chibnall and Nolan [1924].

† Original sample prepared by Chibnall [1924].

Isolation of a small amount of polysaccharide. A collected sample of 50 g. of "soluble" protein was boiled for 2 hours under reflux (carbon dioxide excluded) in 500 cc. water containing 25 g. crystalline barium hydroxide. The hot mixture was filtered, the residue washed with hot water, and the filtrate and washings neutralised to litmus with sulphuric acid. The precipitated barium sulphate was centrifuged off, washed, and the clear liquid and washings were collected. The "reducing sugar" before hydrolysis with 0.7 % HCl was 0.16 g. and after hydrolysis 1.30 g. The increase was equivalent to 2.3 % of the original protein.

The remainder of the solution was treated with a saturated solution of lead acetate until precipitation was complete. The mixture could not be completely separated on the centrifuge, and it was necessary to treat the cloudy liquid with charcoal and filter. The clear filtrate was then treated with ammonia and lead acetate until precipitation was complete [Rimington, 1929]. The precipitate was centrifuged off, washed several times with a dilute solution of ammonia and then filtered under suction. The residue was redissolved by grinding with dilute acetic acid, a small amount of insoluble pigment removed by filtration, and the lead-sugar complex reprecipitated by adding ammonia. It was washed as before and then decomposed under pressure with hydrogen sulphide in the usual way. The precipitated lead sulphide was washed with hot water and the extract and washings were collected. The reducing sugar before hydrolysis with 0.7 % HCl was negligible, and after hydrolysis was 0.60 g., equivalent to 1.2 % of the original protein. To the remainder of the solution sufficient sulphuric acid was added to make the concentration 10 %, followed by 10 cc. of a 30 % solution of mercuric sulphate in 10 % sulphuric acid, to precipitate most of the nitrogen [Greenwald, 1930]. The mixture was cooled in ice and the sulphuric acid removed quantitatively by the addition of a hot saturated solution of barium hydroxide. The precipitate was removed by filtration and the filtrate

freed from mercury by means of hydrogen sulphide. The solution at this stage contained: total solids, 0.8 g.; total nitrogen, 0.009 g.; reducing sugar after hydrolysis, 0.3 g. It was next evaporated under reduced pressure to 15 cc., and the slight turbidity removed by filtration. To the filtrate were added 4 volumes of methyl alcohol followed by 4 volumes of ether and the solution was kept for 5 days. The precipitate, a white powder, was filtered off, washed with ethyl alcohol and kept for several days *in vacuo* over sulphuric acid.

The material (0.24 g.) had no definite m.p., but darkened slowly above 180°. (Found: ash, 11.5 %; ash-free, C, 43.9; H, 6.5 %. A polysaccharide requires C, 44.4; H, 6.1 %.) The following results are quoted ash-free: N, 1.2 %; $[\alpha]_D - 37.0^\circ$; reducing sugar after hydrolysis with 0.7 % hydrochloric acid, 48.3 %; total polysaccharide by the colorimetric method of Tillmans and Philippi [1929], 100 %. The small yield (1 %) and low nitrogen content of the polysaccharide, together with the relative ease with which it is set free from the protein on alkaline hydrolysis, did not suggest that it was an amino-trisaccharide of the type recently isolated by Levene and Mori [1929] from ovalbumin and ovomucoid, and by Rimington [1929] from both the albumin and globulin of horse's blood-serum. To obtain further evidence the following experiments were made.

50 mg. of the polysaccharide were hydrolysed with 0.7 % hydrochloric acid (5 cc.) for 2 hours on a boiling water-bath. The solution was neutralised with sodium acetate and treated with the requisite quantities of phenylhydrazine hydrochloride and sodium acetate, heating for 1 hour on a boiling water-bath. On cooling an orange-coloured crystalline precipitate with the appearance of an osazone separated out. This precipitate was filtered, washed and dried on porous tile, *in vacuo*, over sulphuric acid: m.p. 128–130°. There was not sufficient material for further characterisation.

Another 50 mg. were hydrolysed with 10 % sulphuric acid for 2 hours, the acid removed with barium hydroxide in the usual way and the clear solution treated with salicylaldehyde [Irvine and Earl, 1922]. No insoluble crystalline precipitate of salicylidene-glucosamine was formed, showing the probable absence of glucosamine.

From these results it seems probable that the polysaccharide is nitrogen-free, and that on acid hydrolysis it gives 50 % of reducing sugar.

The amount present (3–4 %) is only about one-fifth of the total nitrogen-free material which the soluble proteins must contain, and no further investigation has been made into its constitution.

Amino-acid analysis. Sample O, which contained (ash-free) 14 % N, was considered sufficiently pure for the usual Van Slyke analysis. The results are given in Table VI, together with those of certain other "soluble" leaf-proteins. Dicarboxylic acid-N was determined by the method of Damodaran [1931], some of whose results are also quoted. The analysis of the cocksfoot again illustrates the very close agreement in amino-acid composition which has been found in all the leaf-proteins so far analysed. The agreement is particularly

Table VI. *Van Slyke analyses of cocksfoot and certain other "soluble" leaf-proteins (in percentages of total nitrogen; nitrogen figures corrected for solubility of bases).*

	Cocksfoot	<i>Zea Mays</i> *	Spinach†	<i>Medicago sativa</i> *	Runner bean‡
Amide-N	5.9	7.4	6.9	5.5	5.7
Humin-N	6.5	4.6	2.5	2.7	4.2
Arginine-N	14.3	14.7	13.8	15.3	16.1
Histidine-N	5.3	4.7	3.9	3.1	6.2
Lysine N	8.3	8.8	9.6	10.0	10.1
Cystine-N	0.0	0.8	1.3	0.8	
Mono-amino-N	56.0	55.8	58.1	58.6	53.8
Non-amino-N	3.7	2.0	2.6	3.2	3.4
	99.7	98.8	98.7	99.2	99.5
Total basic-N	27.9	29.0	28.6	29.2	32.4
Dicarboxylic acid-N	19.9	21.9	17.7	20.1	20.8
% N in protein (ash-free)	14.0	14.4	16.25	15.8	15.85

* Chibnall and Nolan [1924].

† Chibnall [1924].

‡ Chibnall and Grover [1926].

close in the case of *Zea Mays*, which also belongs to the Gramineae and contains about the same amount of N-free impurity.

In one respect however the cocksfoot protein differs from the others, and that is in the apparent absence of cystine. The sulphur content of the phosphotungstic acid precipitate was estimated in two ways. (i) After removal of phosphotungstic acid with amyl alcohol-ether and treating the bases with the Benedict-Denis reagent in the usual way [Van Slyke, 1915]. (ii) By the modified method of Plimmer and Lowndes [1927], in which the estimation was carried out without the previous removal of the phosphotungstic acid. This result is in harmony with that of Aitken [1930] who was unable to detect cystine in either cocksfoot or perennial ryegrass after hydrolysis of the whole grass with 20 % HCl.

The sulphur content of "soluble" protein E was 0.75 % and of O 1.0 %. Proof that the major part of the sulphur in preparation E was organic sulphur was obtained in the following way. The protein (1 g.) was hydrolysed with 20 % HCl (50 cc.) for 16 hours, excess acid removed by concentration *in vacuo*, and the acid humin filtered off and washed. The ash weight of the acid humin was 0.11 %. Assuming that this was all calcium sulphate the sulphur is equivalent to 0.03 %. The filtrate and washings were treated with barium chloride and HCl in the usual way. No precipitate of barium sulphate was obtained, showing the absence of inorganic sulphate. These results demonstrate that the major part of the sulphur in preparation E was organic sulphur, and unless this was derived from some N-free impurity, it follows that the protein must have contained a non-basic sulphur-containing amino-acid such as methionine. This interesting result will be investigated in greater detail when further supplies of grass proteins are available.

SUMMARY.

Various attempts have been made to prepare proteins in good yield from grasses. The original ether method of Chibnall was unsuccessful, but by substituting ether-water for ether good yields of protein have been obtained in some cases, but not in others.

The protein preparations have a low N-content (12·3–14 %), and contain varying amounts of N-free impurities which are difficult to remove. A polysaccharide is present in small amount, but the constitution of the major part of the impurity has not yet been determined.

A preparation containing 14 % of N has been analysed by the method of Van Slyke. The amino-acid composition was very similar to that of other leaf-proteins, but the presence of cystine could not be demonstrated, although the protein contained 1·0 % of sulphur.

Another preparation containing 0·75 % of sulphur gave only small amounts of inorganic sulphur, suggesting the presence in the protein of a non-basic sulphur-containing amino-acid such as methionine.

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REFERENCES.

- Aitken (1930). *Biochem. J.* **24**, 250.
Chibnall (1923). *J. Biol. Chem.* **55**, 333.
— (1924). *J. Biol. Chem.* **61**, 303.
— and Grover (1926). *Biochem. J.* **20**, 108.
— and Nolan (1924). *J. Biol. Chem.* **62**, 173.
Damodaran (1931). *Biochem. J.* **25**, 2123.
Greenwald (1930). *J. Biol. Chem.* **88**, 1.
Hanes (1929). *Biochem. J.* **23**, 99.
Irvine and Earl (1922). *J. Chem. Soc.* **121**, 2376.
Levene and Mori (1929). *J. Biol. Chem.* **84**, 49.
MacLean (1919). *Biochem. J.* **13**, 135.
Plimmer and Lowndes (1927). *Biochem. J.* **21**, 247.
Rimington (1929). *Biochem. J.* **23**, 430.
Tillmans and Philippi (1929). *Biochem. Z.* **215**, 36.
Van Slyke (1915). *J. Biol. Chem.* **23**, 411.