in edible oil to light brown color results into loss of up to 80.00% of phytic acid. Frying to brown color (well-done Puri), the loss of phytic acid is 85%, which shows that for preparing well-done Puri further loss of phytic acid is not significant.

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Registry No. Phytic acid, 83-86-3.

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Amino Acid Composition of Malts: Effect of Germination and Gibberellic Acid on Hulled and Hulless Barley and Utility Wheat

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Malts of hulled barley Harrington, hulless barley Scout, and utility wheat Glenlea showed a decrease in the glutamic acid as compared to aspartic acid and lysine values, which increased with extended germination from 2 to 5 and 8 days. Inverse and statistically significant coefficients of correlation between glutamic and aspartic acids ($r = -0.72$, $P < 0.01$) and between glutamic acid and lysine values ($r = -0.78$, $P < 0.01$) were obtained. The values for proportion of total essential to total amino acids of ungerminated Harrington and Glenlea cultivars increased by 3–7% due to germination, with a negligible increase shown by the malts of hulless barley Scout. The chemical score for threonine was lower than that of lysine and isoleucine, except for the 2-day malts. A consistent increase in the score for lysine with germination was observed in all malts, except for the 2-day Scout malts.

INTRODUCTION

Amino acid composition of various cereal grains as affected by germination has been the subject of several investigations (Dalby and Tsai, 1976; Wu and Wall, 1980; Wu, 1982, 1983), emphasizing changes in essential amino acids, especially lysine. Robbins and Pomeranz (1971) studied changes in the amino acid composition of barley malts. The effect of gibberellic acid (GA) and nutritional status of malt proteins has not been studied so far. Information regarding the amino acid compositon of hulless barley cv. Scout, and utility wheat cv. Glenlea (*Triticum aestivum* L.) is not available. The hulless barley and the utility wheat cultivars are low in price and high in protein content. These are mainly used as feed grain (Crop Research '82, 1982). Their potential for malting for food purposes has been reported by Singh and Sosulski (1985). The purpose of this investigation was to study the changes in the amino acids of these cultivars as influenced by germination and GA treatment. Changes in the proportion of total essential (E) to total amino acids (T) in protein (E/T %) and chemical score (WHO, 1973) profile of essential amino acids are reported.

MATERIALS AND METHODS

Grain samples of two-row hulled barley Harrington, hulless Scout and utility wheat Glenlea cv. were obtained from the Research Farm of the University of Saskatchewan. The samples were cleaned and stored in air-tight containers for use as desired.

Malting. A weighed sample (60 g) of each variety was steeped to 44% moisture at 15 °C changing water every

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Table I. Amino Acid Composition of Barley and Wheat Cultivars

amino acid, g/16 g N	hulled, Harrington	hulless, Scout	wheat, Glenlea
Try	1.1	1.0	1.0
Lys	3.3	3.1	2.5
His	2.4	2.2	2.2
NH_3	2.1	1.9	2.2
Arg	4.7	4.6	4.4
Asp	5.7	5.5	6.1
Thr	2.1	2.1	1.7
Ser	4.3	4.3	4.8
Glu	26.4	27.0	32.0
Pro	11.7	12.4	10.2
Gly	3.8	3.6	4.1
Ala	3.9	3.7	3.4
$^1/_2$Cys	2.2	2.2	2.2
Val	4.8	4.9	4.0
Met	2.0	2.1	1.7
Ile	3.2	3.4	3.2
Leu	7.1	7.0	6.5
Tyr	3.2	3.2	2.9
Phe	5.5	5.5	4.6
total[a]	99.5	99.9	99.7
Kjeldahl N,[b] %	2.4	2.7	2.4

[a] As is. [b] Dry basis.

24 h, followed by an air rest of 1 h. Gibberellic acid was applied by steeping in 1 ppm solution for the terminal 4 h with concurrent controls. Germination was conducted for 2,5, and 8 days in the perforated plastic bottles at 15 °C and a relative humidity of 95%. The green malts were dried in an air oven at 50 °C for 20 h followed by kilning at 85 °C for 4 h. The roots were removed by rubbing with hand and sifting. The samples were kept in air-tight containers.

Amino Acid Analyses. Germinated as well as ungerminated samples of each cultivar were powdered in the UDY cyclone mill (Boulder, CO). For hydrolysis, 20 mg of the sample in duplicate was taken in ampules to which 3.5 mL of 6 N HCl was added and the contents mixed, evacuated, and sealed. Hydrolysis was carried out at 110 °C in an air oven for 22 h. After removal from the oven and cooling to 20 °C, the ampules were broken open, evaporated to dryness under reduced pressure at 55 °C, and dissolved in 10 mL of pH 2.2 citrate buffer. The insoluble residue from the hydrolyzed solutes was removed by filtration through glass wool. For determination of tryptophan, hydrolysis of the samples was carried out with barium hydroxide as described by Sosulski and Sarwar (1973).

Amino acid analyses were performed on a Beckman 119 BL automatic amino acid analyzer (Palo Alto, CA). Aliquots (100 μL) of the hydrolysates in duplicate were loaded automatically on the columns of the instrument for separation of acidic, neutral, and basic amino acids, respectively. In the case of tryptophan, a short column was used as described by Tkachuk and Irvine (1969).

An electronic integrator (Spectra Physics, Bedford, MA) was used to process the data, and the results are expressed in grams of amino acid/16 g of N. Recoveries were made concurrent with the analysis of the samples. Kjeldahl nitrogen was determined by the procedures of the Association of Official Analytical Chemists (1980) and reported on a moisture-free basis.

The amino acid composition data were used to compute the nutritional value of the protein based on the provisional amino acid scoring pattern (WHO, 1973). The nutritional parameters included proportion of total essential (E) to total amino acids (T) in the protein (E/T %) and chemical score data. The relationship between glutamic and aspartic acids and lysine values for the grains and the malts was determined by calculating the coefficients of correlation (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Kjeldahl nitrogen of Harrington barley and Glenlea wheat was about the same, but lower than that of hulless Scout barley (Table I). The total nitrogen content of the malts with and without GA was expected to decrease as a result of the formation of the new tissues, particularly rootlets. Expressed on the kernel weight basis, negligible changes in the nitrogen contents were caused by germination with and without GA treatment (Tables II and III). This was due to the simultaneous decrease in kernel weight caused by respiration and removal of rootlets (Singh and Bains, 1977). Hwang and Bushuk (1973) reported a minor decrease in protein content of flour from wheat steeped and then germinated up to 8 days. Dalby and Tsai (1976) observed a steady increase in the total protein (db) of

Table II. Effect of Germination on the Amino Acid Composition of Malts

	days germinated								
	hulled, Harrington			hulless, Scout			wheat, Glenlea		
amino acid, g/16 g N	2	5	8	2	5	8	2	5	8
Try	1.4	1.4	1.4	1.2	1.1	1.2	1.6	1.6	1.7
Lys	3.6	3.6	3.7	2.8	3.0	2.9	2.9	3.5	3.6
His	2.4	2.4	2.3	2.0	2.1	2.0	2.5	2.6	2.6
NH_3	2.1	2.0	1.9	1.1	1.3	1.2	2.1	1.8	1.8
Arg	4.7	4.8	4.9	4.3	4.3	4.5	5.0	5.3	5.3
Asp	5.9	8.1	8.0	4.9	5.3	5.7	5.1	8.2	8.0
Thr	3.3	2.4	2.5	1.7	1.6	1.6	3.0	2.4	2.4
Ser	4.2	3.9	3.8	4.1	4.1	4.1	4.5	4.3	4.3
Glu	23.4	19.6	16.1	26.0	24.5	24.0	28.1	24.1	23.3
Pro	11.1	10.5	11.9	10.9	11.5	11.4	10.0	9.6	10.2
Gly	3.8	3.7	3.7	3.0	3.0	3.0	4.0	4.1	4.1
Ala	4.5	4.3	4.3	3.8	3.9	4.1	4.0	4.4	4.4
$^1/_3$Cys	2.3	2.2	2.2	1.8	1.7	1.6	2.2	2.2	2.2
Val	5.3	5.4	5.3	4.7	4.7	4.8	4.3	5.0	4.9
Met	1.4	1.4	1.4	1.5	1.5	1.3	1.7	1.8	1.8
Ile	3.6	3.5	3.5	3.4	3.4	3.5	3.6	3.6	3.6
Leu	7.4	7.0	6.8	6.7	6.7	6.7	7.0	6.9	6.9
Tyr	3.6	3.5	3.5	2.9	3.0	3.0	3.0	3.5	3.6
Phe	5.9	5.7	5.5	5.1	5.3	5.2	4.7	5.0	5.2
total[a]	99.9	95.4	92.7	91.9	92.0	91.8	99.3	99.9	99.9
Kjeldahl N,[b] %	2.3	2.3	2.4	2.7	2.7	2.7	2.4	2.4	2.4

[a] As is. [b] Dry basis.

Table III. Effect of Germination and Gibberellic Acid (GA) on the Amino Acid Composition of Malts

	days germinated								
	hulled, Harrington			hulless, Scout			wheat, Glenlea		
amino acid, g/16 g N	2	5	8	2	5	8	2	5	8
Try	1.3	1.2	1.4	1.1	1.2	1.2	1.5	1.5	1.6
Lys	3.3	3.4	4.0	2.6	3.0	3.2	3.0	3.3	3.6
His	2.2	2.2	2.2	2.0	2.1	2.1	2.6	2.1	2.6
NH_3	2.2	2.0	1.9	1.0	1.0	1.0	2.1	2.1	2.5
Arg	4.6	4.5	4.5	4.2	4.2	4.2	5.0	5./	5.1
Asp	6.0	8.0	8.9	5.9	6.7	6.8	5.3	8.0	9.3
Thr	3.2	2.3	2.4	1.4	1.4	1.4	3.0	2.4	2.4
Ser	4.1	3.8	3.4	4.1	4.0	3.9	4.8	4.5	4.1
Glu	19.9	16.5	13.8	25.3	23.0	20.9	25.1	21.4	19.0
Pro	10.7	10.5	10.0	11.0	10.7	10.6	10.6	10.5	9.8
Gly	3.7	3.5	3.5	3.0	3.1	3.1	4.3	4.2	4.1
Ala	4.3	4.2	4.4	3.8	4.1	4.2	4.0	4.3	4.2
$^1/_2$Cys	2.4	2.3	2.1	2.1	2.0	1.9	2.4	2.3	2.4
Val	5.1	5.0	5.0	4.7	4.8	4.8	4.7	5.0	4.9
Met	1.7	1.6	1.6	1.6	1.5	1.6	1.8	1.8	1.8
Ile	3.7	3.6	3.5	3.4	3.5	3.5	3.8	3.8	3.6
Leu	7.1	6.6	6.2	6.5	6.4	6.2	7.0	6.9	6.4
Tyr	3.4	3.4	3.4	3.4	3.5	3.3	3.6	3.9	3.5
Phe	5.6	5.3	5.0	5.1	5.1	5.1	5.3	5.4	5.2
total[a]	94.5	89.9	87.6	92.2	91.4	89.3	99.9	99.0	95.8
Kjeldahl N,[b] %	2.4	2.4	2.4	2.7	2.7	2.7	2.4	2.4	2.4

[a] As is. [b] Dry basis.

wheat, barley, triticale, rye, and rice with progressive germination.

Amino Acid Composition. The amino acid composition of Harrington and Scout barley and Glenlea wheat differed largely in respect of aspartic acid, glutamic acid, and proline content (Table I). The amount of proline was maximum in Scout proteins. Differences in the values for the rest of the amino acids were marginal in the case of Harrington and Scout barleys. Glenlea wheat had the highest glutamic and aspartic acids contents, despite similar nitrogen content to that of Harrington. In addition, Glenlea proteins were higher in serine and glycine and lower in lysine, valine, leucine, and phenylalanine than those of the barley.

Changes in the amino acid composition of barley and wheat malts germinated for 2–8 days with and without GA are summarized in Tables II and III. The analyses accounted for 87.6–99.9% of the total nitrogen in the malts. However, from the Scout malts with and without GA treatment, lower recoveries than those of Harrington and Glenlea malts were obtained. Vose and Youngs (1978) attributed the lower recoveries in barley malts to metabolites, presumably comprising nonprotein nitrogen. Calculated on the basis of amino acid values of protein, a decrease in glutamic acid and proline contents with concomitant increase in aspartic acid of the 2-day Harrington malt was observed (Table II). Changes in the profile of these and the rest of the amino acids of Harrington malts corroborated the findings of Robbins and Pomeranz (1971) for the 2-day-germinated Piroline and Dickson barley malts. Extending germination of Harrington barley to 5 and 8 days caused a decrease in glutamic acid values associated with increase in aspartic acid contents. The proline contents of the 2- and 5-day Harrington malts, however, decreased as compared to the ungerminated control. The values increased for the 8-day malts. Glenlea wheat malt proteins showed a similar pattern of changes in glutamic acid, aspartic acid, and proline contents. Proteins of the 2-day Scout malts exhibited relatively smaller decreases in glutamic acid and larger in proline contents as compared to Harrington and Glenlea malts, and corresponding ungerminated controls. Extended germination of Scout barley to 5 and 8 days decreased the glutamic acid accompanied by increased aspartic acid values. The values for glutamic acid were higher and for aspartic acid lower than those of the corresponding Harrington and Glenlea malts. Probably, less degradation of the storage proteins markedly high in glutamic acid (Pomeranz et al., 1972) accounted for the relatively less availability of free α-glutamic acid for transformation into aspartic acid. This view was strengthened by the inverse and highly significant value for correlation coefficient ($r = -0.72$, $P < 0.01$) between these amino acids. Wu (1982) observed a decrease in the prolamine and glutelin fractions of triticale protein on germination with an increase in the aspartic acid and a decrease in the glutamic acid and proline contents. GA treatment increased the aspartic acid and decreased glutamic acid contents of malts. Changes in the values for histidine, arginine, serine, glycine, alanine, and half-cystine due to germination from 2 to 8 days with and without GA were negligible.

Essential Amino Acid Composition. On germination, the values for lysine increased in the control and GA-treated malts of Harrington barley and Glenlea wheat (Tables II and III). However, some decrease in lysine was observed in the malts of hulless barley Scout. An increase in lysine content of cereals on germination has been reported by Dalby and Tsai (1976) and by Robbins and Pomeranz (1971). The trend in the lysine and glutamic acid values was similar for the Harrington and Glenlea malts, whereas decreased lysine values and higher glutamic acid values were obtained for the hulless Scout malts. An inverse and significant correlation ($r = -0.78$, $P < 0.01$) was obtained between lysine and glutamic acid values. Interestingly, the values for the rest of the essential amino acids in the 2-day Scout malts decreased as compared to the ungerminated controls (Tables I–III). Changes in the essential amino acid values for the 5- and 8-day malts of this variety were negligible. The phenylalanine + tyrosine values of Harrington malts decreased as compared to those of Scout and Glenlea malts. Tryptophan contents were generally higher in the 2-day malts with and without GA as compared to those of the ungerminated grain (Tables I–III). The values leveled off as germination was extended to 5 and 8 days. The amount of threonine and leucine decreased with extended germination from 2 to 8 days.

Table IV. Effect of Germination and Gibberellic Acid (GA) on the Chemical Score[a] of Malts

essential amino acid, g/16 gN	chemical score, days germinated											
	hulled, Harrington				hulless, Scout				wheat, Glenlea			
	0	2	5	8	0	2	5	8	0	2	5	8
control												
Ile	(72)	(74)	(75)	(76)	(76)	(83)	(82)	(82)	(85)	(82)	(79)	(78)
Leu	120	114	113	110	118	122	122	122	125	120	113	112
Lys	(76)	(76)	(80)	(83)	(72)	(70)	(75)	(73)	(66)	(68)	(79)	(80)
Met + Cys/2	114	91	93	94	116	97	93	85	120	107	105	104
Phe + Tyr	129	129	131	129	129	129	133	132	127	116	123	126
Thr	(61)	(88)	(67)	(70)	(61)	(54)	(50)	(51)	(57)	(89)	(68)	(67)
Val	98	99	106	105	101	105	104	106	94	90	100	97
Try	103	119	125	126	93	122	111	122	106	152	154	153
GA												
Ile		(78)	(80)	(78)		(83)	(84)	(84)		(82)	(81)	(79)
Leu		112	110	104		118	115	112		113	110	105
Lys		(72)	(78)	92		(65)	(74)	(79)		(66)	(73)	(81)
Met + Cys/2		104	105	100		108	101	101		109	105	111
Phe + Tyr		125	128	124		137	136	134		126	131	126
Thr		(88)	(67)	(70)		(44)	(44)	(60)		(84)	(67)	(68)
Val		98	102	102		104	105	106		92	98	98
Try		114	112	131		111	119	120		134	133	146

[a] (mg AA/g EAA × 100)/(mg AA in whole egg/g EAA in whole egg). AA = amino acid, E = essential. Values in parentheses show the limiting amino acids.

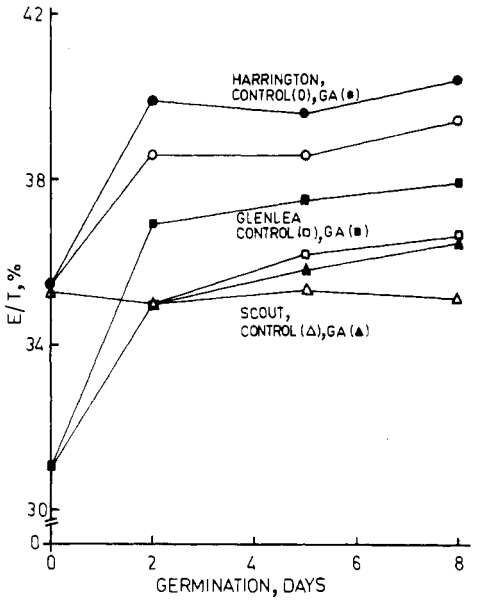

Figure 1. Effect of germination and gibberellic acid (GA) on the total essential to total amino acid proportion (E/T %) of the malts.

The effect of germination and GA on the values for isoleucine in the various malts was negligible.

Amino Acid Composition and Browning. Kilning is an essential step for producing flavoring and color components in malt (Kaminski et al., 1981) involving Maillard reactions. According to Finot et al. (1982) and Hurrell et al. (1983), destruction of lysine and various other essential amino acids occurred as a result of these reactions involving dicarbonyls and aldehydes and the amino group of lysine. The results in Tables II and III, however, indicated that lysine was negligibly involved in the Maillard reactions during the kilning of these malts. The development of flavoring and color compounds seemed to be the artifact of carmelization of the reducing sugars formed during germination.

E/T Proportion and Chemical Score. The nutritional quality of a protein principally depends on its amino acid composition. As compared to the value (51.3%) for the whole egg reference protein, values of E/T % for barley and utility wheat were lower by 16% and 20%, respectively (Figure 1). The 2-day Harrington and Glenlea malts showed a spectacular increase in the E/T % values as compared to those of ungerminated controls. The values further increased by the GA treatment. However, the E/T % values for Scout barley were negligibly affected by malting as compared to those of Harrington and Glenlea malts. Extending germination to 5 and 8 days caused marginal changes in the E/T % values. The Harrington malts had higher E/T % values than those of hulless Scout barley.

The chemical score values in parentheses (Table IV) indicated that the lower the values of the score, the more limiting the essential amino acid. Threonine seemed to be the first limiting amino acid, the next being lysine and isoleucine in the malts. Unlike the malts of hulless Scout, the nutritional status of Harrington and Glenlea malts improved more by germination than GA. The results showed a considerable effect of germination on the amino acid composition of the malts. A substantial increase in E/T % values and chemical scores occurred on germination. The differential behavior of the hulless barley in this respect as compared to the hulled barley and Glenlea wheat seemed noteworthy.

Registry No. L-Glu, 56-86-0; L-Asp, 56-84-8; L-Lys, 56-87-1; L-Thr, 72-19-5; L-Ile, 73-32-5; gibberellic acid, 77-06-5.

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Aerobic Formation of Keto Diene from Linoleic Acid Catalyzed by One of the Two Forms of Lipoxygenase Isolated from Bengal Gram (*Cicer arietinum*)

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Lipoxygenase from Bengal gram (*Cicer arietinum*) has been resolved into two active forms. One of them catalyzes the formation of conjugated diene hydroperoxide from linoleic acid while the other forms keto diene among other products. The aerobic formation of keto diene as one of the major products in a lipoxygenase reaction is unique. This suggests that the two forms of the enzyme present in Bengal gram are distinct.

INTRODUCTION

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12), which catalyzes the hydroperoxidation of fatty acids containing *cis,cis*-1,4-pentadiene system, has been well studied in soybean and shown to exist in multimolecular forms (Axelrod, 1974). They exhibit differences in their pH–activity profile, substrate specificity, and the positional specificity (Axelrod, 1974). These forms have also been resolved by HPLC and shown to be antigenically distinct (Ramadoss and Axelrod, 1982). Multiple forms of this enzyme have been reported in pea (Erriksson and Svensson, 1970; Reynolds and Klein, 1982) and also in a few other species [winged bean (Truong et al., 1982); cowpea (Truong and Mendoza, 1982); wheat (Hsieh and McDonald, 1984); rice (Shoji et al., 1983)].

Some lipoxygenases, besides catalyzing the hydroperoxidation reaction, form other secondary products such as keto diene (Vioque and Holman, 1962). Originally these were shown to be generated only anaerobically (Garssen et al., 1971; Garssen et al., 1972) but were later reported to occur also under aerobic condition (Pistorius, 1974; Hurt and Axelrod, 1977). However, this reaction was not well characterized.

We report here the presence of two forms of lipoxygenase in Bengal gram, one of which catalyzes the aerobic formation of keto diene as a major product. This kinetic feature clearly distinguishes one form from the other.

MATERIALS AND METHODS

Materials and Chemicals. Seeds of Bengal gram were obtained from Seed Corp. of India, Mysore, India. Linoleic acid was obtained from Nucheck Prep. Inc. Minnes. DEAE-Sephadex was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Hydroxylapatite was prepared according to the method of Tiselius as described by Bernardi (1971). A stock solution of 10 mM sodium linoleate in Tween-20 was prepared as described by Axelrod et al. (1981).

Isolation of the Enzyme. Bengal gram flour (50 g) was stirred with 250 mL of 50 mM borate buffer, pH 8.0, for 30 min at 4–6 °C. The extract was centrifuged at 12000*g* for 20 min. The enzyme in the supernatant was purified by ammonium sulfate fractionation and DEAE-Sephadex chromatography. The active fraction from this was resolved into two activities on hydroxylapatite column. The two forms were further purified by rechromatography on hydroxylapatite column followed by molecular sieving on Sephadex G-200. The purified forms appeared to be homogeneous as judged by electrophoresis on SDS–polyacrylamide gel. Details of the purification procedure and molecular properties of the enzyme will be presented elsewhere. The two forms of the enxyme were designated as BGL_1 and BGL_2 according to the order of their elution from the hydroxylapatite column. The final preparations of the enzyme were in 50 mM sodium phosphate buffer, pH 6.8, containing 1 mM EDTA and 40% glycerol and stored at −20 °C.

The enzyme was assayed either by following the oxygen consumption on a Gilson oxygraph or by the appearance of conjugated diene absorbing at 234 nm on a Beckman Model 26 spectrophotometer.

One unit of enzyme is defined as the utilization of 1 μmol of substrate or the formation of 1 μmol of product per minute under the assay conditions.

Protein was determined according to Lowry et al. (1951) with bovine serum albumin as standard.

For the determination of K_m for linoleic acid, linear regression analysis of the data was done on Hewlett-Packard HP 33E programmable scientific calculator. Parameters for Hofstee plot were used to get the apparent K_m.

Isolation of Products. Linoleic acid (2 μmol) was incubated separately with 3 units of either BGL_1 or BGL_2 in 20 mL of sodium phosphate buffer, pH 6.5. The reaction was carried out for 4 min at 4 °C. After acidification with 2 M citric acid, the products were extracted twice with 2 vol of hexane–ether mixture (80:20, v/v). The combined extracts were washed with distilled water until neutral.

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