

Table 1. Autoantibodies in gastric juice, as shown by immunofluorescence. 0, negative; $\pm$, equivocal; W+, weakly positive; +, positive; ++, strongly positive.

To gastric parietal cell		To thyroid acinar cell	
Serum	Gastric juice	Serum	Gastric juice
<i>Pernicious anemia</i>			
+	+	0	0
+	+	0	0
++	+	+	0
++	0	W+	0
<i>Atrophic gastritis</i>			
++	++	W+	0
+	$\pm$	++	0
+	$\pm$	+	0

positive staining with the conjugated antiserum to IgG; in three out of four subjects positive staining occurred with antisera to both IgG and IgA (Fig. 2). With whole human serum, both of these conjugates gave single, appropriate lines of precipitation in immunoelectrophoresis. To confirm this apparent absence of a contaminating antibody to IgG in the conjugated antiserum to IgA, sera and gastric juices were absorbed with sufficient rabbit antiserum to human Fc (5) to suppress completely all IgG antibody detectable by immunofluorescence. The conjugated antiserum to IgA was absorbed with an amount of human IgG (6) sufficient to neutralize any contaminating antiserum to IgG present in the conjugated antiserum to IgA. Neither of these maneuvers separately, or combined, suppressed staining with the conjugated antiserum to IgA. These preliminary results show that the gastric parietal cell antibody in both serum and gastric juice is of both IgG and IgA type, the former probably predominating.

In human saliva, colostrum, and lacrimal secretions the predominant immunoglobulin is IgA, whereas in serum, IgG is the major γ -globulin. In bile and in small intestinal secretions, IgG predominates but the ratio of IgG to IgA is lower than in serum. It has been suggested that either a preferential secretion or local production in adjacent lymph nodes may account for some of the globulins present in these fluids (2), and the work of Tomasi *et al.* (7) suggests that there may be local synthesis of IgA in the salivary gland; this is predominantly 11S γ -globulin, a polymer which has been found in saliva and colostrum but is not present in serum. No similar studies of gastric secretion have been reported. Our own data do not help to resolve the question whether parietal-cell anti-

bodies are elaborated in the gastric mucosa or not, since IgG and IgA components are present in both gastric juice and serum. Synthesis in the gastric mucosa might be one explanation of why the parietal cell antibody is found in gastric juice but the thyroid acinar cell antibody is not. An alternative explanation is that some factors in the gastric mucosa determine that of the two autoantibodies only the parietal cell antibody will pass across the gastric mucosa to the gastric lumen (8).

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9. We thank Dr. R. Wistar for preparation of antisera to human sera, Cohn's fraction II, human Fc piece, and purified human γ G globulin. Type-specific antisera to γ G and γ A globulin were supplied by Hyland Laboratories. This investigation was supported by NIH grant AM-06971-02 and by a USPHS postdoctoral fellowship grant (J.M.F.).

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Second Mutant Gene Affecting the Amino Acid Pattern of Maize Endosperm Proteins

Abstract. *The mutant floury-2 results in the production of maize endosperm proteins with an altered amino acid pattern. The lysine concentration is high, approximately equal to that in mutant opaque-2, and the methionine concentration is higher than in any other stock tested. Other mutants of similar phenotype, opaque-1, floury-1, and soft-starch do not cause major changes in amino acid pattern.*

The drastic change in amino acid pattern of endosperm proteins effected by the *opaque-2* mutant of maize has been reported (1). The effectiveness of *opaque-2* maize in supporting the growth of weanling rats has also been reported (2).

That maize endosperm proteins are deficient in lysine and tryptophan encourages the search for stocks with higher contents of these amino acids. The rationale for investigating originally the amino acid pattern of *opaque-2* and other mutants of like phenotype—pronounced opacity of the endosperm in contrast with the translucence of normal endosperms—was based on the conjecture that such mutants might lack the ability to produce the zein fraction of endosperm proteins. If so, and if there were compensatory synthesis increasing the amount of the other major protein fractions (acid-soluble and glutelin), an increased lysine and tryptophan content would result

since the lysine and tryptophan content of zein is very low (3). None of the mutants is completely blocked in the ability to produce zein (4), but the amount present in *opaque-2* is significantly reduced (1). In addition starch-gel electrophoresis shows that four components of normal zein are missing from *opaque-2* zein (4).

Five different mutants of similar phenotype, *opaque-1* (o_1), *opaque-2* (o_2), *floury-1* (fl_1), *floury-2* (fl_2), and *soft-starch* (h) were available. Their isolation and mode of inheritance has been reported by Emerson, Beadle, and Fraser (5). The opaque and floury mutants were tested initially, and h was tested subsequently. We now present the amino acid patterns for all five mutants and show that in addition to *opaque-2*, the *floury-2* mutant also has an altered amino acid pattern and higher lysine and methionine concentrations.

The seeds analyzed were mature, air-

Table 1. Amino acids in the defatted endosperms of normal and five mutant stocks (expressed as grams per 100 g of protein).

Amino acid	W64A+	W64Ao ₂	o ₁	fl ₁	fl ₂ *	fl ₂ †	h
Lysine	1.6	3.7	1.7	1.8	3.2	3.4	1.8
Tryptophan‡	0.3	0.7	0.6	0.6	0.6	0.9	0.5
Histidine	2.9	3.2	2.3	3.2	2.0	2.4	2.7
Arginine	3.4	5.2	3.3	3.7	4.6	4.3	3.8
Aspartic acid	7.0	10.8	6.1	4.9	7.4	10.9	7.0
Glutamic acid	26.0	19.8	21.5	20.2	17.5	20.6	23.7
Threonine	3.5	3.7	3.3	3.1	3.0	3.6	3.6
Serine	5.6	4.8	4.9	4.7	4.2	5.3	5.7
Proline	8.6	8.6	9.4	11.9	7.6	10.0	10.4
Glycine	3.0	4.7	2.9	2.8	3.0	3.7	3.4
Alanine	10.1	7.2	8.2	7.7	7.3	8.6	9.4
Valine	5.4	5.3	4.7	4.4	4.8	5.6	5.0
Cystine	1.8	[0.9]§	2.2		2.0	1.6	1.5
Methionine	2.0	1.8	2.2	2.5	3.0	3.4	2.8
Isoleucine	4.5	3.9	4.2	3.8	3.8	4.2	4.3
Leucine	18.8	11.6	15.4	15.1	12.7	13.9	16.8
Tyrosine	5.3	3.9	5.0	4.9	4.3	4.7	5.6
Phenylalanine	6.5	4.9	5.7	5.3	4.8	5.4	6.2
Percent protein							
	12.7	11.1	10.4	10.8	13.6	13.6	10.8

* Grown 1958, analyzed 1964. † Grown 1964, analyzed 1965. ‡ Analyzed by the Spies and Chambers method O (7) by J. M. Concon. § Other analyses of *opaque-2* stocks have given cystine values equal to or greater than normal.

dried seeds produced by self-pollination. The endosperms were separated from the embryos and pericarps, ground in a burr mill to a particle size of 0.025 cm, and extracted with hexane (b.p. 65° to 67°C) in a Soxhlet apparatus for 36 hours. The protein content (nitrogen $\times$ 6.25) was measured by micro-Kjeldahl assays. Ground, defatted endosperm was hydrolyzed with a 4000-fold excess of 6N HCl by refluxing at 110°C for 24 hours with norleucine as an internal standard. Portions (1 ml) of hydrolyzed protein (0.5 mg of protein per milliliter) were then placed on the long and short columns of a Spinco automatic amino acid analyzer.

The amino acid concentrations for a normal (nonmutant) inbred stock and the five mutants are shown in Table 1. The normal inbred was W64A. The *opaque-2* (o₂) stock was a mutant that occurred in the W64A stock and presumably differs from W64A only at the *opaque-2* locus. The analyses for these two stocks give a valid comparison of the changes in amino acid patterns attributable to the *opaque-2* gene. We have analyzed three *opaque-2* mutants from independent mutational events in diverse backgrounds. The differences from normal noted here—increased lysine, arginine, aspartic acid, and glycine together with decreased glutamic acid, alanine, leucine, tyrosine, and phenylalanine—are typical of all *opaque-2* stocks. No such rigorous comparison is possible for the other

four mutants, but their amino acid concentrations, relative to those of W64A normal offer reasonable indications of the effect of the mutant gene in question.

Apart from *opaque-2*, only *floury-2* has a major effect on amino acid pattern. For *floury-2*, two analyses of the same line are given. One sample was grown in 1958 and analyzed in 1964, the other was grown in 1964 and analyzed in 1965. For the 1965 analysis, the total recovery was higher (111.7 percent) than the 1964 analysis (95.1 percent), and hence the concentrations of most amino acids are higher. It is apparent that the concentration of lysine is approximately twice that of normal, *opaque-1*, *floury-1* or *soft-starch* and nearly as high as *opaque-2*. The protein content of this *floury-2* line is higher than that of the *opaque-2* line. Thus 100 g of *floury-2* endosperm contains 0.45 g of lysine (from the mean value of the two analyses) compared with 0.41 g for the *opaque-2* line. Further, *floury-2* has a higher methionine concentration (3 to 3.4 g per 100 g of protein) than any line (normal or mutant) tested heretofore. This mutant may be valuable nutritionally in diets where corn is supplemented with kidney beans or soy beans which are low in this amino acid (6).

The enhanced lysine content of *opaque-2* stocks results in part from a higher lysine content of the zein (1). Preliminary investigations indicate that the lysine content of *floury-2* zein (0.3

g per 100 g zein) is only one-third of that in *opaque-2* zein. In addition, starch-gel electrophoresis of *floury-2* zein gives a pattern different from that of normal and of *opaque-2* zein (4). Apparently the biochemical basis of elevated lysine content in *floury-2* is different from that in *opaque-2*, and this suggests the possibility that, in the double mutant stocks now being derived, there may be higher amounts of lysine than in either mutant alone.

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Biosynthesis of Histones and Acidic Nuclear Proteins under Different Conditions of Growth

Abstract. The incorporation of uniformly labeled L-lysine-C¹⁴ into the normal and regenerating rat liver, into Novikoff hepatoma histones, and into acidic nuclear proteins was studied. In rat liver, different histone fractions incorporate labeled lysine to a different extent. Such differences become less obvious in regenerating liver, and they are even less so in Novikoff hepatoma. In the hepatoma cells the ratio of the biosynthesized acidic nuclear proteins to histones was altered.

Since the DNA in mammalian chromosomes appears to be associated with histones, Stedman and Stedman (1) suggested that histones may act as gene regulators or suppressors. The possibility that histones may act as gene