5. More evidence of shrunken-floury's effect on protein synthesis.

A supposed effect of the gene sh^{fl} on protein synthesis was reported in the 1955 News Letter. The evidence was morphological in nature and involved abnormalities in the endosperm, aleurone, and in microsporogenesis. Proof of this supposition lay in a chemical comparison of the proteins in sh^{fl} and Sh^{fl} (normal) tissue.

Since determinations of total nitrogen in mutant and normal kernels failed to reveal a significant difference, an attempt was made to fractionate the proteins, making use of their varying solubilities. In this way a somewhat more refined comparison of the proteins of the two kernel classes is possible.

Mutant and normal kernels were classified from self- or sib- pollinated ears produced on plants heterozygous for sh^{fl}. Only ears with an extreme expression of shrunken-floury were used. Those with segregating modifiers which bring the expression of sh^{fl} toward normal were avoided in these tests. The kernels were finely ground and samples of two to four grams were weighed out. These were placed in 125 ml. Erlenmeyer flasks for extraction. The samples were successively extracted with water, 1.0 N potassium chloride, 80% ethyl alcohol, and 0.2% sodium hydroxide. Hence, the protein fractions will be referred to as water, salt, alcohol, and base soluble, and insoluble proteins. Twenty-five ml. of solution were used for each gram of the sample, and the extraction was continued for 24 hours. Kjeldahl nitrogen determinations were made for each fraction and these values were converted to protein by multiplying by the 6.25 factor. Since the separate ears varied in the percentage of kernel weight which was protein, a better comparison was obtained by computing the percentage of the total protein which resided in the particular protein fraction. Table 1 gives the results of five ears whose mutant and normal kernel classes were fractionated as above. The values cited are average values for two replications.

Significant differences between the nitrogen content of sh^{fl} and Sh^{fl} kernels exist in the water, salt, and alcohol soluble fractions. The differences within the base soluble and insoluble fractions are not significant. The shrunken-floury class contains about 1.74 times as much water soluble "protein" as the normal class. Differences of the same type occur in the salt soluble fraction, but the factor is 1.60. These differences are compensated for in the alcohol soluble fraction where the shrunken-floury class has a little more than one-half the protein of the normal class.

Table 1. Comparison of the protein fractions of sh^{fl} and Sh^{fl} Kernels.

Protein Fraction	Ear Number	sh^{fl}*	Sh^{fl}*	sh^{fl}/Sh^{fl} Ratio
Water Soluble Fraction	1	15.48%	9.70%	1.60
	2	18.65	12.47	1.50
	3	19.95	12.60	1.57 m = 1.74**
	4	21.86	10.41	2.10
	5	22.78	11.77	1.93

	1	10.58%	6.75%	1.57	
	2	12.35	9.86	1.25	
Salt Soluble Fraction	3	10.36	8.44	1.23	m = 1.60**
	4	12.53	51.84	2.15	
	5	12.57	6.92	1.82	
	1	22.66%	28.61%	0.79	
	2	15.48	29.82	0.52	
Alcohol Soluble Fraction	3	17.06	31.38	0.54	m = 0.56**
	4	23.20	43.69	0.53	
	5	14.57	33.32	0.44	
	1	26.88%	31.89%	0.84	
	2	27.12	27.63	0.98	
Base Soluble Fraction	3	22.66	25.94	0.87	m = 0.97
	4	27.63	23.12	1.20	
	5	25.05	26.57	0.94	
	1	24.35%	23.19%	1.05	
	2	25.47	21.78	1.17	
Insoluble Fraction	3	29.56	21.69	1.36	m = 1.13
	4	14.75	16.86	0.88	
	5	24.90	20.93	1.19	

*Percentage of the total nitrogen appearing in the given fraction.
**Significantly different from 1.0.

One interpretation which might be placed on these results is that the gene sh^{fl} is blocking the conversion of the simpler proteins (water and salt soluble) to the more complex (alcohol soluble) proteins. It is likely that the water and salt soluble fractions contain proteins of lower molecular weight than the protamines of the alcohol fraction, and it is possible that scme of these simpler components are unconverted substrates of the alcohol soluble proteins. Proof of this will require a more involved chemical treatment, but it seems certain that sh^{fl} is involved in protein metabolism. It exerts its effect at sometime after the incorporation of nitrogen and it hinders the production of certain of the more complex proteins.

Shrunken-floury has been tested for allelism with a number of endosperm mutants and was found to be allelic with a gene in the Co-op stock labelled "sh_4" Singleton. This symbol is apparently tentative and, I suggest the locus be called shrunken-floury due to the obvious floury nature of the kernel.

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