pairing of bases, is shown generally by our results. The base composition of all the DNA preparations correspond to the excess adenine-thymine (AT) class and show surprisingly little variation over a wide range of plant types. In this respect the plant DNA preparations resemble the AT pattern of the DNA from many animal tissues (Laland *et al.* 1952; Wyatt, 1951*a*) except for their consistently higher 5-methylcytosine content.

In contrast, DNA preparations from bacterial sources exhibit a wide range of compositions, from the extreme AT types to the extreme guanine-cytosine (GC) class with the apparent absence of 5-methylcytosine (Laland *et al.* 1952; Sherratt & Thomas, 1953; Smith & Wyatt, 1951).

SUMMARY

1. The mixed nucleic acids were extracted from five species of leaves by 10% (w/v) NaCl at 100°. After incubation with alkali, PNA and DNA fractions were isolated.

2. Quantitative analyses for purines and pyrimidines of the nucleic acid fractions were made. The PNA fractions had no unusual features. The DNA fractions were all in the excess adenine-thymine class and had a relatively high content of 5-methylcytosine.

The authors wish to thank Professor W. Charles Evans for his interest and encouragement. We also thank the Agricultural Research Council for their financial support.

REFERENCES

- Brown, G. L. & Watson, M. (1953). *Nature, Lond.*, **172**, 339.
- Chargaff, E., Crampton, C. F. & Lipshitz, R. (1953). *Nature, Lond.*, **172**, 289.
- Davidson, J. N., Leslie, I. & Waymouth, C. (1949). *Biochem. J.* **44**, 5.
- Davidson, J. N. & Smellie, R. M. S. (1952). *Biochem. J.* **52**, 594.
- Deriaz, R. E., Stacey, M., Teece, E. G. & Wiggins, L. F. (1949). *J. chem. Soc.* p. 1222.
- Dorner, R. W. & Knight, C. A. (1953). *J. biol. Chem.* **205**, 959.
- Eggman, L., Singer, S. J. & Wildman, S. G. (1953). *J. biol. Chem.* **205**, 969.
- Feulgen, R., Behrens, M. & Mahdihassan, S. Z. (1937). *Hoppe-Seyl. Z.* **246**, 203.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Holden, M. (1952). *Biochem. J.* **51**, 433.
- Laland, S. G., Overend, W. G. & Webb, M. (1950). *Acta chem. scand.* **4**, 885.
- Laland, S. G., Overend, W. G. & Webb, M. (1952). *J. chem. Soc.* p. 3224.
- Lusena, C. V. (1951). *Cereal Chem.* **28**, 400.
- Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 401.
- Mirsky, A. E. & Pollister, A. W. (1946). *J. gen. Physiol.* **30**, 117.
- Osborne, T. B. & Campbell, G. F. (1900). *J. Amer. chem. Soc.* **22**, 379.
- Parker, G. (1952). *Biochem. J.* **51**, 389.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
- Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
- Sherratt, H. S. A. & Thomas, A. J. (1953). *J. gen. Microbiol.* **8**, 217.
- Smith, J. D. & Wyatt, G. R. (1951). *Biochem. J.* **49**, 144.
- Takasugi, N. (1943*a*). *J. chem. Soc. Japan*, **64**, 869.
- Takasugi, N. (1943*b*). *J. chem. Soc. Japan*, **64**, 1375.
- Takasugi, N. (1944). *J. chem. Soc. Japan*, **65**, 333.
- Watson, J. D. & Crick, F. H. C. (1953). *Nature, Lond.*, **171**, 737.
- Williams, R. F. (1945). *Aust. J. exp. Biol. med. Sci.* **23**, 213.
- Wyatt, G. R. (1951*a*). *Biochem. J.* **48**, 581.
- Wyatt, G. R. (1951*b*). *Biochem. J.* **48**, 584.

The Amino Acid Content of the Proteins of Barley Grains

BY B. F. FOLKES AND E. W. YEMM
Department of Botany, University of Bristol

(Received 2 May 1955)

In his classical researches on the cereal proteins, Osborne (1895*a*) found that barley grains contained four distinct classes of protein. Three of these, albumin, globulin and prolamins (hordein), he separated from the grain and showed by elementary analyses to be distinct and reproducible fractions of the protein reserves. The fourth type of protein, glutelin, was only partially soluble in dilute acids and alkalis and Osborne was unable to obtain reasonably pure and reproducible pre-

parations of it. Subsequently, Larmour (1927) and Csonka & Jones (1929) described preparations of barley glutelin (hordenin), but in neither case was the reproducibility of the fraction fully demonstrated.

More recent studies by means of the ultracentrifuge and by electrophoresis have extended our knowledge of the homogeneity of some of the protein fractions. For example, Quensel & Svedberg (1938) found that, while hordein behaved

as a homogeneous protein, both the albumin and the globulin fractions were polydisperse. The globulin fraction has since been further examined by Sverborn, Danielsson & Svedberg (1944) and by Danielsson (1949), and four distinct barley globulins are now recognized. Biserte & Scriban (1950) found that under their conditions hordein also was not homogeneous, and it seems likely therefore that this protein resembles the prolamins which Scallet (1947) found to be an association-dissociation complex. Under certain conditions of concentration, pH and ionic strength, this complex could behave as a homogeneous protein during electrophoresis.

In the present work, interest was centred on the amino acid metabolism associated with the mobilization of the proteins of the grain during germination (Yemm, 1949; Folkes, 1951). Such a study required the separation of the grain proteins into fractions which were broadly reproducible and which were sufficiently free of carbohydrate contaminants to permit of amino acid analyses. Osborne's fractions of albumin, globulin and hordein, while recognized to be physically heterogeneous, were deemed to be sufficiently constant in chemical composition for this purpose. Moreover, their clear-cut physical properties facilitated small-scale determinations of their relative proportions in the grain (cf. Bishop, 1929). In the absence of well-established methods for the preparation of hor-

dein, the methods of Csonka & Jones (1929) were further investigated with a view to obtaining reproducible preparations. For this purpose the amide content of the protein fractions was used as a rapid means of characterization. The storage proteins of cereal grains differ widely in their amide contents and this has often been exploited to distinguish between them (cf. Bishop, 1928; Rose & Anderson, 1936; Blish & Sandstedt, 1929).

Specimens of all four protein fractions were prepared and their amino acid content was determined, chiefly by microbiological assay, by the methods of Yemm & Folkes (1953). These data serve to extend the very incomplete analyses of barley proteins previously available, which have been summarized by Bishop (1928).

EXPERIMENTAL

Methods of analysis

Determination of amide content of crude protein preparations. Amide content was determined by the method of Yemm & Folkes (1953), involving hydrolysis in 2N-H₂SO₄. Estimation of the resulting NH₃ was by vacuum distillation into dilute acid followed by treatment with Nessler reagent. When this method was applied to crude protein preparations containing appreciable amounts of carbohydrate contaminants, difficulty was experienced; there was a progressive increase with time in the colour intensity of distillates after treatment with Nessler reagent. This was eventually traced to the presence in the distillate of

Table 1. *Correction of estimates of amide content for furfural produced during acid hydrolysis*

Mixed solutions of sugars and (NH₄)₂SO₄, or aqueous extracts of various barley tissues, were heated with 2N-H₂SO₄ for 3 hr. under conditions for the total hydrolysis of amides. Ammonia was then distilled off *in vacuo* and estimated colorimetrically 10, 20 and 30 min. after the addition of Nessler reagent. The 30 min. estimate and a 0 min. estimate (obtained by extrapolation) are shown in the table.

A duplicate sample was first steam-distilled to free it from furfural before ammonia was removed by vacuum distillation (see Experimental section). Here the ammonia was estimated 30 min. after the addition of Nessler reagent and is recorded in the last column of the table.

All results are expressed as apparent μ g. ammonia N/ml. solution. In the tissue extracts this is approximately equal to μ g. N/20 mg. dry tissue.

		Estimates of ammonia N after acid hydrolysis		
		Corrected		
		Uncorrected. 30 min. reading	0 min. reading (by extrapolation)	30 min. reading after steam distillation
2.24 μ g. N/ml. plus:		Sugar-ammonia solutions		
5 mg. glucose/ml.		2.49	2.29	2.25
5 mg. sucrose/ml.		2.58	2.26	2.24
1 mg. xylose/ml.		5.34	2.25	2.23
		Aqueous extracts of barley tissues		
Embryos from 13-day-old seedlings		6.40	5.87	5.69
Endosperms from 6-day-old seedlings		7.05*	6.15	6.21
		7.16	6.12	6.22
Leaves from mature plants		7.39*	6.69	6.68
		7.39	6.78	6.71

* Duplicate estimates.

furfural, formed from carbohydrates during acid hydrolysis of the protein preparation: a close correlation ($r=0.995$; $n=9$; $P \leq 0.001$) could be demonstrated between the rate of increase of colour intensity and the amount of furfural present in the distillate (as determined by the colorimetric method of Youngburg & Pucher, 1924). Two ways of overcoming this difficulty were developed. One was to extrapolate the rectilinear graph of colour intensity against time to zero time. An alternative and more satisfactory method was to remove furfural by steam distillation of the acidic hydrolysate before transferring the residue to the vacuum-distillation apparatus and making alkaline for the distillation of NH_3 . Steam distillation for 5 min. from a Kjeldahl flask was sufficient to remove all the furfural, and this method was adopted for routine determination of amide content of all except relatively carbohydrate-free protein preparations. A comparison of both methods of correction is given for mixtures of sugars and $(\text{NH}_4)_2\text{SO}_4$ and for extracts of plant material in Table 1. It can be seen that the steam distillation method is the more reliable.

For the purpose of characterization of the protein fractions the amide content is usually expressed as amide N as % of the total protein N. The estimates of amide content so obtained are thus largely independent of non-nitrogenous contaminants, especially when mild conditions, such as those described above, are used for the hydrolysis. Under more severe conditions of hydrolysis additional ammonia may be derived from the breakdown of amino acids and this is probably influenced by the presence of carbohydrates (Chibnall, 1939, p. 282; Yemm, 1950). For this reason, the results of earlier workers, who used strong acid hydrolysis, are of less value for characterization and are not directly comparable with the present results.

Total nitrogen. This was determined by Kjeldahl incineration followed by steam distillation and treatment with Nessler reagent as described by Yemm & Folkes (1953).

Analysis of protein preparations and estimations of amino acid content were by the methods of Yemm & Folkes (1953); all of the amino acids with the exception of alanine and threonine were determined by microbiological assay, and a specific decarboxylase (Gale, 1945) was used to give additional estimates of glutamic acid. Alanine and threonine were estimated by the methods described by Folkes (1953).

Separation and characterization of hordenin

The method of Csonka & Jones (1929) was adopted as a basis for experiments on the preparation of hordenin from barley flour. This method entailed the extraction of the glutelin in ethanolic NaOH (0.05N-NaOH in 50% v/v aqueous ethanol), and its precipitation by adjusting the pH to 6.8. The subsequent fractionation with $(\text{NH}_4)_2\text{SO}_4$, used by Csonka & Jones, was, however, omitted in the present work because of the difficulty of quantitative estimation of protein N in the presence of this reagent. Instead, the protein was repeatedly dissolved in ethanolic NaOH and reprecipitated at pH 6.4 with HCl, samples being taken for the determination of amide content after each precipitation. The results of a preliminary investigation are given in Table 2. In this experiment, the albumin, globulin and non-protein fractions were first removed by extraction with 5% (w/v) K_2SO_4 solution. The flour was then extracted

Table 2. *Extraction of barley flour and the isoelectric precipitation of hordenin at varying concentrations of ethanol*

Whole grains of barley were ground in an adjustable cone mill. The flour was extracted exhaustively at the centrifuge with 5% (w/v) K_2SO_4 and then with 0.05N-NaOH in 50% (v/v) ethanol. A balance sheet for the total N and the amide N extracted is shown in the upper part of the table.

Portions of the ethanolic NaOH extract were made to different ethanol concentrations and adjusted to pH 6.4 with dilute HCl. The precipitated protein was redissolved in ethanolic NaOH and again precipitated with HCl at the same ethanol concentration as before. The total N and amide N of successive precipitates are shown in the lower part of the table.

Extraction	mg. N/g. flour		Amide N Total N (%)
	Total N	Amide N	
Soluble in 5% K_2SO_4	5.04	0.342	6.8
Soluble in ethanolic NaOH	7.62	1.37	18.0
Insoluble residue	2.70	0.214	7.9
Sum of fractions	15.4	1.93	—
Whole flour (direct estimate)	15.3	1.92	—
Precipitates from fraction soluble in ethanolic NaOH			
55% (v/v) ethanol: precipitate 1	1.86	0.190	10.2
precipitate 2	1.44	0.144	10.0
45% (v/v) ethanol: precipitate 1	1.94	0.234	12.1
precipitate 2	1.68	0.170	10.1
precipitate 3	1.42	0.144	10.1
35% (v/v) ethanol: precipitate 1	3.92	0.652	16.6
precipitate 2	3.54	0.538	15.2

with ethanolic NaOH, and portions of the alkaline extract were adjusted to various ethanol concentrations and then fractionated by repeated precipitations at pH 6.4. It can be seen from Table 2 that the first precipitate from a solution containing 55 % (v/v) ethanol had amide N approx. 10 % of total N, and this was relatively unchanged on reprecipitation. On the other hand, the precipitate from 45 % ethanol reached this amide content only after one reprecipitation and similar treatment with 35 % ethanol left a precipitate whose amide content was 15 %. Since it is known that hordein has an amide content of approx. 23 % (Bishop, 1928), these results suggested that the precipitate was severely contaminated with this alcohol-soluble protein at low concentrations of ethanol, but that at higher concentrations of ethanol the precipitate was a reproducible fraction, capable of reprecipitation without change of amide content. This distinctive fraction is considered to be barley glutelin, hordenin.

prolonged exposure to the ethanolic NaOH solution and loses the property of precipitation at the isoelectric point. Presumably this modification involves hydrolysis, but no increase in free NH_3 can be detected in alkaline solutions of the protein. A similar phenomenon has been reported for glutenin from wheat by Blish (1925).

Results of a further experiment, given in Table 3, demonstrate that, provided the hordenin is exposed to the alkali for not more than 1–2 hr., almost quantitative separations of hordein and hordenin can be made. In this experiment, the hordenin was precipitated from a solution containing 45 % ethanol. Here, as in the previous experiment, the amide content of the precipitate was nearly 12 %, although, after solution in fresh ethanolic NaOH and reprecipitation, the amide content fell to 10 %. The fraction left in solution by this reprecipitation can be estimated from the differences between the first and second precipitates. Its high amide content, similar to that of

Table 3. *Separation of hordein and hordenin from barley flour*

Endosperms of barley grain were ground in a ball mill and then extracted as described in Table 2. The fraction soluble in ethanolic NaOH was further fractionated by repeated precipitation of the hordenin at pH 6.4 from 45 % (v/v) ethanol. The amide content of successive precipitates is shown below. Precautions were taken in this experiment to avoid prolonged exposure of the proteins to alkaline conditions.

Extraction	mg. N/g. flour		Amide N Total N (%)
	Total N	Amide N	
Soluble in 5% K_2SO_4	5.12	0.482	9.4
Soluble in ethanolic NaOH	15.4	2.93	19.0
Insoluble residue	1.99	0.116	5.8
Sum of fractions	22.5	3.53	—
Endosperm flour (direct estimate)	22.5	3.46	—
Fractionation of fraction soluble in ethanolic NaOH			
Unprecipitated at pH 6.4	10.15	2.25	22.2
Precipitated at pH 6.4 (precipitate 1)	5.50	0.652	11.8
Sum (cf. total extract, above)	15.65	2.90	—
Reprecipitation of precipitate 1			
Precipitate 2	4.75	0.476	10.0
Precipitate 3	4.25	0.422	9.9
Differences between successive precipitates			
Precipitate 1 – precipitate 2	0.75	0.176	23
Precipitate 2 – precipitate 3	0.50	0.054	11

However, if the ethanolic NaOH extracted only hordein (amide content 23 %) and hordenin (amide content 10 %) it should be possible to calculate from the amide content of the mixture the relative proportions of the two components. On this count, the hordenin fraction in this experiment should contain approx. 2.94 mg. N/g. flour. In fact, none of the fractions which were precipitated and had an amide content of about 10 % of the total N, exceeded 1.86 mg. N/g. flour. Later experiments revealed that hordenin is appreciably modified on

the fraction left after the first precipitation, indicated that this was hordein, the main contaminant. Further reprecipitation results in some loss of hordenin; this is shown by the low amide content of the material remaining in solution after the third precipitation, as calculated from the difference between the second and third precipitates. This experiment seems to establish with reasonable certainty that the ethanolic NaOH extract contains only two types of protein, hordein and hordenin. Within the experimental error of the

determinations, which may be judged from the agreement between the sum of fractions and the direct estimates, these two protein fractions are shown to have amide contents of approx. 23 and 10% respectively and to occur in quantities which agree closely with those calculated in the manner described above from the amide content of the ethanolic NaOH extract, namely 10.65 mg. hordein N and 4.75 mg. hordenin N/g. flour.

Preparation of proteins for analysis

Specimens of albumin, globulin and hordein were prepared by methods closely similar to those of Osborne (1895*a*) and the procedure is only briefly summarized here. A more detailed description of the preparation of hordenin is given, as this has not been previously described and the volumes employed are important; attempts to carry out the preparation with a smaller ratio of solvent to flour resulted in specimens of hordenin severely contaminated with hordein. The whole barley flour used in these preparations was obtained from grain of high nitrogen content (2% N) by grinding in a rotary hammer mill.

Albumin and globulin. Barley flour was shaken with 5 times its weight of 5% (w/v) K_2SO_4 for 30 min. and then centrifuged off and re-extracted in the same way. The combined extracts were filtered and then saturated with $(NH_4)_2SO_4$. The precipitate was filtered off, redissolved in 3% K_2SO_4 , and then dialysed against tap water and finally distilled water. The precipitated globulin was centrifuged off and the solution filtered through a pad of paper pulp. From this solution the albumin was coagulated by heating to 80° in the presence of 0.01M sodium acetate buffer (pH 4.6). Both proteins were washed with distilled water, twice with ethanol, once with ether and finally dried at 80°. Preparation I from 300 g. flour gave 0.57 g. albumin and 1.57 g. globulin. Preparation II was more carefully purified, in that the globulin precipitate was redissolved in 5% K_2SO_4 , filtered and redialysed. The albumin solution was also redialysed and filtered before the albumin was coagulated. 600 g. flour gave 0.82 g. albumin and 0.39 g. globulin. The low yield of globulin was probably due to the difficulty of completely redissolving the precipitated protein after the first dialysis.

Hordein. The extracted residue from preparation I above was heated to 55° with 1.5 l. 70% (v/v) ethanol for 30 min., with occasional shaking, and then centrifuged off rapidly. The decanted supernatant was somewhat cloudy because hordein came out of solution as the solvent cooled. It was therefore reheated before it was filtered. The filtrate was concentrated *in vacuo* to one-third of its original volume and then refrigerated to complete the precipitation

of the protein. The precipitate was centrifuged off and extracted twice with a total of 1 l. of hot 70% ethanol. The combined extracts were reheated and poured into 7 l. of absolute ethanol to precipitate the hordein. The centrifuged precipitate was washed twice with ethanol and once with ether and dried at 80°. Total weight from 300 g. flour was 6.40 g.

Hordenin. 560 g. flour was extracted by shaking for 15 min. with 2.4 l. 5% K_2SO_4 and then centrifuging. This was repeated twice with K_2SO_4 and then once with distilled water. All these extracts were rejected. The residue was then similarly extracted four times with 0.05N-NaOH in 50% (v/v) ethanol. The combined extracts were filtered through a pad of paper pulp, previously washed with 50% ethanol, and then made up to 24 l. and 55% (v/v) ethanol content by addition of 8.5 l. ethanol and 6 l. distilled water. This dilution was necessary to prevent the separation of hordein at the next step, which was to adjust the pH to 6.4–6.5 at the glass electrode by the addition of 2N-HCl (approx. 50 ml.). The whole procedure was performed as quickly as possible so that the pH adjustment was complete not more than 3 hr. after the start of the ethanolic NaOH extraction. The dense precipitate of hordenin was allowed to settle for 2 hr. and was then separated by decanting and centrifuging. The separated protein was pounded in a mortar to break up lumps and then stirred mechanically for 30 min. with 1.6 l. ethanolic NaOH. The slightly cloudy solution was centrifuged and a slight amorphous residue separated. To the clear solution were added 3.6 l. ethanol and 2.8 l. water to bring the ethanol content to 55%. The pH was then adjusted to 6.4 and the precipitate allowed to settle. The solid, collected by decanting and centrifuging, was washed twice with ethanol and once with ether and dried at 80°. During this final washing the product became contaminated with a small amount of glass wool, but the low ash content of the preparation (see Table 4) shows this to be of negligible importance. Total yield was 4.39 g.

RESULTS

General analysis of protein preparations

Results of analyses of total N, ash and amide content of the various protein preparations are given in Table 4. From this it is apparent that the preparations II of albumin and globulin are purer than preparations I. This is evident from the very low total N of globulin I, indicative of carbohydrate contamination, and from the slightly lower amide content of albumin I which may be caused

Table 4. *Analysis of protein preparations*

The values (%) are for moisture-free preparations.

	Albumin		Globulin		Hordein	Hordenin
	I	II	I	II		
Total N	15.3	15.3	11.9	16.9	16.6	15.5
Ash	—	0.4	—	3.6	0.2	2.4
Total N (calc. for ash-free sample)	—	15.3	—	17.5	16.6	15.9
Amide N (as % total N)	5.8	5.9	5.1	5.1	23.0	10.3

by globulin contamination. For this reason preparations II were used as far as possible for the analysis of amino acid content described in the next section.

Amino acid content of the proteins

The results of the analyses of amino acid content are given in Table 5. Those for albumin are based throughout on preparation II. Globulin II was also mainly used, but the data for glycine, alanine, tryptophan, serine and threonine are derived from globulin I.

and manometrically by glutamic acid decarboxylase, although the results for albumin are an exception; it seems probable from a detailed examination of these analyses that a value of 8.2% glutamic acid N is not inconsistent with either set of data. As in the analyses of the protoplasmic proteins, estimates of tyrosine in both acid and alkaline hydrolysates agree fairly closely; such differences as do appear may be attributed to experimental error rather than to any systematic differences in losses under the two hydrolysis procedures.

Table 5. *Amino acid content of the protein preparations*

	Hordein		Hordenin	Globulin	Albumin	Protoplasmic protein, barley seedling (Yemm & Folkes, 1953)
	Present work	Others (Bishop, 1928)				
Amide (mild hydrolysis)	23.0	—	10.3	5.1	5.9	4.8
Aspartic acid	1.2	0.81	4.7	5.6	8.0	6.6
Glutamic acid { assay { decarboxylase	23.0* 23.6	22.85–23.9	{ 11.6 11.8	{ 6.8 7.1	{ 8.7 7.7	8.0
Proline	15.3*	4.16–9.72	6.6*	2.7*	4.2*	3.9
Glycine	1.7*	0.00	5.2	10.7	6.7*	6.9
Alanine	2.2	0.39–1.22	6.6	0.65	7.2	8.3
Valine	3.5	0.09–0.97	4.9	4.1	5.8	5.1
Leucine	4.6	3.52–4.35	{ 5.8 3.5	{ 4.5 2.2	{ 5.7 4.1	{ 6.0 4.5
Isoleucine	3.6		2.7	2.1	3.0	3.1
Phenylalanine	3.6	2.48–2.71	1.9	1.5	2.7	2.3
Tyrosine { acid hydrol. { alk. hydrol.	1.6 1.6	0.75–1.80	{ 1.9 1.9	{ 1.3 2.2	{ 2.7 2.2	
Tryptophan (alk. hydrol.)	0.70	0.36–0.85	1.1	0.65	1.3	1.9
Serine	3.2	0.08	4.2	3.9	4.1	3.5
Threonine	1.9	—	3.1	2.4	3.4	4.0
Cystine + cysteine	1.5	1.00–1.05	0.90*	2.6	1.5	0.9
Methionine	0.75	—	1.1	0.90	1.4	1.2
Lysine	0.80*	0.00	4.8*	6.3*	7.9*	7.9
Histidine	2.2*	0.80–2.02	4.3	3.1*	4.3*	4.2
Arginine	6	4.04–5.88	12	22	13	15
Insoluble humin	0.85	—	3.9	1.4	1.9	4.3
Ammonia (acid hydrol.)	23.0	20.8–23.3	11.5	8.0	7.0	5.8
Total in acid hydrolysate	100.8†	—	99.4†	91.6†	100.1†	101.5†

* Based on duplicate assays.

† Amide and tryptophan are omitted from this total. A mean value has been taken for glutamic acid.

All of the analyses were carried out alongside those of the protoplasmic proteins of barley already described by Yemm & Folkes (1953). The comments made in that paper on the probable accuracy of the estimates apply equally here. In general, the standard error of individual determinations is unlikely to be greater than about 4%, but losses during hydrolysis may be severe in the estimation of cystine and cysteine and somewhat less extensive with methionine, threonine and serine. The analyses of arginine are considerably less reliable than those of the other amino acids.

As can be seen from Table 5, satisfactory agreement is, in the main, apparent between the estimates of glutamic acid by microbiological assay

For three of the protein preparations analysed in the present investigation it was possible to account for 99–101% of the total nitrogen in terms of amino acids, ammonia and humin estimated in the acid hydrolysate. A similar result was obtained in the analyses of the protoplasmic proteins. The position of the globulin, in which less than 92% of the N can be accounted for, is therefore exceptional. The result suggests that some nitrogen-containing compound other than those estimated may be present in the globulin fraction. No unknown spot could be detected by ninhydrin on two-dimensional paper chromatograms of the hydrolysate run in phenol saturated with water and in butanol-acetic acid-water (2:1:1 by vol.) and tests for

hydroxyproline by the method of McFarlane & Guest (1939) were negative. No suggestion can therefore be put forward as to the nature of this unknown component. However, assuming that purified barley globulin has a nitrogen content of 18.1% (Osborne, 1895*a*) and that the unknown component accounts for 8% of the protein N, its nitrogen content would be about 15%.

The fairly complete recoveries of total N noted for the other three protein fractions allow estimates to be made of their probable nitrogen contents when free of carbohydrates and other contaminants. Thus 'pure' preparations of hordein, hordenin and albumin are likely to have nitrogen contents of 17.5, 17.4 and 16.7% respectively. It is noteworthy that Osborne (1895*a*) found 17.4 and 16.8% as the nitrogen contents of his purest preparations of hordein and albumin.

DISCUSSION

Few previous analyses of the proteins of barley grains are available for comparison with the present results. Only hordein has been extensively analysed, and for this component results, obtained chiefly by the ester distillation method, have been reviewed by Bishop (1928) and are given, expressed on a percentage nitrogen basis, in Table 5. As might be expected from the methods used, the greatest differences as compared with the present analyses are apparent for monoamino monocarboxylic acids; reasonable agreement is, in general, found for amide and the basic and acidic amino acids. No previous analyses are available for the other protein fractions except for results by the indirect method of Van Slyke (1911). These results, while recognized to be unreliable, are of interest in connexion with the published methods for the preparation of hordenin. As already noted in this paper, if hordenin is to be prepared uncontaminated with hordein, care must be taken to carry out the isoelectric precipitation from a volume of ethanolic sodium hydroxide which is large enough to retain in solution all the hordein present. In their preparation, Csonka & Jones (1929) used a volume which was only about one-quarter of this, and while subsequent precipitations with ammonium sulphate may have resulted in further purification, it seems probable from the low lysine N (3.4% of the protein N) and high ammonia N (16.1%) content of their preparation that it was severely contaminated with hordein. In contrast, Larmour (1927) used barley flour from which the hordein had already been extracted by hot ethanol and there was thus no danger of contamination, although the protein may have been somewhat denatured. His values for lysine N and ammonia N by the Van Slyke method were 5.3 and 11.4%, quite close to

those given for hordenin in Table 5 (i.e. 4.8 and 11.5%).

When the four types of protein from barley grains are compared with one another, it is clear that hordein and hordenin are markedly different from the albumin and globulin. These latter resemble much more closely the protoplasmic protein from young barley seedlings described by Yemm & Folkes (1953) and this is especially true of the albumin preparation (see Table 5). Osborne (1909) has pointed out that most of the albumin and globulin of the wheat grain are present in the embryo, and are probably physiologically active proteins; the relatively much smaller amounts in the endosperm may be derived from the protoplasmic structures of this tissue. From analyses performed in this laboratory it is likely that the same is true for barley. Moreover, the close association of diastatic properties with the albumin of barley malt, noted by Osborne (1895*b*), and the similar physical properties (e.g. isoelectric point, heat coagulation) of albumin and the protoplasmic proteins further support this idea. In contrast, hordein and hordenin may be considered as relatively inert storage products.

It can be seen from Table 3 that over 90% of the total nitrogen of the grain can be extracted by 5% potassium sulphate solution followed by ethanolic sodium hydroxide. Of these extracts, it has been shown that the ethanolic sodium hydroxide extract may be cleanly fractionated into hordein and hordenin, while the extract in potassium sulphate solution contains albumin, globulin and non-protein nitrogenous fractions. The small proportion of nitrogen left unextracted in the flour has a low amide content similar to that of the physiologically active proteins, and it seems probable that this represents protoplasmic protein, either denatured or trapped in unruptured cells. Some support for the latter view is given by the fact that this fraction is larger when the flour is ground in an adjustable cone mill, which is less efficient than a ball mill (cf. Tables 2 and 3).

The nitrogenous constituents of barley grains can therefore be analysed into two types of storage proteins, hordein and hordenin, together with smaller amounts of physiologically active proteins and non-protein compounds. Upon germination of the grain, practically all of these constituents are broken down; the products are translocated to the growing embryo and are there incorporated into protoplasmic proteins and, to a much smaller extent, non-protein substances (Folkes, Willis & Yemm, 1952). The major changes are therefore the conversion of the storage proteins into physiologically active, protoplasmic proteins. The latter have already been examined by Yemm & Folkes (1953) and have been shown to vary little in

composition, whether derived from cytoplasm or chloroplasts or from seedling or mature tissues. It is therefore possible to estimate the nature and extent of the amino acid interconversions involved in germination by a comparison of the amino acid contents of the storage and protoplasmic proteins. A similar comparison has already been made by Chibnall (1939) from rather incomplete analyses of the proteins of wheat, and the present results largely confirm his conclusions.

It can be seen from Table 5 that hordein and hordenin are far richer than the protoplasmic proteins in amide, glutamic acid and proline. Indeed over 60% of the hordein N is accounted for by glutamic acid and amide (probably associated as glutamine) and proline residues. On the other hand, the storage proteins are markedly deficient in aspartic acid, alanine, glycine and the basic amino acids lysine and arginine. Smaller deficiencies in isoleucine, tryptophan and threonine may also be noted. It is therefore reasonable to suppose that, during the germination of the barley grain, there is an overall synthesis of these amino acids, the nitrogen for which is derived almost wholly from glutamine and proline residues in the storage proteins. By measurements of the quantitative changes in the various protein fractions during germination of barley grain, more precise estimates of the interconversion of amino acids can be made; their significance in relation to the metabolism of the seedling will be considered more fully in a later paper.

SUMMARY

1. Preparations are described of the four chief protein fractions of barley grains, i.e. albumin, globulin, prolamin (hordein) and glutelin (hordenin). One of these, hordenin, is prepared by a new method which was tested in a series of experiments involving isoelectric reprecipitation of the protein from solutions of different ethanol content.

2. Analyses of amino acid content of the various preparations have been made, chiefly by microbiological assay. The results are compared with an analysis of the protoplasmic protein of barley seedlings which has been previously described.

3. While the albumin and the globulin closely resemble the protoplasmic proteins in their amino acid content, hordein and hordenin differ widely from it. The significance of these results is discussed with reference to the status of the albumin and the globulin and to the amino acid interconversions involved in the germination of the barley grain.

We are indebted to Dr A. J. Willis and Professor M. Skene for their helpful criticism of the manuscript.

REFERENCES

- Biserte, G. & Scriban, R. (1950). *Bull. Soc. Chim. biol., Paris*, **32**, 959.
 Bishop, L. R. (1928). *J. Inst. Brew.* **34**, 101.
 Bishop, L. R. (1929). *J. Inst. Brew.* **35**, 316.
 Blish, M. J. (1925). *Cereal Chem.* **2**, 127.
 Blish, M. J. & Sandstedt, R. M. (1929). *J. biol. Chem.* **85**, 195.
 Chibnall, A. C. (1939). *Protein Metabolism in the Plant*. New Haven: Yale University Press.
 Csonka, F. A. & Jones, D. B. (1929). *J. biol. Chem.* **82**, 17.
 Danielsson, K. E. (1949). *Biochem. J.* **44**, 387.
 Folkes, B. F. (1951). *Biochem. J.* **49**, xxvii.
 Folkes, B. F. (1953). *Analyst*, **78**, 496.
 Folkes, B. F., Willis, A. J. & Yemm, E. W. (1952). *New Phytol.* **51**, 317.
 Gale, E. F. (1945). *Biochem. J.* **39**, 46.
 Larmour, R. K. (1927). *J. agric. Res.* **35**, 1091.
 McFarlane, W. D. & Guest, G. H. (1939). *Canad. J. Res. B*, **17**, 139.
 Osborne, T. B. (1895a). *J. Amer. chem. Soc.* **17**, 539.
 Osborne, T. B. (1895b). *J. Amer. chem. Soc.* **17**, 587.
 Osborne, T. B. (1909). *The Vegetable Proteins*, 1st ed. London: Longmans Green.
 Quensel, O. & Svedberg, T. (1938). *C.R. Lab. Carlsberg, Sér. chim.* **22**, 441.
 Rose, R. C. & Anderson, J. A. (1936). *Canad. J. Res. C*, **14**, 109.
 Sävborn, S., Danielsson, K. E. & Svedberg, T. (1944). *Svensk kem. Tidskr.* **56**, 75.
 Scallet, B. L. (1947). *J. Amer. chem. Soc.* **69**, 1602.
 Van Slyke, D. D. (1911). *J. biol. Chem.* **10**, 15.
 Yemm, E. W. (1949). *New Phytol.* **48**, 315.
 Yemm, E. W. (1950). *Proc. Roy. Soc. B*, **136**, 632.
 Yemm, E. W. & Folkes, B. F. (1953). *Biochem. J.* **55**, 700.
 Youngburg, G. E. & Pucher, G. W. (1924). *J. biol. Chem.* **61**, 741.