Protein Metabolism in Developing Endosperms of High-Lysine and Normal Barley

P. R. SHEWRY, H. M. PRATT, M. M. LEGGATT, and B. J. MIFLIN, Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, England

Cereal Chem. 56(2):110-117

ABSTRACT

In barley cv. Bomi synthesis of hordein occurred between two and five weeks after anthesis, and this fraction accounted for approximately 50% of the total nitrogen of the mature endosperm. In the high-lysine mutant Risø 1508, however, hordein accounted for only 15% of the mature endosperm N and synthesis was essentially complete at four weeks. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing of the hordein fractions demonstrated only minor changes in the relative polypeptide compositions of both varieties during endosperm development. In Risø 1508 the amounts of nonprotein nitrogen (NPN) and salt-soluble proteins increased until three and four weeks, respectively, whereas in Bomi the amount of NPN fell and salt-soluble protein increased between two and three weeks, after which the amounts of both fractions remained constant. In Risø 1508 these fractions accounted for about 18 and 25% of the mature endosperm N compared with 9 and 12% in Bomi. The amount of glutelin N per endosperm was similar in both lines and represented about 20% of the total N in Bomi and 29% in Risø 1508. Incorporation of radioactivity from $^{14}CO_2$ into the N fractions of the developing endosperms was consistent with the changes in total amounts. Risø 1508 showed an increased incorporation of $^{14}CO_2$ in lipids, and further investigation of this fraction showed that the mutant had increased total amounts of both neutral and polar lipids. It was concluded that the Risø 1508 mutation acts throughout endosperm development and affects the synthesis of the major components of the seed—proteins, lipids, and carbohydrates.

The storage protein of barley grain is an alcohol-soluble protein (prolamin) termed hordein. This is the major nitrogenous fraction of the endosperm, accounting for up to 50% of the total nitrogen at grain maturity (Shewry et al 1978b). The unusual amino acid composition of this fraction, notably the very low lysine, is responsible for the poor nutritional quality of the grain as feed for monogastric animals. In the high-lysine barley mutant Risø 1508, produced by mutagenesis of the Scandinavian variety Bomi (Ingversen et al 1973), the increased lysine content results from depressed synthesis of hordein and an increase in other more lysine-rich protein fractions and in free amino acids (Brandt 1975, 1976; Ingversen et al 1973; Shewry et al 1978b). It is, therefore, important to understand the regulation of synthesis of hordein and the other N fractions of the seed if a rational approach to improving the quality of grain protein, either by agronomic practices or by breeding, is to be adopted.

Previous studies showed the general pattern of development in barley endosperms regarding the overall amino acid composition of the grain (Pomeranz 1973; Pomeranz and Robbins 1972; Pomeranz et al 1974, 1976, Smith 1972), and changes in the total levels of the various fractions (Bishop 1930, Brandt 1976, Briedert and Schon 1974, Ivanko 1971, Munck 1972), their amino acid composition (Brandt 1976, Ivanko 1971), and the nature of their component polypeptides (Briedert and Schon 1974, Brandt 1976, Munck 1972). Although the general conclusion can be drawn that hordein synthesis starts later and goes on longer than that of other fractions, the absolute amounts of hordein produced differ considerably, probably because some workers have used techniques that fail to completely extract this fraction. This can prevent accurate conclusions about the polypeptide and amino acid compositions of both the hordein and glutelin fractions and, for example, about the nature of the high-lysine mutation in Risø 1508 (Miflin and Shewry 1977, Shewry et al 1978b). Use of such procedures may account for conflicting reports of the presence (Briedert and Schon 1974) or absence (Brandt 1976) of changes in the hordein polypeptide pattern during grain development. Also previous studies have given little insight into the dynamics of the various groups, particularly possible protein turnover.

We reinvestigated the changes in protein fractions of developing barley endosperms using extraction procedures that reliably extract approximately 95% of the hordein polypeptides (Shewry et al 1978b). Improved gel separation and quantification procedures more accurately determined changes in the hordein polypeptide composition. We fed $^{14}CO_2$ to the developing grain to study the start and rate of synthesis and possible turnover of the various fractions. Comparison of Risø 1508 and Bomi gave further details of the effects of the high-lysine mutation, both during grain development and in the mature seed.

MATERIALS AND METHODS

Na_2 $^{14}CO_3$ (S.A. 59.3 mCi/mmol) was obtained from the Radiochemical Centre, Amersham.

Plant Growth

Seeds of Bomi and Risø 1508 (obtained from Risø) were grown in 13-cm pots (four plants per pot) under a glass canopy during the summer of 1975.

Ears were checked for anthesis every two days and 10 heads from each variety (main tillers from at least five different pots) were harvested at weekly intervals from two weeks after anthesis to grain maturity (seven weeks). Seeds from the central parts of the spikes were removed and the endosperms hand-dissected, frozen in liquid N_2, lyophilized, and stored desiccated at −15°C.

Isotope Administration

$^{14}CO_2$ was administered to 10 heads of each variety at weekly intervals from two to five weeks after anthesis. The heads were main tillers of 10 individual plants from at least five different pots. Each head was placed in a glass tube (25 × 1.7 cm) sealed at the top with a rubber bung and around the stem at the bottom with a split bung and cotton wool. $^{14}CO_2$ was liberated from a vial of Na_2 $^{14}CO_3$ (50 μCi) in the tube by addition of lactic acid through a Pasteur pipette fixed in a hole in the top bung. Feeding was under natural light supplemented with 1,000-W mercury vapor lamps. After 1 hr the tube contents were evacuated into 10% KOH, the tubes removed, and the plants replaced under normal growing conditions. Five heads were harvested and the endosperm dissected out, as described above, 24 hr after termination of isotope administration. The remaining five heads were harvested at grain maturity (seven weeks after anthesis).

Protein Extraction

Endosperms were ground either in a pestle and mortar (^{14}C-labeled seeds) or in a Glen Creston hammer mill to pass a 0.5-mm sieve.

Duplicate 1-g samples (0.5 g at two weeks after anthesis) were extracted sequentially in magnetically stirred screw-capped 50-ml

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centrifuge tubes (Kφie and Nielsen 1977) with the following solvents:

1. Butan-1-ol (two 15-ml volumes for 30 min each at 20° C) and petroleum ether (40–60° boiling range) (15 ml for 30 min at 20° C) to extract lipids.
2. 0.5*M* aqueous NaCl (three 15-ml volumes for 1 hr each at 20° C) to extract salt-soluble N.
3. 55% (v/v) aqueous propan-2-ol + 2% (v/v) 2-mercaptoethanol (three 15-ml volumes for 1 hr each at 60° C) to extract hordein.
4. 0.5*M* borate buffer, pH 10, + 1% (w/v) sodium dodecyl sulfate (SDS) + 1% (v/v) 2-mercaptoethanol (three 15-ml volumes for 1 hr each at 20° C) to extract glutelin.

The protein-containing extracts (2, 3, 4) were each made to 50 ml and aliquots removed for determination of total Kjeldahl N and (where necessary) radioactivity.

Aliquots (5 ml) of salt-soluble fractions were mixed with 1.25 ml of 25% (w/v) trichloroacetic acid (TCA) and allowed to stand overnight at 4° C. After centrifugation the precipitates (salt-soluble protein) and supernatants (nonprotein nitrogen) were assayed for total Kjeldahl N.

Extracts 3 and 4 were dialyzed against water to remove solvents and buffer salts, lyophilized, and pyridylethylated as described previously (Shewry et al 1978a).

Protein Hydrolysis

Samples (100 mg) of milled endosperms were hydrolyzed with 10 ml of 6*N* HCl under N_2 in sealed tubes at 110° C for 21 hr (Kirkman 1974). Samples (10 mg) of alkylated protein fractions were hydrolyzed similarly but with addition of 0.1% 2-mercaptoethanol to protect methionine.[1]

[1]M. A. Kirkman, unpublished data.

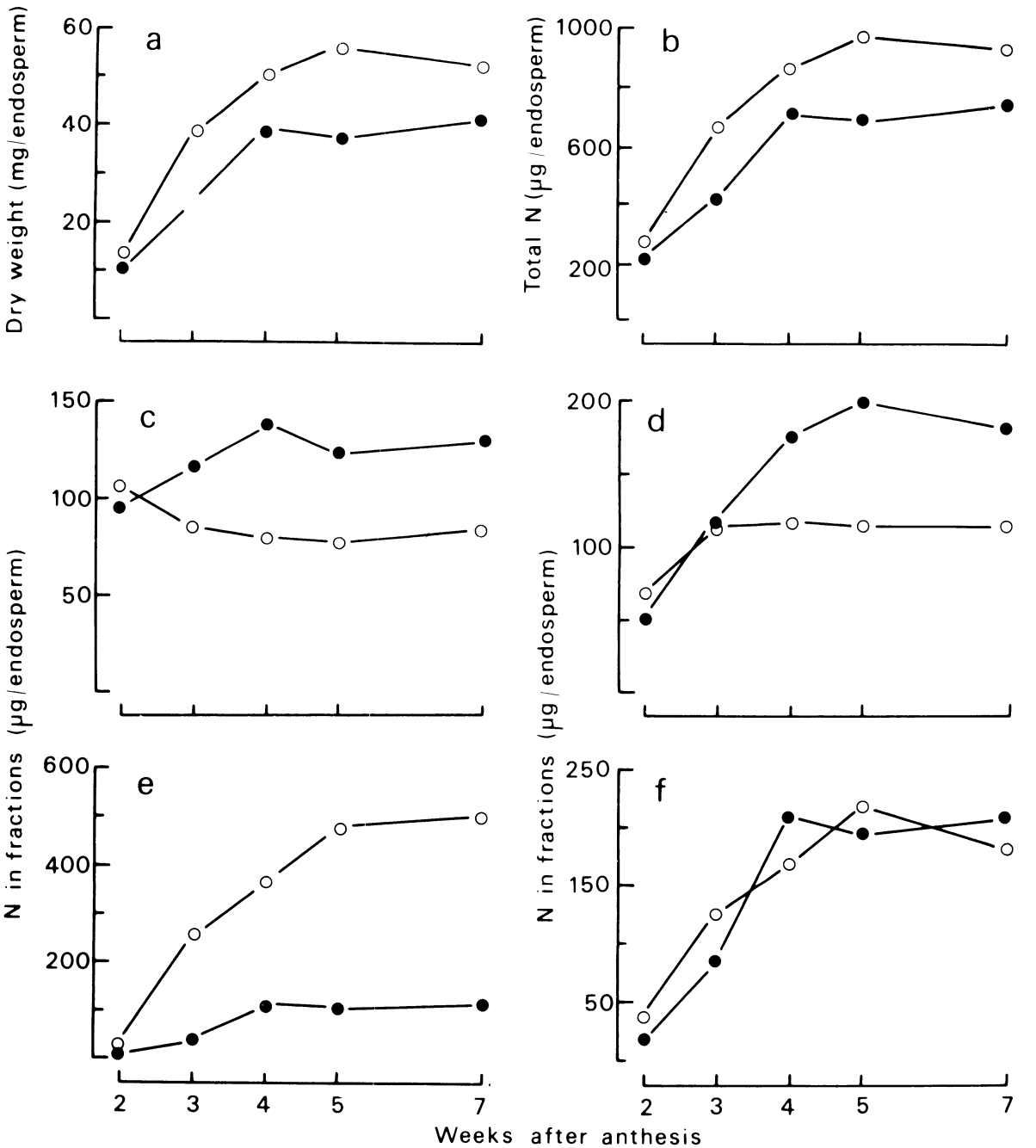

Fig. 1. Changes in dry weight, total N, and N fractions of developing endosperms of Bomi (o) and Risφ 1508 (•). **a**, dry wt; **b**, total N; **c**, TCA-soluble salt-soluble N; **d**, TCA-precipitable salt-soluble N; **e**, hordein N; **f**, glutelin N. Average percentage error from the mean of duplicate extractions and determinations was 3% for **c**, and 4–5% for **d**, **e**, and **f**.

Amino Acid Analysis

Amino acids were determined with a Technicon TSM-1 as described previously (Shewry et al 1978b), but with detection of all amino acids at 410 nm (Rokushika et al 1977). Reproducibility of the determination was within ±5%.

Protein Separation

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 12.5% acrylamide slab gels at pH 8.9 was as described previously (Shewry et al 1978a).

For quantification the gels were fixed overnight in 10% TCA and then washed with distilled water for 4 × 1 hr before scanning at 460 nm in a Beckman Gel Scanner (modified from Righetti et al 1977).

Isoelectric focusing (IEF) in 5% polyacrylamide slab gels was performed as described previously (Shewry et al 1978a).

Lipid Extraction and Fractionation

Total oil was extracted from milled endosperms with petroleum ether (40–60° b.r.) and determined gravimetrically.

Total lipids were extracted with water-saturated butanol (Fishwick and Wright 1977) and separated into neutral and polar fractions (Nichols 1964).

Liquid Scintillation Counting

Nonaqueous samples were counted in toluene-based scintillant containing 0.5% (w/v) 2,5-diphenyloxazole (PPO) and 0.03% (w/v) 1,4-*bis*-2-(5-diphenyloxazoleyl)-benzene. Aqueous samples were counted in scintillant containing 0.3% (w/v) PPO and 25% (v/v) Triton X-100 in xylene (Anderson and McClure 1973). Residues were solubilized with 2*N* NaOH, diluted with water, and aliquots counted with Tritosol (Fricke 1975). Results were corrected for quenching by addition of an internal standard.

TABLE I
Amino Acid Composition of Whole Endosperms and Albumin and Globulin Fractions of Bomi and Risφ 1508

	Whole Endosperm										Albumin + Globulin Fraction					
	Bomi					Risφ 1508					Bomi			Risφ 1508		
	Weeks after Anthesis															
Amino Acid[a]	2	3	4	5	7	2	3	4	5	7	2	4	7	2	4	7
Lysine	4.8	3.0	3.0	3.0	2.9	5.2	5.0	5.1	4.6	4.2	5.4	4.2	4.0	6.3	4.7	4.5
Histidine	1.8	2.1	2.3	2.5	2.2	2.6	2.2	2.8	2.5	2.5	2.1	1.9	2.3	2.4	2.3	2.6
Arginine	4.2	3.3	4.0	3.6	4.5	3.7	4.1	4.4	4.4	4.5	4.9	5.6	7.1	5.4	5.9	6.6
Aspartic acid[b]	8.2	6.7	5.2	5.3	5.1	11.3	10.9	9.7	9.0	6.7	8.8	8.2	8.2	9.4	8.0	8.4
Threonine	4.2	3.6	4.0	3.7	3.9	5.8	4.6	4.4	4.7	3.9	5.0	4.7	4.1	4.9	4.8	4.8
Serine	5.4	6.3	5.2	5.2	5.0	6.7	6.0	5.0	5.2	5.2	6.4	5.8	5.0	5.8	6.0	5.9
Glutamic acid[b]	19.8	22.3	22.5	23.0	21.8	12.6	14.1	15.4	15.6	17.4	13.8	14.0	13.9	10.1	14.8	14.6
Proline	7.7	14.2	14.2	14.0	15.4	9.4	16.8	8.7	9.7	9.6	7.7	8.5	8.4	5.3	8.5	7.6
Glycine	7.4	7.1	6.2	6.3	5.7	7.0	10.2	7.6	9.3	7.5	8.3	9.1	9.6	10.0	10.0	9.7
Alanine	12.0	6.6	6.3	5.4	5.4	11.6	11.3	7.5	7.9	6.6	8.5	8.1	7.9	9.8	8.2	7.6
Cysteine[c]	0.5	1.1	0.6	0.7	1.1	0.6	0.4	0.5	0.7	0.9	2.8	4.6	5.3	1.5	3.2	2.8
Valine	6.3	5.9	6.0	6.7	6.4	6.5	7.4	8.0	7.1	6.6	6.6	6.1	6.5	7.1	5.9	6.8
Methionine	2.1	1.9	2.2	1.4	1.6	2.3	1.8	2.6	2.2	2.6	2.1	1.9	1.7	1.8	1.8	1.8
Isoleucine	3.3	3.7	3.7	4.1	3.9	4.2	3.2	3.8	3.5	4.0	4.0	3.4	3.3	4.6	3.2	3.5
Leucine	6.6	6.9	7.2	8.3	8.0	6.6	6.8	7.4	7.4	9.8	8.0	7.8	7.1	8.8	6.9	6.8
Tyrosine	2.4	2.3	2.9	2.2	2.9	1.7	2.0	3.3	2.5	3.8	2.4	3.1	2.7	2.9	3.0	2.7
Phenylalanine	3.3	3.1	4.4	4.5	4.2	2.2	3.1	3.8	3.6	4.2	3.2	3.0	2.9	3.9	2.8	3.3

[a] Results are expressed as mole %.
[b] Include amides.
[c] Determined as either half-cystine (whole endosperms) or pyridylethylcysteine (albumin + globulin fraction).

TABLE II
Amino Acid Composition of Hordein and Glutelin Fractions from Developing Endosperms of Bomi and Risφ 1508

	Hordein						Glutelin					
	Bomi			Risφ 1508			Bomi			Risφ 1508		
	Weeks after Anthesis											
Amino Acid[a]	2	4	7	2	4	7	2	4	7	2	4	7
Lysine	1.5	0.8	0.8	5.2	1.9	2.1	6.1	7.6	7.3	6.6	6.8	5.4
Histidine	2.1	1.7	2.3	1.3	3.1	2.3	2.6	2.9	3.6	2.0	3.0	3.1
Arginine	2.8	2.6	3.0	4.3	2.6	3.7	5.1	4.9	5.6	6.3	5.4	5.6
Aspartic acid[b]	2.7	1.6	2.2	8.8	3.3	4.0	8.8	7.5	8.2	8.5	7.5	8.7
Threonine	3.4	2.8	3.6	5.3	5.0	4.8	5.6	4.8	4.0	5.6	5.1	5.3
Serine	5.1	4.9	4.2	7.6	6.7	6.1	4.2	4.3	4.0	8.0	4.6	5.4
Glutamic acid[b]	29.7	29.9	29.7	16.2	21.8	23.9	10.8	12.4	12.8	10.6	12.5	13.4
Proline	21.6	21.0	18.4	6.6	13.3	14.2	6.9	6.6	7.7	6.2	7.0	5.9
Glycine	4.2	3.5	3.7	10.3	9.3	6.4	9.0	9.8	9.8	6.8	10.5	10.0
Alanine	2.8	1.8	3.2	8.8	4.6	4.8	9.2	8.7	8.3	9.0	7.9	8.6
Cysteine[c]	2.0	2.4	3.0	0.8	2.7	2.8	1.0	1.0	1.1	1.2	1.2	1.1
Valine	3.7	5.8	4.7	5.9	6.4	5.2	9.6	9.7	7.5	6.7	8.3	7.7
Methionine	1.4	1.2	1.4	1.9	1.7	1.8	0.6	0.9	0.6	1.8	1.0	1.4
Isoleucine	3.2	3.9	3.3	3.5	3.3	3.7	5.3	5.1	5.2	4.7	5.0	3.9
Leucine	6.6	7.8	7.1	7.6	7.0	8.2	9.7	9.1	9.6	9.0	9.2	7.5
Tyrosine	2.5	2.8	3.4	2.3	3.9	2.6	1.3	0.6	Trace	3.0	1.1	3.0
Phenylalanine	4.7	5.4	6.0	3.6	3.4	3.4	4.2	4.1	4.7	4.0	3.9	4.0

[a] Results are expressed as mole %.
[b] Include amides.
[c] Determined as pyridylethylcysteine.

RESULTS

Changes in Nitrogen Fractions

Endosperms were analyzed at two, three, four, five, and seven weeks (grain maturity) after anthesis. The dry weight and total N of the endosperms increased for about five weeks (Fig. 1a, b) and were higher in Bomi than in Risø 1508 throughout development. When expressed on a dry weight basis the N contents of the two varieties were similar, decreasing from approximately 2 mg/g at two weeks to 1.7–1.8 mg/g at three weeks and remaining at this level until maturity.

The amounts of N fractions of the endosperms are shown in Fig. 1c–f. In Bomi nonprotein nitrogen (NPN) (Fig. 1c) decreased between two and three weeks while salt-soluble protein (Fig. 1d) increased between two and three weeks and then remained constant. In Risø 1508, however, the synthesis of both of these fractions was extended: to three weeks for NPN and four weeks for salt-soluble proteins. The final amounts of NPN and salt-soluble protein in Risø 1508 represented 17.6 and 25.0% of the total N compared with 8.9 and 12.1% in Bomi.

In Bomi the hordein fraction was synthesized between two and five weeks, with only a slight increase between five weeks and grain

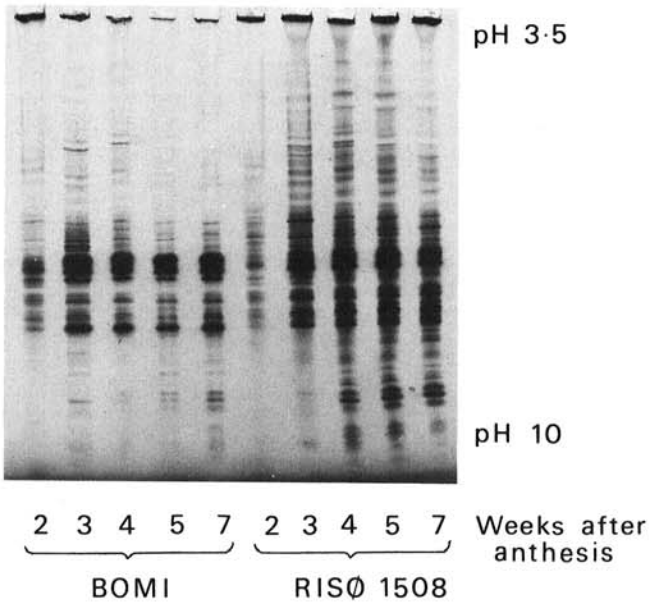

Fig. 2. Isoelectric focusing (pH range 3.5–10) of pyridylethylated albumin + globulin fractions from developing endosperms of Bomi and Risø 1508.

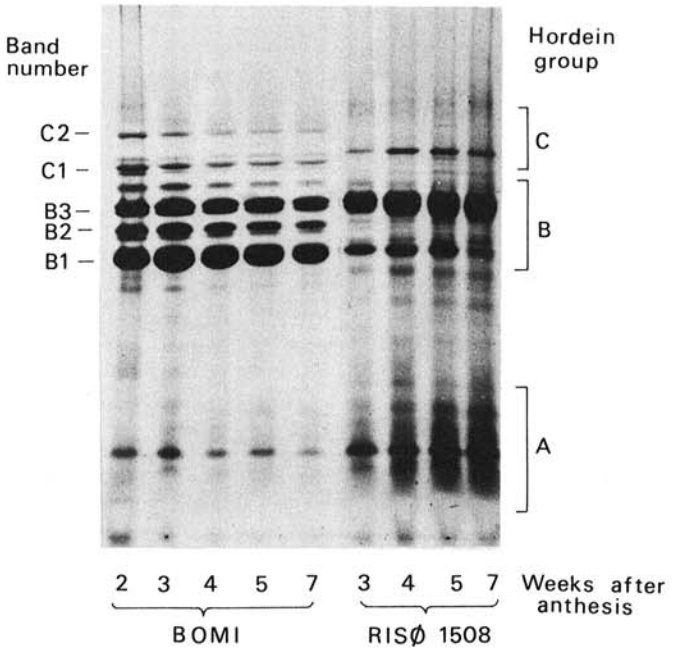

Fig. 3. SDS-PAGE of pyridylethylated hordein from developing endosperms of Bomi and Risø 1508. The hordein band numbers correspond to those used in Fig. 5.

TABLE III
Total Counts per Minute Recovered from $^{14}CO_2$-Fed Developing Endosperms of Bomi and Risø 1508[a]

Variety	Time of Administration (weeks)	Time of Harvest	Total cpm Recovered/100 Endosperms[b] ($\times 10^{-6}$)	Range (±)[c] (%)
Bomi	2	24 hr	39.9	1.0
		7 weeks	60.1	5.2
	3	24 hr	66.1	5.5
		7 weeks	86.9	2.0
	4	24 hr	63.8	0.1
		7 weeks	95.1	1.3
	5	24 hr	4.5	0.1
		7 weeks	6.3	1.0
Risø 1508	2	24 hr	80.0	5.0
		7 weeks	10.6	1.7
	3	24 hr	28.2	1.1
		7 weeks	38.1	<0.1
	4	24 hr	53.1	0.1
		7 weeks	51.0	0.6
	5	24 hr	12.6	1.0
		7 weeks	3.7	0.3

[a] Isotope was administered at two, three, four, and five weeks after anthesis and the endosperms harvested either after 24 hr or at grain maturity (seven weeks).
[b] Mean of duplicate analyses.
[c] Difference between duplicate and mean expressed as percentage of the mean.

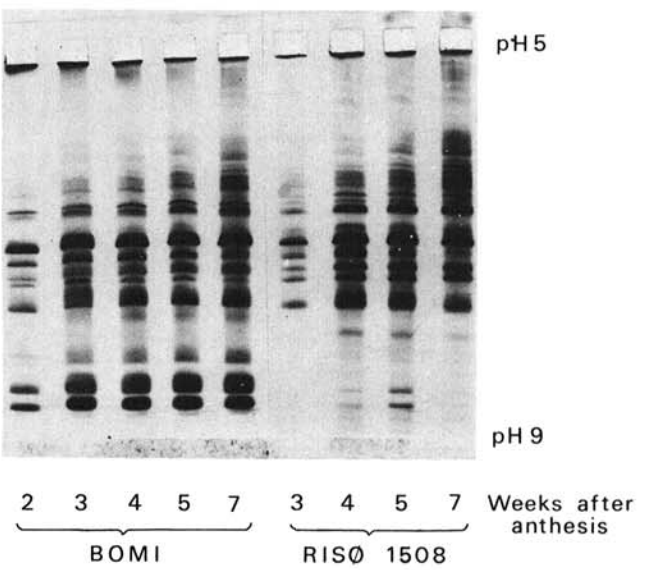

Fig. 4. Isoelectric focusing (pH 5–9) of pyridylethylated hordein from developing endosperms of Bomi and Risø 1508.

maturity (Fig. 1e). This fraction represented half of the total N of the mature endosperm compared with Risø 1508 where synthesis was complete at four weeks and the final amount was only 15.5%.

The amount of glutelin per endosperm was approximately the same in both varieties and synthesis was complete at four weeks. In the mature endosperm of Bomi this fraction accounted for 19.5% of the total N and in Risø 1508 for 28.7%.

The residual fraction accounted for only 1–4% of the total N with the exception of the two week old Risø 1508 endosperms where it was 11%.

Amino Acid Analysis of Protein Fractions

Amino acid analyses of milled whole endosperms and the extracted protein fractions are given in Tables I and II. Free amino acids were not determined, since Brandt (1975, 1976) previously made a detailed study of this fraction.

The whole endosperms of Bomi showed decreases between two and three weeks in the relative amounts of lysine, aspartate, and alanine and an increase in the relative amount of proline. The composition changed little between three weeks and maturity. In Risø 1508 there were decreases between two and seven weeks in aspartate and alanine and a slight decrease in lysine.

The salt-soluble fractions (Table I) from Risø 1508 and Bomi had similar amino acid composition with increases in the relative amount of cysteine but little other change during development. The analyses, which were made on pyridylethylated samples and are thus much more reliable (Friedman et al 1970), also showed relatively more cysteine in Bomi.

The hordein fractions (Table II) of Bomi had high glutamate (Ω 30%) and proline (Ω 20%) and, except for a decrease in lysine between two and four weeks (1.5 to 0.8%), there was little change during development. The hordein fractions from four and seven week old Risø 1508 were similar in composition with slightly higher lysine (2%) and lower glutamate (22–24%) and proline (13–14%) than those in Bomi. At two weeks, however, the composition was considerably different with over 5% lysine, less glutamate and proline, and more alanine, glycine, and aspartate.

The glutelin fractions (Table II) of both varieties had relatively high lysine (5–8%) and low glutamate (10–13%) and proline (6–8%) with little change during development.

Polyacrylamide Gel Electrophoresis

The reduced and pyridylethylated salt-soluble protein fractions were separated by IEF in the pH range 3.5–10 (Fig. 2). Both varieties had complex polypeptide patterns and differed in relative band intensity, with notably more alkaline polypeptides in Risø 1508. The relative amounts of alkaline polypeptides also increased in both varieties during grain development.

The reduced and alkylated hordein fractions were separated by SDS-PAGE at pH 8.9 (Fig. 3) and by IEF in the pH range 5–9 (Fig. 4). The SDS-PAGE separations were quantified by scanning gels fixed with TCA. This method, unlike the scanning of gels stained with Coomassie blue or other protein stains, gives results that are not affected by the amino acid composition of the polypeptides. The hordein polypeptides were classified into A, B, and C groups as previously described (Køie et al 1976).

SDS-PAGE of the Bomi hordein fractions (Fig. 3) indicated little change from three weeks after anthesis to grain maturity, although there appeared to be some quantitative differences at two weeks. This was confirmed by gel-scanning (Fig. 5a), which demonstrated a decrease in the relative amount of high molecular weight C polypeptides between two and three weeks and a corresponding increase in B bands. From three weeks to grain maturity, there were only minor changes in the relative amounts of the major B polypeptides. IEF (Fig. 4) also showed changes in polypeptide composition between two and three weeks.

Separation of the hordein fraction from 2 week old Risø 1508 by either SDS-PAGE or IEF showed no protein bands and this fraction is, therefore, omitted from Figs. 3 and 4. SDS-PAGE of the hordein from mature endosperms of Risø 1508 showed a polypeptide composition very different from that of Bomi. This has been described previously (Shewry et al 1977, 1978b) and will not be further discussed here. The relative polypeptide composition in Risø 1508 also changed during grain development, especially between five and seven weeks when gel scanning (Fig. 5b) demonstrated an increase in the relative amount of low molecular weight A hordein (from 11 to 21%) and decreases in the B polypeptides. IEF of these fractions (Fig. 4) showed an increase in acidic polypeptides, which correspond to A hordeins (Shewry et al 1978a) between five and seven weeks. These separations also confirmed that Risø 1508 had only trace amounts of alkaline polypeptides (Miflin and Shewry 1977).

SDS-PAGE of the glutelin fractions showed a complex banding pattern similar to that described previously (Shewry et al 1978b); no major changes during development were observed.

Metabolism of $^{14}CO_2$

Duplicate samples of the $^{14}CO_2$ fed endosperms were ground and the protein fractions extracted and assayed for total N and radioactivity. The recovery of N in the fractions was similar to that

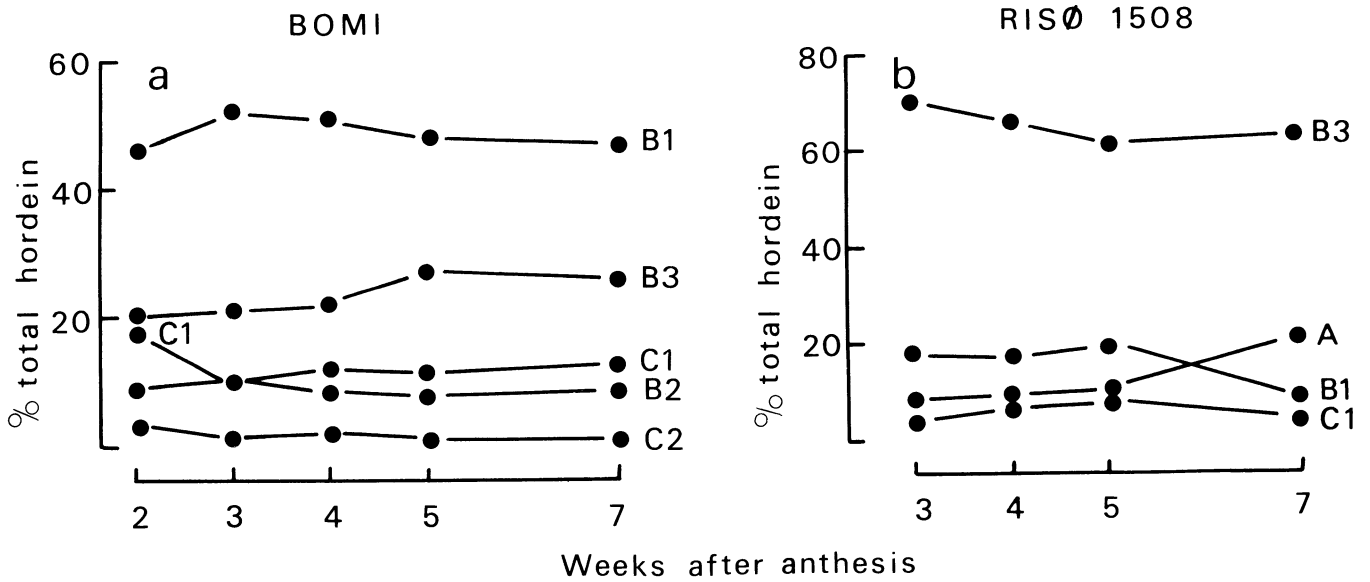

Fig. 5. Relative polypeptide composition of hordein fractions from developing endosperms of Bomi and Risø 1508. The hordein band numbers correspond to those used in Fig. 3. The other bands each corresponded to less than 1% of the total fractions throughout the development. Results are the mean of four gel scans of each sample.

shown in Fig. 1 and is therefore not presented. The total radioactivity recovered is given in Table III. In all cases, the values for the duplicate samples differed less than 6% from the mean. Figure 6 shows the radioactivity recovered in the fractions expressed as a percentage of the total radioactivity recovered. Major differences between the two varieties were apparent both 24 hr after isotope administration and in mature grain.

Radioactivity in the lipid fractions (Fig. 6a) of both varieties was greater in mature grain than at 24 hr after the isotope was administered. There was much greater incorporation into this fraction in Risφ 1508 than in Bomi, especially in the endosperms fed at three, four, and five weeks and harvested at maturity.

Radioactivity in the TCA-soluble part of the salt-soluble fraction (includes free amino acids, organic acids, and sugars) (Fig. 6b) was greater in Risφ 1508 than in Bomi. It increased during development to 75 and 66% of the total from five week old endosperms of Risφ 1508 and Bomi, respectively, harvested 24 hr after administration. Radioactivity recovered in this fraction decreased by half or more between 24 hr after administration and grain maturity.

The radioactivity recovered in the TCA-precipitable part of the

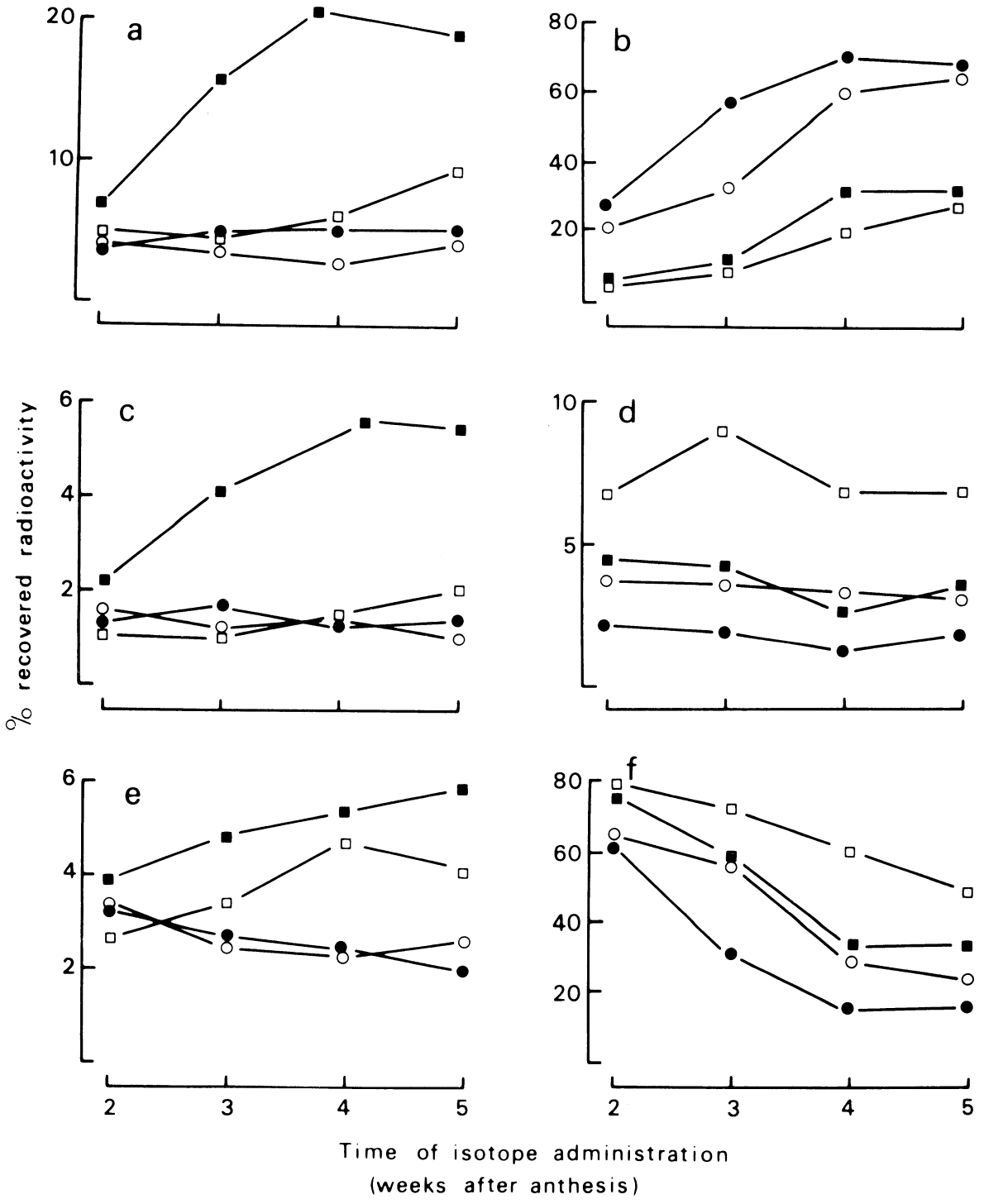

Fig. 6. Recovery of radioactivity in the fractions of developing endosperms of Bomi (open symbols) and Risφ 1508 (closed symbols). Isotope was administered two, three, four, and five weeks after anthesis and the endosperms harvested after 24 hr (circles) or at grain maturity (squares). **a**, lipids; **b**, TCA-soluble salt-soluble fraction; **c**, TCA-precipitable salt-soluble fraction (albumin + globulin); **d**, hordein; **e**, glutelin fraction; **f**, residue. The average percentage error from the mean of duplicate extractions and determinations was 1–2% for **a** and **f** and 4–5% for **b**, **c**, **d**, and **e**.

salt-soluble fraction (salt-soluble proteins) (Fig. 6c) did not change during development of Bomi, being 2% or less of the total at both 24 hr after administration and in mature grain. In Risø 1508 the radioactivity recovered 24 hr after administration was similar to that in Bomi. The radioactivity in the mature grain, however, increased during development to over 5% in grain fed at four and five weeks.

Incorporation into hordein (Fig. 6d) was greater in Bomi at all stages of development, the maximum (9.1%) being in grain fed at three weeks and analyzed at maturity. In both varieties, incorporation was two times greater at maturity than at 24 hr after administration.

The glutelin fraction extracted using borate buffer + SDS + 2-mercaptoethanol contained some carbohydrate, so incorporation of radioactivity into this fraction (Fig. 6e) was probably not solely due to glutelin synthesis. In seeds fed at three, four, and five weeks, however, incorporation was greater in Risø 1508 than in Bomi and increased between 24 hr and grain maturity.

Incorporation into the residue (Fig. 6f), which contains most of the starch, was greater in Bomi than in Risø 1508. It was also greater in mature grain than in seeds harvested 24 hr after isotope administration. In both varieties, however, it decreased during grain development.

Investigations on the Lipid Fraction

Determination of the total oil content of mature endosperms (by extraction with petroleum ether) showed that the mutant had twice as much (3.6%) as the normal variety (1.8%).

When total lipids extracted from mature ^{14}C-labeled endosperms ($^{14}CO_2$ administered at three weeks) with water-saturated butanol were fractionated into polar and neutral fractions, the radioactivity recovered in both fractions was greater in Risø 1508 than in Bomi: 12.3% compared with 1.4% in neutral lipids and 1.4% compared with 0.5% in polar lipids. The phosphate content of the polar lipid fraction was also about three times greater in Risø 1508, indicating increased total phospholipid.

DISCUSSION

The pattern of development of the different protein fractions in the normal variety Bomi is similar to that reported by previous workers (Bishop 1930, Brandt 1976). There is no evidence for any great change in either the amino acid or polypeptide composition of hordein or the amino acid composition of glutelin. Our results on hordein agree with those of Brandt (1976) but differ in that we found little change in the glutelins. We ascribe this difference to the use of our improved extraction procedures. In contrast, the albumin and globulin fractions show changes in both the polypeptide and amino acid composition (notably an increase in cysteine) during development. Ivanko (1971) reported a similar increase in the cysteine content of his albumin fraction, although the reason for this remains uncertain.

In Bomi the relative amount of radioactivity incorporated in the albumin and globulin fraction is similar at all stages of development, although there is no net increase in the total N content of the fraction after two weeks. This suggests that some turnover takes place. In Risø 1508, however, incorporation is the same as in Bomi 24 hr after administration but is considerably greater in mature grain (seven weeks). This is consistent with the continued increase in this fraction during development and suggests that turnover may be arrested by the mutation.

In comparing Bomi and Risø 1508, the results with mature seeds agree with previous studies (Shewry et al 1977, 1978b). The mutation is operating in the same way throughout development and there is no evidence for an arrest of the normal pattern at any given stage. The most marked difference between the two lines is in the incorporation of $^{14}CO_2$ into lipids, the increase being ninefold in the neutral lipid fraction of the mutant. There have been previous reports (Munck 1976, Tallberg 1977) of increased total lipid in seed of Risø 1508, and the authors have ascribed this to the larger embryo. Our results, however, show that the major reason for this is increased endosperm lipid.

These results emphasize that the Risø 1508 mutation not only affects total prolamin synthesis but also involves major changes in endosperm metabolism affecting proteins, lipids, and carbohydrates. Since the Risø 1508 character is reported to be controlled by a single recessive gene (Doll 1973), these results indicate that the mutation is pleiotropic and suggest that the possibility of increasing the seed size and thus the yield of the mutant may be limited.

ACKNOWLEDGMENTS

The authors thank H. Doll and his colleagues at Risø (B. Køie, G. Nielsen, A. Brandt) for seed of Risø 1508 and Bomi and for useful discussion. We also thank J. M. Hill for advice and assistance with amino acid analysis. This work was supported by EEC Grant 473.

LITERATURE CITED

ANDERSON, L. E., and McCLURE, W. O. 1973. An improved scintillation cocktail of high-solubilising power. Anal. Biochem. 51:173.

BISHOP, L. R. 1930. The composition and determination of the barley proteins. III. Fourth report on barley proteins. The proteins of barley during development and storage and in the mature grain. J. Inst. Brew. 36:336.

BRANDT, A. 1975. In vivo incorporation of ^{14}C-lysine into the endosperm proteins of wild type and high-lysine barley. FEBS Lett. 52:288.

BRANDT, A. 1976. Endosperm protein formation during kernel development of wild type and a high-lysine barley mutant. Cereal Chem. 53:890.

BRIEDERT, D., and SCHON, W. J. 1974. Biochemische Probleme bei der Qualitatservbesserung der Getreideproteine. Gottinger Pflanzenzuchter-Seminar 1:89.

DOLL, H. 1973. Inheritance of the high-lysine character of a barley mutant. Hereditas 74:293.

FISHWICK, M. J., and WRIGHT, A. J. 1977. Comparison of methods for the extraction of plant lipids. Phytochemistry 16:1507.

FRICKE, U. 1975. Tritosol: A new scintillation cocktail based on Triton X-100. Anal. Biochem. 63:555.

FRIEDMAN, M., KRULL, L. H., and CAVINS, J. F. 1970. The chromatographic determination of cystine and cysteine residues as S-β-(4-pyridylethyl) cysteine. J. Biol. Chem. 245:3868.

INGVERSEN, J., KØIE, B., and DOLL, H. 1973. Induced seed protein mutant of barley. Experimentia 29:1151.

IVANKO, S. 1971. Changeability of protein fractions and their amino acid composition during the maturation of barley grain. Biol. Plant. 13:155.

KIRKMAN, M. A. 1974. Comparative determination of protein amino acids in plant materials by automated cation-exchange and gas-liquid chromatography of the amino acid N-heptafluorobutyryl, n-propyl-esters. J. Chromatogr. 97:175.

KØIE, B., INGVERSEN, J., ANDERSEN, A. J., DOLL, H., and EGGUM, B. O. 1976. Composition and nutritional quality of barley proteins, p. 55. In: Evaluation of Seed Protein Alterations by Mutation Breeding. IAEA: Vienna.

KØIE, B., and NIELSEN, G. 1977. Extraction and separation of hordeins, p. 25. In: MIFLIN, B. J., and SHEWRY, P. R. (eds.). Techniques for the Separation of Barley and Maize Seed Proteins. CEC Eur 5687e, Luxenbourg.

MIFLIN, B. J., and SHEWRY, P. R. (eds.). 1977. Techniques for the Separation of Barley and Maize Seed Proteins. CEC Eur 5687e, Luxembourg.

MUNCK, L. 1972. Improvement of nutritional value in cereals. Hereditas 72:1.

MUNCK, L. 1976. Developing the concept of quality in barley breeding programs for malt and feed, p. 526. In: GAUL, H. (ed.). Barley Genetics III. Garching.

NICHOLS, B. W. 1964. Separation of plant phospholipids and glycolipids, p. 321. In: JAMES, A. T., and MORRIS, L. J. (eds.). New Biochemical Separations. Van Nostrand: London.

POMERANZ, Y. 1973. A review of proteins in barley, oats, and buckwheat. Cereal Sci. Today 18:310.

POMERANZ, Y., and ROBBINS, G. S. 1972. Amino acid composition of maturing barley. Cereal Chem. 49:560.

POMERANZ, Y., ROBBINS, G. S., and GILBERTSON, J. T. 1976. Protein content and amino acid composition of tissue components in developing barley. J. Food Sci. 41:283.

POMERANZ, Y., WESENBERG, D. M., ROBBINS, G. S., and GILBERTSON, J. T. 1974. Changes in amino acid composition of maturing Hiproly barley. Cereal Chem. 51:635.

RIGHETTI, P. G., GIANAZZA, E., VIOTTI, A., and SOAVE, C. 1977. Heterogeneity of storage proteins in maize. Planta 136:115.

ROKUSHIKA, S., MURAKAMI, F., and HATANO, H. 1977. Single-wavelength detection for amino acid analysis. J. Chromatogr. 130:324.
SHEWRY, P. R., ELLIS, J. R. S., PRATT, H. M., and MIFLIN, B. J. 1978a. A comparison of methods for the extraction and separation of hordein fractions from 29 varieties. J. Sci. Food Agric. 29:433.
SHEWRY, P. R., HILL, J. M., PRATT, H. M., LEGGATT, M. M., and MIFLIN, B. J. 1978b. An evaluation of techniques for the extraction of hordein and glutelin from barley seed and a comparison of the protein composition of Bomi and Risø 1508. J. Exp. Bot. 29:677.
SHEWRY, P. R., PRATT, H. M., CHARLTON, M. J., and MIFLIN, B. J. 1977. Two-dimensional separation of the prolamins of normal and high lysine barley (*Hordeum vulgare* L.). J. Exp. Bot. 28:597.
SMITH, D. B. 1972. The amino acid composition of barley grain protein during development and germination. J. Agric. Sci. 78:265.
TALLBERG, A. 1977. The amino-acid composition in endosperm and embryo of a barley variety and its high lysine mutant. Hereditas 87:43.

[Received April 27, 1978. Accepted July 21, 1978]