AMINO ACIDS AND PROTEIN SYNTHESIS IN DEVELOPING WHEAT ENDOSPERM

By A. C. Jennings* and R. K. Morton*

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Summary

The amino acid composition of several protein fractions of developing wheat endosperm (cv. Gabo, grown in 1960 in Adelaide) was determined by ion-exchange chromatography. There were considerable differences in the compositions of the fractions extracted by pyrophosphate, acetic acid, and by sodium hydroxide. The composition of the fraction extracted by acetic acid remained relatively constant during development whereas there were changes in the compositions of the fractions extracted by pyrophosphate buffer, and by sodium hydroxide.

The amino acid composition of the total protein body fraction, at day 18 and at day 46 after flowering, showed little change during development. However, there was considerable difference in composition between the total protein body fraction and a fraction of small protein bodies obtained by gradient centrifugation.

The results suggest that the amino acid composition of the protein bodies may change during growth and development of the protein body. The number of protein bodies increase during development of the endosperm; the increase in the amount of residues of amino acids in the protein body fraction during endosperm development confirms that the protein bodies are the sites of accumulation of storage proteins in endosperm.

I. Introduction

During the development of wheat endosperm, there is rapid synthesis of proteins, which may be fractionated by successive extraction with sodium pyrophosphate, acetic acid, and sodium hydroxide (Graham, Morton, and Simmonds 1963). Jennings and Morton (1963) have described the changes in the major nitrogenous constituents during grain development. This paper compares the changes in the amino acid composition of the non-protein amino acid pool of the endosperm with the changes during development in the amino acid composition of the protein fractions, and also of the protein bodies and of the "soluble" (high-speed supernatant) proteins (Graham *et al.* 1962; Graham, Morton, and Raison 1963; Jennings, Morton, and Palk 1963). The protein bodies are the sites of accumulation of the storage proteins of endosperm (Graham *et al.* 1962; Graham, Morton, and Raison 1963).

II. Materials and Methods

Triticum vulgare cv. Gabo was grown in field plots during 1960; the grain was harvested and the endosperm was dissected from the grain as already described (Jennings and Morton 1963).

Proteins were extracted from dried endosperm by successive treatments with 0·01M sodium pyrophosphate, 0·05N acetic acid, 0·1N sodium hydroxide (Graham,

* Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide.

Morton, and Simmonds 1963), and the non-protein nitrogen fractions were prepared by dialysis of the sodium pyrophosphate extracts (Graham, Morton, and Simmonds 1963).

Endosperm tissue was homogenized and fractionated by differential centrifugation to obtain protein bodies and high-speed supernatant proteins as described by Graham, Morton, and Raison (1963), but modified (J. K. Raison, unpublished data) to obtain the total protein bodies (i.e. precipitate obtained at 10,000 *g*) and the total soluble protein (i.e. supernatant obtained at 105,000 *g*). Amino acids, other than cystine, cysteine, methionine, and proline, were estimated after hydrolysis of the material in 6N HCl for 20 hr at 110°C in an evacuated sealed tube (Moore and Stein 1954). An automatic, 9-column amino acid analyser (Simmonds and Rowlands 1960) was used. Proline in the hydrolysates was estimated as described by Chinard (1952). Cystine (and cysteine) were estimated as cysteic acid, and methionine was estimated as methionine sulphone after treatment of the protein with performic acid (Schram, Moore, and Bigwood 1954) before hydrolysis, essentially as described by Simmonds (1962).

III. Results

The changes during grain development in the amino acid composition of the various fractions studied are shown in Tables 1–6. In Tables 1, 3, and 5 the composition at each stage of development (i.e. at 14, 18, 32, and 46 days after flowering) is given as the amino acid nitrogen as a percentage of the total amino acid nitrogen of the sample; Tables 2, 4, and 6 give the amount of amino acid residues in the endosperm for each stage of development. Tables 1 and 2 show the results for the whole endosperm, the non-protein nitrogen fraction, and the total protein. Tables 3 and 4 give the results for the protein fractions extracted by successive treatments with pyrophosphate, acetic acid, and sodium hydroxide. Tables 5 and 6 show the changes in the amino acid composition of the protein body fraction and of the soluble protein (high-speed supernatant) fraction.

Because of the loss of amide nitrogen from the protein stored in 0·1N NaOH, the values obtained for amide nitrogen in the protein fraction extracted with 0·1N NaOH were made equal to the values for glutamic acid. Although there may also be loss of cystine and, to a lesser extent, of methionine in this protein fraction, no correction was applied to the values obtained, and these are probably low.

The soluble protein fraction contained detergent ("Nonidet P 40"; see Graham, Morton, and Raison 1963). This was partly removed by dialysis. It has been found that digestion of amino acids with 6N HCl at 110°C for 20 hr in the presence of 2% "Nonidet P 40" results in greater destruction of amino acids than in the absence of the detergent; there is preferential loss of glutamic acid. However, because of the uncertainty as to the amount of detergent actually present in the supernatant protein fractions at the time of digestion, and the uncertainty concerning the influence of other non-nitrogenous materials present in the extracts, the results are given without correction for possible losses, except that the value for glutamic acid was corrected by making it equal to the value for amide (see Table 5).

TABLE 1

CHANGES DURING DEVELOPMENT IN THE AMINO ACID COMPOSITION OF WHOLE ENDOSPERM OF WHEAT (CV. GABO, 1960 HARVEST), AND OF THE NON-PROTEIN AND PROTEIN NITROGEN FRACTIONS

Amino acid nitrogen as a percentage of total nitrogen. Coefficient of variation for more than two analyses given in parenthesis. n.d., not determined

Amino Acid	Whole Endosperm				Non-protein Nitrogen Fraction				Total Protein*			
Days after flowering:	14	18	32	46	14	18	32†	46	14	18	32†	46
Aspartic acid	5·7	3·9	2·8	2·6	6·6(8·1)	7·3(6·6)	—	13·6(8·0)	5·4	3·5	—	2·4
Threonine	2·5	1·9	1·9	1·7	1·5	1·3	—	1·2	2·8	1·9	—	1·7
Serine	4·4	4·1	4·0	3·4	6·7(6·3)	9·6	—	3·1(3·7)	3·6	3·4	—	3·5
Glutamic acid	12·4	14·7	19·0	19·5	20·0	20·4(11·1)	—	19·1(8·7)	9·8	13·9	—	19·5
Glycine	4·8	5·3	3·9	3·7	5·8	7·9(6·4)	—	5·4(6·4)	4·2	4·9	—	3·7
Alanine	7·5	5·1	4·0	2·8	11·2	13·1(9·8)	—	9·3	6·2	4·0	—	2·7
Valine	3·8	3·6	3·4	3·4	2·2(1·7)	2·5	—	1·2	4·3	3·7	—	3·4
Isoleucine	2·5	2·1	2·2	2·0	1·0	0·79	—	0·66	3·0	2·3	—	2·0
Leucine	4·8	4·6	4·9	4·6	0·79(10·7)	0·47	—	0·66	6·1	5·1	—	4·7
Tyrosine	1·4	1·1	1·3	1·4	1·5	1·3	—	1·7	1·4	1·1	—	1·4
Phenylalanine	2·0	2·2	2·7	2·8	1·8	1·6	—	0·62	2·1	2·3	—	2·8
Lysine	6·9	6·0(9·5)	3·7(3·2)	2·8(3·6)	4·3	1·9	—	2·2	7·7	6·5	—	2·9
Histidine	3·6	3·7(2·2)	3·6	4·0	4·4(6·2)	3·7(3·7)	—	4·1	3·2	3·7	—	4·0
Amide nitrogen	11·6	17·6	17·7	18·2	18·6	14·8	—	20·0	9·3	18·0	—	18·2
Arginine	10·2	8·4	7·8	7·7	3·9(12·2)	4·6(10·3)	—	9·9	12·3	8·8	—	7·7
Proline	7·7	8·0(0·8)	9·6(2·1)	11·3	4·7	4·0	—	2·4	8·7	8·5	—	11·5
½ Cystine	1·2	1·2(4·2)	1·3	1·6	n.d.	n.d.	—	n.d.	n.d.	n.d.	—	n.d.
Methionine	2·2	1·8	1·1	1·4	n.d.	n.d.	—	n.d.	n.d.	n.d.	—	n.d.

* Estimated by difference between values for whole endosperm material and the non-protein nitrogen fraction.

† Amino acid content not determined for day 32.

TABLE 2

AMINO ACID RESIDUES IN WHOLE ENDOSPERM OF WHEAT (CV. GABO, 1960 HARVEST) AND IN THE PROTEIN AND NON-PROTEIN NITROGEN FRACTIONS DURING DEVELOPMENT OF ENDOSPERM

Results are given as μg per grain

Amino Acid	Whole Endosperm				Non-protein Nitrogen Fraction				Total Protein*			
Days after flowering:	14	18	32	46	14	18	32†	46	14	18	32†	46
Aspartic acid	34	57	86	147	9·7	11·9	—	14	24	45	—	134
Threonine	13	24	53	84	2·0	1·8	—	1·1	11	22	—	83
Serine	20	46	94	147	7·6	12·0	—	2·3	12	34	—	145
Glutamic acid	82	240	664	1218	33	38	—	22	49	203	—	1197
Glycine	14	38	61	101	4·3	6·4	—	2·7	9·3	32	—	99
Alanine	27	46	77	97	10	13	—	5·7	17	32	—	92
Valine	19	45	92	161	2·8	3·5	—	1·1	17	42	—	160
Isoleucine	15	31	69	109	1·5	1·3	—	0·65	13	29	—	109
Leucine	28	66	146	253	1·1	0·75	—	0·65	27	65	—	252
Tyrosine	12	23	56	109	3·1	2·9	—	2·3	8·6	20	—	107
Phenylalanine	15	42	109	195	3·5	3·3	—	0·80	12	39	—	195
Lysine	23	49	65	88	3·5	1·7	—	1·2	19	47	—	87
Histidine	8	21	45	88	2·8	2·4	—	1·6	5·6	19	—	86
Amide nitrogen	10	36	77	141	3·8	3·4	—	2·8	5·8	32	—	138
Arginine	21	41	83	146	2·0	2·6	—	3·3	19	39	—	142
Proline	39	98	254	530	5·8	5·5	—	2·0	3·3	93	—	528
½ Cystine	6	15	37	79	—	—	—	—	—	—	—	—
Methionine	15	30	39	87	—	—	—	—	—	—	—	—
Nitrogen	72	178	380	677	18	20	19	12	54	158	361	665

* Estimated by difference between values for whole endosperm material and the non-protein nitrogen fraction.

† Amino acid content not determined for day 32.

TABLE 3

CHANGES DURING DEVELOPMENT IN THE AMINO ACID COMPOSITION OF THE PYROPHOSPHATE-SOLUBLE, ACETIC ACID-SOLUBLE, AND SODIUM HYDROXIDE-SOLUBLE PROTEINS EXTRACTED FROM ENDOSPERM OF WHEAT (CV. GABO, 1960 HARVEST)

Amino acid nitrogen as a percentage of total nitrogen. Coefficient of variation for more than two analyses given in parenthesis. n.d., not determined

Amino Acid	Pyrophosphate-soluble Proteins				Acetic Acid-soluble Proteins				Sodium Hydroxide-soluble Proteins			
Days after flowering:	14	18	32	46	14	18	32	46	14	18	32	46
Aspartic acid	4·6	5·0(4·3)	4·5	4·6	2·0	1·8	1·9(7·8)	2·0(1·7)	5·7	6·5	3·6	3·3
Threonine	2·5	2·7(6·1)	2·4	2·5	1·6	1·6	1·5(3·3)	1·4	2·7	3·1	2·4	2·0
Serine	3·8	3·6(9·9)	3·3	3·4	3·6	3·9	3·6(3·4)	3·3	3·8	4·1	3·8	3·4
Glutamic acid	12·4	11·5	14·2	13·5	23·0	22·9	23·3(3·9)	24·0(3·4)	12·0	10·6	15·6	17·7
Glycine	4·2	4·7	4·7	5·4	3·0	3·7	2·9(2·3)	2·9(5·8)	5·7	6·1	6·0	5·4
Alanine	4·9	5·1	4·6	5·0	2·1	1·9	2·2(6·0)	2·2(6·0)	5·3	5·7	4·7	4·1
Valine	5·6	5·1	5·4	4·2	3·2	3·2	3·1(3·0)	3·0	3·9	4·7	3·7	3·6
Isoleucine	2·2	2·4	2·3	2·0	2·4(9·4)	2·2	2·5(3·7)	2·5	2·7	3·0	2·5	2·4
Leucine	4·4	4·0	4·9	4·4	4·7(1·2)	4·4(4·8)	4·7(2·0)	4·8(0·8)	5·7	6·0	5·3	5·1
Tyrosine	2·0	1·5	1·5	1·3	1·7	1·5(6·0)	1·7	1·7(8·6)	1·9	1·8	1·9	1·8
Phenylalanine	2·5	2·1	2·3	2·0	3·4(3·7)	2·8	3·1	3·1	2·4	2·3	3·0	2·7
Lysine	7·9	7·8	4·5(4·5)	3·9	1·4(9·2)	1·3	1·1	1·2(10·4)	8·3	8·6	4·8	4·4
Histidine	3·7	4·4	4·4	3·8	3·2(10·4)	3·8(3·3)	3·4(9·1)	4·1(4·6)	3·7	3·7	3·5	3·4
Amide nitrogen	13·3	12·3	12·0	11·2(2·5)	20·9	19·5(10·2)	20·2	19·4	12·0	10·6	15·6	17·7
Arginine	11·1	12·7	11·8	12·3(6·1)	6·1	6·6(8·0)	5·9(1·2)	5·6	11·9(10·1)	13·0	10·1(3·5)	9·9
Proline	6·7	6·3(2·4)	8·2	8·2	12·5(1·1)	12·0(2·6)	11·2(4·5)	11·7(2·6)	6·4(1·0)	4·5	7·3(3·7)	8·0
½ Cystine	1·9	2·4(1·5)	2·4	2·9	n.d.	1·2(2·4)	1·5	1·2(3·4)	0·16	0·32	0·10	0·17
Methionine	1·5	1·6	1·6	1·9	n.d.	0·88	1·2	0·94	0·94	1·5	1·3	1·5

TABLE 4

AMINO ACID RESIDUES OF THE PYROPHOSPHATE-SOLUBLE, ACETIC ACID-SOLUBLE, AND SODIUM HYDROXIDE-SOLUBLE PROTEINS EXTRACTED FROM DEVELOPING ENDOSPERM OF WHEAT (cv. GABO, 1960 HARVEST)

Results are given as μg per grain

Amino Acid	Pyrophosphate-soluble Proteins				Acetic Acid-soluble Proteins				Sodium Hydroxide-soluble Proteins			
Days after flowering:	14	18	32	46	14	18	32	46	14	18	32	46
Aspartic acid	1·6	15	19	31	3·2	8·4	26	51	13	30	34	59
Threonine	0·72	7·1	8·9	15	2·2	6·6	17	33	5·4	13	20	32
Serine	0·88	8·1	11	17	4·4	14	37	65	6·7	15	27	46
Glutamic acid	4·6	38	67	92	41	120	350	702	31	54	167	359
Glycine	0·69	6·8	9·7	18	2·4	8·5	20	38	6·5	13	28	48
Alanine	0·96	9·3	12	21	2·1	5·4	18	36	7·5	16	28	46
Valine	1·6	13	20	24	4·3	13	36	68	7·9	19	30	56
Isoleucine	0·71	6·9	9·5	13	3·8	10	32	63	6·1	14	23	42
Leucine	1·4	12	20	29	7·4	20	62	122	12·8	27	49	91
Tyrosine	0·93	6·4	9	13	3·9	9·6	32	63	6·2	12	26	47
Phenylalanine	1·05	7·4	13	17	6·8	17	53	102	6·9	14	36	63
Lysine	1·46	13	11	15	1·3	3·4	8·4	18	10·7	22	25	44
Histidine	0·46	5·1	7·0	10	2·0	7·1	18	42	3·3	6·8	13	24
Amide nitrogen	0·62	5·0	7·0	10	4·6	13	38	70	3·8	6·7	21	46
Arginine	1·25	13	17	28	3·3	11	27	49	9·3	20	33	60
Proline	1·87	16	29	44	17	47	127	258	12·5	17	59	122
½ Cystine	0·57	6·3	9·1	17	—	4·9	18	27	0·32	1·3	0·87	2·7
Methionine	0·55	5·0	7·2	14	—	4·7	18	28	2·4	7·8	14	31
Nitrogen	4·1	36	51	81	19	57	163	318	28	56	116	220

The recovery of nitrogen as amino acids in relation to nitrogen of the sample used for amino acid separation and estimation varied from about 71 to 98%. All values given are adjusted to a recovery of 95%.

The amino acid compositions given are means of replicates. Where more than two analyses were obtained, the coefficient of variation is given in Tables 1, 3, and 5.

TABLE 5

CHANGES DURING DEVELOPMENT IN THE AMINO ACID COMPOSITION OF THE PROTEIN BODY FRACTION AND OF THE HIGH-SPEED SUPERNATANT PROTEIN FRACTION OF DEVELOPING WHEAT ENDOSPERM (CV. GABO, 1960 HARVEST)

Results are given as amino acid nitrogen as a percentage total nitrogen. Coefficient of variation for more than two analyses given in parenthesis. For details of preparation of fractions, see text. n.d., not determined

Amino Acid	Protein Body Fraction			Supernatant Fraction‡	
Days after flowering:	18*	18†	46†	18	46
Aspartic acid	5·1(2·2)	1·4	1·5	5·0(6·4)	4·2(5·4)
Threonine	2·4(1·1)	1·6	1·5	2·7	2·9(9·9)
Serine	4·0(4·6)	3·2	3·2	3·3(3·6)	3·5(5·7)
Glutamic acid	13·7(0·6)	20·4	21·4(3·5)	11·2§	13·1§
Glycine	5·0(3·0)	3·6	3·4	4·8	5·0(4·9)
Alanine	5·2	2·0	1·6	4·9(2·1)	4·5(5·1)
Valine	4·2(3·5)	4·3	3·7	3·8	5·2(11·1)
Isoleucine	2·9(2·6)	2·3	2·3	2·6	2·5(3·3)
Leucine	5·6(4·7)	4·2	4·5	4·6	5·4(6·5)
Tyrosine	1·9(4·3)	1·1	0·89	1·7(1·2)	1·9
Phenylalanine	2·6(1·8)	2·2	2·2	2·2(4·8)	2·3(6·5)
Lysine	6·3(4·5)	2·1	2·1	8·3(1·7)	7·4
Histidine	4·3(2·7)	2·7(4·0)	2·3	5·2	5·4
Amide nitrogen	11·6	20·2(0·8)	19·9	11·2	13·1(14·4)
Arginine	12·8(9·3)	6·5(7·9)	6·3	15·0(2·7)	11·7
Proline	7·4(2·9)	13·6	14·3	4·3	7·0
½ Cystine	n.d.	2·6(4·5)	2·8(7·5)	2·8(7·0)	n.d.
Methionine	n.d.	1·2	1·0	1·7	n.d.

* Fraction of protein bodies isolated and purified as described by Graham, Morton, and Raison (1963).

† Total protein bodies obtained by centrifuging at 10,000 *g* for 30 min.

‡ Supernatant remaining after centrifuging at 105,000 *g* for 90 min.

§ Values for glutamic acid were unreliable, and were adjusted to equal the value for amide.

For other determinations, the difference between duplicate estimations, expressed as a percentage of the mean, was within the range 0–35%, and it was usually less than 15%.

The results given here are from separate experiments on different samples of material from the same harvest. In the fractionation used for results given in Tables 5 and 6, ribosomal and other material which sediments on centrifuging to

obtain the supernatant material was discarded. At day 18, 51% of the non-dialysable nitrogen was associated with the protein body fraction and 38% with the soluble protein fraction; at day 46, 57% was associated with the protein body fraction and 17% with the soluble protein fraction.

TABLE 6

CHANGES DURING DEVELOPMENT IN THE AMINO ACID RESIDUES IN THE TOTAL PROTEIN BODY FRACTION AND IN THE HIGH-SPEED SUPERNATANT PROTEIN FRACTION OF WHEAT ENDOSPERM (CV. GABO, 1960 HARVEST)

Results are given as μg per grain

Amino Acid	Protein Body Fraction*		Supernatant Fraction†	
Days after flowering:	18	46	18	46
Aspartic acid	9·5	46	24	38
Threonine	9·1	40	11	23
Serine	16·2	76	12	24
Glutamic acid	152	745	62‡	132‡
Glycine	12	53	12	22
Alanine	8·3	31	15	25
Valine	25	100	16	40
Isoleucine	15	70	13	22
Leucine	28	137	22	48
Tyrosine	9·9	39	12	24
Phenylalanine	18	88	14	26
Lysine	7·7	37	23	37
Histidine	6·9	29	10	19
Amide nitrogen	19	86	8	16
Arginine	15	66	25	36
Proline	77	375	18	53
½ Cystine	16	77	12	—
Methionine	9·1	36	10	—
Nitrogen	81	378	60	110

* Obtained by centrifuging at 10,000 g for 30 min.

† Supernatant remaining after centrifuging at 105,000 g for 90 min.

‡ The values for glutamic acid are calculated from the adjusted values given in Table 5.

IV. Discussion

(a) *Analytical Procedure*

The results presented here were obtained by the procedure of Moore and Stein (1954) by using an automatic 9-column machine developed by Simmonds and Rowlands (1960), who have reported on the precision of the instrument in the amino acid analysis of insulin. The use of this apparatus for amino acid analysis of flour has been described by Simmonds (1962).

As indicated by the coefficients of variation given in Tables 1, 3, and 5, most of the amino acids of the various fractions were determined with considerable precision except for amide. When present in large amounts, this is probably underestimated due to relative overloading of the ion-exchange columns; if the loading is reduced to enable accurate estimation of amide, amino acids in low concentration would not be determined. The fault arises from poor colour development due to insufficient time of heating when a fraction contains a large content of ammonia.

(b) Changes in Amino Acids of Endosperm during Development

Table 1 shows that the amino acid composition of whole endosperm changes considerably during development, due to changes in the composition of both the non-protein nitrogen fraction (pool of free amino acids) and of the protein. Of the free amino acids, aspartic acid and arginine increase in relative amount during development, and lysine and proline decline, whereas most other amino acids, including glutamic acid and amide, show little relative change. The increase in the relative amount of free aspartic acid may possibly be related to the breakdown of leaf proteins, which are typically high in aspartic acid.

In agreement with analyses obtained previously (Jennings and Morton 1963) for cv. Gabo in 1959, the non-protein nitrogen formed a high proportion of the total nitrogen initially (at day 14, 25%) but declined rapidly and was about 2% at maturity (day 46) (see Table 1). As shown in Table 2, and in agreement with the previous findings (Jennings and Morton 1963), the amount of most amino acid residues per endosperm in the pool of free amino acids actually decline during development when protein-bound amino acids increase in amount.

The changes in the composition of the endosperm proteins (Table 1) and in the amount of amino acid residues in the endosperm proteins (Table 2) during development could be due to changes in composition and amount of one or more of the several protein fractions. As shown by Table 3, the change in composition occurs chiefly in the fraction extracted by pyrophosphate buffer and in the fraction extracted by sodium hydroxide. Graham and Morton (1963) have shown that the fraction extracted by pyrophosphate buffer contains a relative predominance of proteins which are fast moving on electrophoresis in starch gel, whereas the proteins subsequently extracted by acetic acid have a relative predominance of slow-moving components. The relative constancy of the amino acid composition of the proteins extracted with acetic acid is consistent with the view that these proteins form a considerable proportion of the storage proteins of wheat endosperm (Graham and Morton 1963; Graham, Morton, and Simmonds 1963). Table 4 shows that the amount of amino acid residues in each of the protein fractions increase considerably during development, the distribution between the proteins extracted by pyrophosphate, acetic acid, and sodium hydroxide reflecting the distribution of total protein nitrogen between these fractions (see also Graham, Morton, and Simmonds 1963; Jennings and Morton 1963).

The supernatant obtained after centrifuging homogenates of endosperm at 105,000 *g* for 90 min contains those proteins which move rapidly on electrophoresis in starch gel, uncontaminated by slow-moving components (Graham, Morton, and

Raison 1963). Similar components appear in the fraction extracted from whole endosperm by pyrophosphate buffer. Comparison of Tables 3 and 5 show that there is a general similarity in the amino acid composition of the supernatant fraction and of the pyrophosphate-soluble fraction, but comparison of the compositions for day 18 and day 46 reveal some differences in trends with time of development. For example, the proportion of lysine nitrogen falls from 7·8 to 3·9% in the pyrophosphate-soluble fraction, but changes little in the supernatant proteins. Since the supernatant protein fraction contains none of the slow-moving electrophoretic components found in the pyrophosphate extract of endosperm, it is considered that the changes in amino acid composition of the supernatant fraction is a truer indication of the relative changes in the composition of the non-storage proteins of endosperm. As shown by Table 6, there is only a slight increase in the amount of each of the amino acid residues of the supernatant fraction between day 18 and day 46, as compared with the changes in the amount of residues in the protein body fraction during this period. It is likely that the proteins of the supernatant are mostly enzymes concerned with cell maintenance and cell expansion. The supernatant may also contain some protein, or proteins, which are intermediates in the synthesis of the storage protein (see Graham and Morton 1963; Graham, Morton, and Raison 1963).

The fraction obtained by centrifuging the homogenates at 10,000 *g* for 30 min contains a large proportion of the total protein bodies of the endosperm. Comparison of Tables 3 and 5 shows that the composition of this fraction changes little between day 18 and day 46 and closely resembles the composition of the acetic acid-soluble proteins at the same periods after flowering. Yet isolated protein bodies are only partly soluble in dilute acetic acid (Graham, Morton, and Raison 1963). Table 5 also gives the amino acid composition of a fraction of protein bodies, separated and purified according to Graham, Morton, and Raison (1963). This fraction contains a predominance of small protein bodies selected from the total protein body fraction. The composition of these bodies differs from that of the total protein body fraction, and more closely resembles the composition of the sodium hydroxide-soluble proteins than that of the acetic acid-soluble proteins at day 18 (see Tables 3 and 5). This suggests that the protein bodies are not necessarily uniform in composition. Indeed, it is possible that the protein bodies may change in composition during their growth.

The marked increase in the amount of amino acid residues in the protein body fraction (Table 6) reflects the increase in the number of protein bodies during development. There is little doubt that these bodies are the sites of accumulation of the storage protein.

Folkes and Yemm (1956) showed that, on germination, the endosperm of barley provided directly over 70% of the amino acid residues of the developing embryo. Moreover, the additional amino acid requirement of the embryo was provided by synthesis from the excess glutamine and proline of the endosperm protein. Thus the function of the cereal endosperm is to provide nutrients for the developing embryo. The change in the amino acid composition of the wheat endosperm during development, and particularly the increase in glutamine (glutamic acid and amide) and in proline is probably therefore a physiological process related to the nutrition of the

embryo. This change appears to be largely brought about by a change in the amount of protein bodies in the endosperm cells. The protein of these bodies is only partly extracted with 0·05N acetic acid but much more completely extracted with 0·1N sodium hydroxide (Graham, Morton, and Raison 1963). Qualitatively, no new protein components appear to be formed after day 12 (Graham and Morton 1963). However, there may be quantitative changes and one or more distinct proteins, rich in glutamine and proline, may tend to form in maturing protein bodies, as indicated by the difference in amino acid composition of the total protein body fraction as compared with that of the isolated, small protein bodies. Thus the results here support other findings which have indicated the considerable importance of glutamine and of glutamic acid in protein metabolism in plants (see Yemm and Folkes 1958; Virtanen 1961) and in wheat in particular (Kretovich, Bundel, and Uspenskaya 1951).

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