out any effect of iron concentration being responsible for the differences seen with the various mixtures.

These observations provide a basis for increasing the sensitivity of the marrow-cell culture method for the study of hemoglobin synthesis, for the assay of erythropoietin in vitro, and for investigations into the mechanism of action of the hormone.

O. GALLIEN-LARTIGUE*
E. GOLDWASSER
Argonne Cancer Research Hospital†,
and *Department of Biochemistry,*
University of Chicago,
Chicago, Illinois

References and Notes

1. S. B. Krantz, O. Gallien-Lartigue, E. Goldwasser, *J. Biol. Chem.* **238**, 4085 (1963).
2. F. W. J. Teale, *Biochim. Biophys. Acta* **35**, 543 (1959).
3. Drabkin's solution: 1 g of $NaHCO_3$, 52 mg of KCN, and 198 mg of $K_3Fe(CN)_6$ per liter.
4. We gratefully acknowledge the capable assistance of Mrs. Sandra Shin and Mr. Edward Morris.

* Present address: College de France, Laboratoire d'Embryologie Experimentale, Paris.
† Operated by the University of Chicago for the United States Atomic Energy Commission.

11 June 1964

Mutant Gene That Changes Protein Composition and Increases Lysine Content of Maize Endosperm

Abstract. *Preliminary tests have shown that the endosperms of maize seeds homozygous for the* opaque-2 *mutant gene have a higher lysine content than normal kernels. As a critical test, a backcross progeny was divided into* opaque-2 *and normal kernels, the endosperms separated, and the amino acids determined. The* opaque-2 *endosperms had a different amino acid pattern and 69 percent more lysine than the normal seeds. The major reason for these changes is the synthesis of proteins with a greater content of basic amino acids in the acid-soluble fraction of the mutant endosperm. This is accompanied by a reduction in the ratio of zein to glutelin.*

Since the development of the copper extraction-fractionation method for separating maize proteins (*1–4*), we have been searching for maize with a lower zein and a higher lysine content. The importance of examining the separated endosperms has been stressed (*5*). Preliminary tests showed that a strain homozygous for the recessive mutant gene, *opaque-2* (o_2), had in the endosperm a lysine content (4 percent of the protein) which was twice that found in typical hybrid corn (*7*). Copper fractionation (*2*) of protein from *opaque-2* endosperm revealed 15.7 percent zein and 42.3 percent glutelin based on total protein (*7*). Endosperms from normal North American and Guatemalan maize lines (*4*) contained 41 to 52 percent zein and 17 to 28 percent glutelin on the same basis. Thus, there is a reversal in the ratio of zein to glutelin in the *opaque-2* endosperm when compared with normal maize lines.

We have now determined the lysine content of endosperms from two additional strains containing the *opaque-2* gene in different genetic backgrounds from each other, and from the *opaque-2* strain first tested. In both strains, the lysine content (3.3 to 4.0 percent) was more than twice that of endosperms from a normal strain used as a control (1.3 percent lysine).

To make a critical test of the hypothesis that the *opaque-2* mutant is responsible for the increased lysine content the normal and *opaque-2* kernels from a single backcross ear ($+/o_2 \times o_2/o_2$) were separated. The endosperms were isolated and defatted as described previously (*1*), and 5 mg of endosperm protein was hydrolyzed at 110°C for 24 hours by refluxing with 20 ml of 6*N* HCl. One-milligram portions of hydrolyzed protein were placed on the short and long columns of a Spinco automatic amino acid analyzer. Norleucine was used as an internal standard.

The amino acid composition of the *opaque-2* and normal endosperms is compared in Table 1. Both types of endosperms contain 8.69 percent crude protein ($N \times 6.25$) on a fat and moisture-free basis (micro-Kjeldahl). Thus, the amino acid contents are directly comparable.

The *opaque-2* endosperm contains 69 percent more lysine than the normal endosperm. The former contains less glutamic acid, alanine, methionine, leucine, and tyrosine, and more lysine, histidine, arginine, aspartic acid, glycine, and cystine than the latter. The same relationship is found when the amino acid compositions of glutelin and zein are compared (*3*).

Copper fractionation (*2*) of duplicate 0.5-g portions of ground, defatted *opaque-2* endosperm, and of a single 0.5-g portion of normal endosperm from the same ear of corn used above,

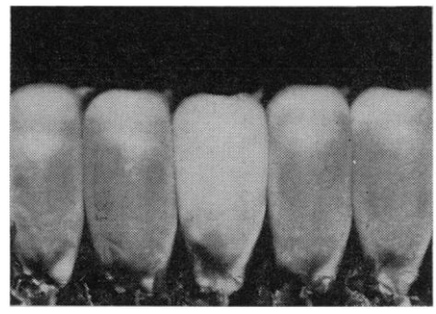

Fig. 1. A row of kernels on an ear of maize showing *opaque-2* mutant (center) and normal kernels.

gave the following distribution based on soluble nitrogen. *Opaque-2*: 35 percent acid-soluble, 26 percent alcohol-soluble (zein), and 39 percent alkali-soluble (glutelin); normal: 34 percent acid-soluble, 37 percent alcohol-soluble (zein), and 29 percent alkali-soluble (glutelin). This confirms the reduction in the zein to glutelin ratio observed previously.

Preliminary data on the basic amino acid and amide ammonia content of the above separated soluble copper fractions show that along with the reduction in the zein to glutelin ratio, important changes occur in the amino acid patterns of the acid-soluble and alcohol-soluble (zein) fractions. We have calculated the ratios of the three basic amino acids and amide ammonia

Table 1. Amino acids in normal and opaque endosperms from the same ear of corn (expressed as grams per 100 g of protein).

Amino acid	Endosperm	
	Opaque	Normal
Lysine	3.39	2.00
Tryptophan*		
Histidine	3.35	2.82
Amide ammonia	3.41	3.28
Arginine	5.10	3.76
Aspartic acid	8.45	6.17
Glutamic acid	19.13	21.30
Threonine	3.91	3.48
Serine	4.99	5.17
Proline	9.36	9.67
Glycine	4.02	3.24
Alanine	6.99	8.13
Valine	4.98	4.68
Cystine	2.35	1.79
Methionine	2.00	2.83
Isoleucine	3.91	3.82
Leucine	11.63	14.29
Tyrosine	4.71	5.26
Phenylalanine	4.96	5.29

* Peptide-bound tryptophan presents a special problem because it is destroyed by hydrolyzing agents (*8*).

using the *opaque-2* values as numerators and the normal values as denominators. The ratios follow. Lysine: acid-soluble, 3.2; zein, 3.0; glutelin, 1.0. Histidine: acid-soluble, 3.4; zein, 1.2; glutelin, 0.9. Arginine: acid-soluble, 2.3; zein, 1.2; glutelin, 1.1. Amide ammonia: acid-soluble, 0.6; zein, 1.8; glutelin, 0.9.

These data suggest that the acid-soluble and zein fractions are quite unlike those of the normal endosperms, whereas the glutelin fractions of the two endosperms are similar with respect to the basic amino acids and amide ammonia. On the basis of these preliminary findings, the increased content of lysine in the *opaque-2* endosperm can be attributed to three factors: (i) increased lysine in the acid-soluble fraction, (ii) increased lysine in the zein fraction, and (iii) reduction in the ratio of zein to glutelin.

The zein fraction of *opaque-2* endosperm contained 0.9 g of lysine per 100 g of protein ($N \times 6.25$), which is more than ten times the amount (0.08 g) found in a composite of zein fractions from U.S. and Guatemalan endosperms (*4*), and three times the amount found in the normal endosperms from the same ear. This finding, together with the 1.8-fold increase in amide ammonia (probably from glutamine) supports the conclusion that *opaque-2* endosperm contains a type of zein that is chemically, and perhaps physically, unlike any described heretofore. Complete amino acid analysis of the copper fractions will be published later.

This is the first demonstration of a radical change in the protein composition of maize endosperm, and is caused by a single mutant gene. In addition to its possible usefulness in studies on protein synthesis, the *opaque-2* gene should permit the development of commercial strains of corn with much higher lysine contents. The value of such corn in human and animal nutrition has been emphasized in previous publications (*4*, *7*).

EDWIN T. MERTZ
LYNN S. BATES
Department of Biochemistry
OLIVER E. NELSON
Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana

References and Notes

1. E. T. Mertz and R. Bressani, *Cereal Chem.* **34**, 63 (1957).
2. E. T. Mertz, N. E. Lloyd, R. Bressani, *ibid.* **35**, 146 (1958).
3. N. E. Lloyd and E. T. Mertz, *ibid.*, p. 156.
4. R. Bressani and E. T. Mertz, *ibid.*, p. 227.
5. E. T. Mertz, *Cereal Sci. Today* **5**, 32 (1960).
6. R. A. Emerson, G. W. Beadle, A. C. Fraser, *Cornell Univ. Agr. Expt. Sta. Mem. No. 180* (1935).
7. E. T. Mertz, *Proc. 18th Ann. Hybrid Corn Industry-Research Conf., Dec. 12 and 13, 1963* (Publ. No. 18, American Seed Trade Association, Washington, D.C.), pp. 7–12.
8. J. R. Spies and D. C. Chambers, *Anal. Chem.* **21**, 1249 (1949).
9. This is journal paper No. 2326, Purdue Agricultural Experiment Station, Lafayette, Ind.

13 April 1964

Nicotinamide Adenine Dinucleotidase Activity in Cells of Tuberculous Animals

Abstract. *There is an increase in the nicotinamide adenine dinucleotidase activity of polymorphonuclear leukocytes, monocytes, and lymphoid cells from tuberculous animals, compared with that of cells from normal animals. There seems to be a correlation between the ability of the animal to develop a progressive disease and its ability to react with an increase in the activity of the enzyme.*

There is an increased activity of nicotinamide adenine dinucleotidase in organs of tuberculous mice with a concomitant reduction in nicotinamide adenine dinucleotide concentration (*1*). These biochemical changes could be elicited by administration of a lipid fraction derived from the tubercle bacilli (cord factor), and the toxicity of this factor could be alleviated by administration of nicotinamide (*2*). Furthermore during the tuberculous infection in mice, soluble dinucleotidase can be detected in their livers and lungs. On the basis of this observation, the enzyme in its soluble form was assumed to come into contact with nicotinamide adenine dinucleotide of the cytoplasm and mitochondria and cause its splitting (*3*).

In the guinea pig, an animal extremely sensitive to infection with tubercle bacilli, the activity of the enzyme is dramatically increased during the infection, whereas in the tuberculous mouse, an animal relatively resistant to infection with tubercle bacilli, such is not the case (*4*). The activity of the enzyme of liver microsomes of infected guinea pigs increases 36-fold while that of liver microsomes of the infected mice is elevated twofold, compared with liver microsomes from normal animals (*3*, *4*).

This report deals with the nicotinamide adenine dinucleotidase activity of polymorphonuclear leukocytes, monocytes, and lymphoid cells from tuberculous and normal guinea pigs, strain C57 black mice, and albino mice.

Albino mice and the C57 strain of black mice, weighing about 20 g, and guinea pigs with an average weight of 400 g, were used as the source of peritoneal polymorphonuclear leukocytes and monocytes, as well as of lymphoid cells from mesenteric and tracheobronchial lymph nodes.

Guinea pigs were infected by intramuscular injection, and the mice by intravenous injection of tubercle bacilli, strain $H_{37}Rv$, grown on Lowenstein-Jensen medium. The amount injected was about 0.1 mg (dry weight).

The establishment of the tuberculous infection was determined by the presence of characteristic lesions in the organs of the animal.

Cells were obtained from mice about 2 weeks after infection and from guinea pigs about 3 weeks after infection.

Polymorphonuclear leukocytes from normal mice were obtained as follows: 3 ml of 0.1 percent glycogen in saline was injected intraperitoneally. Four hours later the mice were killed by decapitation. Cells were collected by washing the peritoneal cavity with Krebs-Ringer phosphate solution supplemented with heparin (2 units/ml). Usually cells from three mice were pooled in siliconized tubes and differential counts showed that 75 percent were polymorphonuclear and 25 percent were mononuclear cells. Polymorphonuclear cells from infected mice were obtained by the same procedure but collected about 16 hours after injection of the irritant.

To obtain polymorphonuclear cells from normal and tuberculous guinea pigs, about 15 ml of 0.1 percent glycogen in saline was injected intraperitoneally. Four hours later 30 ml of heparinized Krebs-Ringer phosphate solution was introduced into the peritoneal cavity, and the exudate was col-