to normal or any mutant except waxy and the amylo-1,6-glucosidase of de*-92. At the same time, the amylose content of the starch produced by de*-92 is only 12% as compared to 25% in normal and 0% in waxy. It is not established, however, that the low glucosyl transferase activity and the resultant low amylose content of the de*-92 stock investigated is conditioned by the de*-92 mutation. It could have its basis in an intermediate waxy allele present in the de*-92 line. See the accompanying report by Warren Bryce for more detailed information on amylo-1,6-glucosidase activity in endosperms of de*-92.

It is not concluded that the low protein content of these mutants indicates inefficiency at a step in transcription or protein synthesis. The shrunken-4 mutant, which also has a low protein content at all stages of development of the seed, appears to be partially blocked in pyridoxal phosphate synthesis as the primary mutational lesion. The lower protein content is a secondary consequence of the lessened availability of pyridoxal phosphate. There are obviously other possible defects in reactions not directly concerned with protein synthesis.

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2. The endosperm proteins of opaque-6.

We reported no change in the amino acid profile of the collective endosperm proteins of opaque-6 (MNL 46:203). A detailed re-examination of the endosperm proteins of the homozygous opaque-6 and normal seeds from the same ear has been done by the modified Osborn-Mendel procedure. Since the results are not in accord with our previous report, we take this opportunity to offer a correction. The results of protein fractionation and amino acid analysis are given in Tables 1 and 2, respectively.

As shown in Table 1, the major differences between the mutant and its normal counterpart occurred in water-soluble and 70 percent ethanol-soluble fractions. The amount of zein (70 percent ethanol-soluble proteins) in normal endosperms was 9.6 mg per endosperm, which constituted 62.6

Table 1

Results of protein fractionation of defatted corn endosperms of o6(R) and its normal counterpart by a modified Osborn-Mendel method

Fraction		Genotype			
		+/-[1]		o6(R)	
		a	b	a	b
H_2O	mg	37.9	0.49	210.7	2.26
	c	3.2		23.4	
	d	3.2		20.7	
H_2O-soluble Protein Nitrogen	mg	33.3	0.43	132.3	1.42
	e	87.9		62.8	
	d	2.8		13.0	
H_2O-soluble Non-protein Nitrogen	mg	4.6	0.06	78.4	0.84
	e	12.1		37.2	
	d	0.4		7.7	
5 percent NaCl	mg	33.8	0.44	60.6	0.65
	c	2.9		6.7	
	d	2.8		6.0	
70 percent Ethanol	mg	750.2	9.60	270.8	2.90
	c	63.8		30.1	
	d	62.6		26.6	
0.2 percent NaOH	mg	356.9	4.57	356.9	3.83
	c	30.3		39.7	
	d	29.8		35.0	
0.2 percent NaOH Precipitate	mg	274.7	3.52	285.1	3.06
	e	77.0		79.9	
	d	22.9		28.0	
0.2 percent NaOH Supernatant	mg	82.2	1.05	71.8	0.77
	e	23.0		20.1	
	d	6.9		7.0	
Total soluble protein	mg	1176.7	15.10	899.0	9.64
	d	98.2		88.3	

Table 1 (Continued)

Fraction		Genotype			
		+/-[1]		o6(R)	
		a	b	a	b
Residue	mg	22.2	0.28	119.7	1.28
	d	1.8		11.7	
Total protein recovered	mg	1198.9	15.38	1018.7	10.92
	d	98.0		95.3	
Total endosperm protein	mg	1223.0	15.65	1069.0	11.47
No. kernels/10 gr.		78.1		93.2	

a - per 10 gr. of endosperm, b - per endosperm, c - percentage of the soluble protein, d - percentage of the total protein

percent of the total endosperm protein, whereas the mutant had a drastic decrease to 2.9 mg per endosperm, which was only 26.6 percent of the total endosperm proteins. In opaque-6, the total water-soluble fraction was increased over its normal counterpart by factors of 4.6 and 5.5 on weight and endosperm basis, respectively. This fraction was distributed among water-soluble proteins, albumins, and the non-protein nitrogen (free amino acids and small peptides).

Differences were also found in 5 percent NaCl-soluble fraction, globulins, and the insoluble proteins. The mutant seeds contained nearly two times more globulin on a weight basis than their normal counterpart. However, this fraction was only a minor portion of the total protein. Therefore, doubling the amount of this fraction in the mutants did not account for a large percent of the total proteins. The protein which could not be extracted by the four solvents utilized was referred to as residue and was found to be higher in mutant than normal by 5.4 times on a weight basis and 4.6 times on a per endosperm basis.

The protein fraction which showed no significant difference between opaque-6 and normal was the 0.2 percent NaOH-soluble fraction. When this fraction was separated into two subfractions, the differences between the mutant and the normal seeds, on a dry weight basis, were still not significant. However, on an endosperm basis, both subfractions in the mutant had less protein than normal because of the reduction of kernel size in the mutant.

The pronounced decrease in the alcohol-soluble fraction and the increase of the water- and salt-soluble fractions have previously been observed in opaque-2, opaque-7, and floury-2.

The amino acid composition analysis of each protein fraction was carried out by ion exchange chromatography following acid hydrolysis. The amino acid content of mutant and normal endosperm is given in Table 2.

Table 2

The amino acid composition of the defatted-endosperms of opaque-6(R) and its normal counterpart

Amino acids	+/-[1]	o6(R)
Lysine	1.55	3.32
Histidine	2.93	3.00
Arginine	2.98	3.93
Aspartic Acid	5.74	9.80
Threonine	3.48	3.65
Serine	5.12	4.78
Glutamic Acid	20.54	22.95
Proline	10.59	7.09
Glycine	2.54	3.38
Alanine	8.24	7.38
Valine	3.23	3.23
Methionine	2.55	3.18
Isoleucine	3.92	3.62
Leucine	15.96	11.76
Tyrosine	4.12	3.80
Phenylalanine	6.51	5.13
Data corrected to 100 percent recovery	101.9	91.5
Actual recovery		

The amino acid composition of the collective endosperm proteins of opaque-6 mutant is rather similar to that of the opaque-2, opaque-7, and floury-2 mutants. The same amino acids (lysine, arginine, aspartate, glycine) are increased as in those mutants. The decreases observed for proline, alanine, and leucine are also characteristic of these other mutants. It is interesting that in this mutant a familiar pattern of amino acid shifts is associated with a lethal seedling condition.

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3. Amylo-1,6-glucosidase activity in maize endosperm.

Amylo-1,6-glucosidase (de-branching enzyme) activity was examined in maize endosperms collected 22 days after pollination, frozen on dry ice, and stored at -20°C. The enzymatic activities in the endosperm of several carbohydrate mutants and in the hybrid W64A x W182E (+) endosperms and pollen were determined using a modification of the procedure described by Nelson and Larner (Analyt. Biochem. 33:87-101, 1970). Their method relies on the incorporation of ^{14}C-glucose into glycogen as a means of quantitatively measuring the de-branching activity. ^{14}C-glucose residues that are incorporated into polysaccharide are precipitated by aqueous ethanol on filter paper, while free glucose molecules are soluble in aqueous ethanol.

The pericarp and embryos were removed from endosperms, which were homogenized (in a ratio of 1 g. fresh weight endosperm/1 ml extraction buffer and 1 g. fresh weight pollen/5 ml extraction buffer) in a Virtis blender in chilled 0.01 M citrate buffer (containing 10^{-3}M dithiothreitol) at pH 6.75. This homogenate was filtered through cheesecloth and centrifuged at 31,000 x g for 20 minutes. A 0% to 70% saturation ammonium sulfate cut was taken and, after centrifugation, the precipitated protein was redissolved in extraction buffer. This preparation, after dialysis for about 14 hours against extraction buffer, was used to assay amylo-1,6-glucosidase activity.

The 400 ul incubation mixture contained 100 ul enzyme prep.; 100 ul of D-glucose-^{14}C-UL solution, (15 uCi and 7.5 umoles/ml); and 200 ul