type in the face of natural randomizing influences. Indeed, among the codes that could be generated by a reassignment of codons, it is doubtful that any would be substantially more protective than the present one. In fact, if the GC content and degree of degeneracy of each amino acid is kept constant, then Table 1 shows that reassignment of codon sets cannot improve the protection of hydrophobic and hydrophilic character.

A different explanation (*4, 27*) for the organization of the code is that it arose through the influence of specific interactions of amino acids and nucleotides before the development of the adaptor RNA. No such chemical affinity has been demonstrated, and such a mechanism cannot adequately explain the fitness of the code to protect amino acid groups in relation to their quantitative importance in proteins.

Sonneborn (*5*) has suggested that protection against lethal mutation may have been the major determinant in the evolution of the code. We suggest, however, that increased accuracy of the systems for gene expression may have been the chief selective influence. The efficiency of cellular processes ultimately depends on the accurate synthesis of enzymes; hence a protective code, apart from its effect on mutation rate, would be advantageous in the same way as a better ribosome or a better sRNA would be. A similar suggestion has been made by Woese (*28*).

Initially, adaptors and the corresponding code were probably nonspecific, recognizing only general aspects of amino acid character. Evolutionary pressures for better enzymes would have selected not only the appropriate amino acid sequences, but also a system for translation that best insured their synthesis. The evolution of the code was thus the development of a larger number of adaptors with greater specificity (*29*) such that related amino acids became associated with closely related adaptors derived from an earlier, less specific one. In this way a genetically protective pattern of coding emerged because of selective pressures for better mechanisms of protein synthesis.

ALFRED L. GOLDBERG
ROBERT E. WITTES
Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts

References and Notes

1. E. Schroedinger, *What Is Life?* (Cambridge Univ. Press, Cambridge, 1944).
2. R. Brimacombe, J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, T. Jaouni, *Proc. Nat. Acad. Sci. U.S.* **54**, 954 (1965); D. Soll, E. Ohtsuka, D. S. Jones, R. Lohrmann, H. Nayatsu, S. Nishimura, H. G. Khorana, *ibid.*, p. 1378 (1965).
3. S. R. Pelc, *Nature* **207**, 597 (1965).
4. C. R. Woese, *Proc. Nat. Acad. Sci. U.S.* **54**, 71 (1965).
5. T. M. Sonneborn, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 377.
6. S. M. Friedman and I. B. Weinstein, *Proc. Nat. Acad. Sci. U.S.* **52**, 988 (1964); J. Davies, W. Gilbert, L. Gorini, *ibid.* **51**, 883 (1965); W. Szer and S. Ochoa, *J. Mol. Biol.* **8**, 823 (1964).
7. See suggestion of A. Rich, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 453.
8. The observed pattern of degeneracy may reflect two distinct mechanisms: (i) lack of complete specificity of the sRNA in reading the third letter, or (ii) the existence of many different codon-specific sRNA molecules. To what extent the two mechanisms apply remains to be seen. Although the two possibilities have different implications with respect to evolution of the code, both have the same genetic effect in stabilizing the phenotype and do not affect our argument.
9. Another protective arrangement of four codons would be of the form *abc, a'bc, abc', a'bc'* and where *a* and *a'* and *b* and *b'* are related by transitions. This pattern would give no protection against transversions, but would afford twice the protection against transitions that the present arrangement does. A code based on this pattern, however, does not exhibit certain correlations characteristic of the present code (see discussion of GC).
10. Abbreviations are G, guanine; C, cytosine; A, adenine; U, uracil; T, thymine; Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Arg, arginine; Asn, asparagine; Gln, glutamine; Gly, glycine; Cys, cystine; Ser, serine; His, histidine; Thr, threonine; Ala, alanine; Pro, proline; Val, valine; Met, methionine; Tyr, tyrosine; Ilu, isoleucine; Leu, leucine; Try, tryptophane; Phe, phenylalanine; Trm, terminating codon (amber and ochre.).
11. D. Krieg, *Progr. Nucleic Acid Res.* **2**, 125 (1963).
12. C. Yanofsky, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 581 (1963).
13. S. Brenner and A. O. W. Stretton, *J. Cell. Comp. Physiol.* **64**, suppl., 43 (1964).
14. W. Kauzmann, in *The Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Ed. (Johns Hopkins Univ. Press, Baltimore, 1954).
15. J. C. Kendrew, *Brookhaven Symp. Biol.* **15**, 216 (1962).
16. M. F. Perutz, J. C. Kendrew, H. C. Watson, *J. Mol. Biol.* **13**, 669 (1965).
17. E. Margoliash and E. L. Smith, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 221.
18. C. Tanford, *J. Amer. Chem. Soc.* **84**, 4240 (1962).
19. A. Orgel and S. Brenner, *J. Mol. Biol.* **3**, 762 (1961).
20. E. Freese, *Proc. Nat. Acad. Sci. U.S.* **45**, 622 (1959).
21. J. B. Stanbury, J. B. Wyngaarden, D. Fredrickson, Eds., *The Metabolic Basis of Inherited Disease* (McGraw-Hill, New York, ed. 2, 1966).
22. The third position is ignored because each codon set contains at least one G or C and one A or U in that position.
23. Since the completion of this manuscript, Martin and Hoyer have reported [*Fed. Proc. Abstr.* **25**, 779 (1966)] the results of agar hybridization with DNA from two different rodents. They have shown that the regions of greatest affinity are those with a high GC content, while the regions of less affinity are lower in GC. These results are consistent with our suggestion that the GC content of codons may play a protective role by increasing genetic stability.
24. E. K. F. Bautz, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 419.
25. N. Sueoka, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 35 (1961).
26. W. M. Fitch, *Proc. Nat. Acad. Sci. U.S.* **52**, 298 (1964).
27. S. R. Pelc and M. G. E. Welton, *Nature* **209**, 868 (1966); M. G. E. Welton and S. R. Pelc, *ibid.*, p. 870.
28. C. R. Woese, *Proc. Nat. Acad. Sci. U.S.* **54**, 1546 (1965).
29. T. H. Jukes, *Amer. Sci.* **53**, 477 (1965).
30. M. Cappechi and J. Adams, personal communication.
31. We thank Dr. J. Beckwith and Professor B. Davis for advice and encouragement. Discussion with Dr. F. Crick has been rewarding. Supported by NSF GB-1307 to B. D. Davis.

15 April 1966

Ozone and Sulfur Dioxide Synergism: Injury to Tobacco Plants

Abstract. *Tobacco plants displayed ozone-type injury when exposed to mixtures of ozone and sulfur dioxide at subthreshold concentrations. The syndrome suggests synergism between ozone and sulfur dioxide that lowers thresholds to injury; exposure to the individual gases at the mixed-gas concentrations caused no symptoms.*

Certain varieties of tobacco are the most sensitive indicators known of the presence of air-polluting ozone, usually displaying symptoms in the field when concentrations exceed 0.05 ppm (parts per million) (*1, 2*). Development of automated methods for measuring ozone, including those based on oxidation of potassium iodide, has facilitated determination of ozone thresholds. However, we have observed and others (*1*) have reported symptoms resembling ozone fleck after indication of concentrations of ozone, in ambient air, as low as 2 parts per 100 million (pphm). Sulfur dioxide causes 100-percent interference with potassium iodide ozone analysis (*3*), although ozone and sulfur dioxide coexist without antagonism in the gaseous phase at concentrations below 1 ppm (*4*). Interference by sulfur dioxide is prevented by placement of a chromium trioxide scrubber at the beginning of the air-sampling train (*3*). Concentrations of sulfur dioxide tend to be higher in late spring and fall than in summer (*5*). We believe that ozone thresholds determined in the field while sulfur dioxide is present are incorrect because of interference by the sulfur dioxide with the measurement of ozone. Furthermore, the apparently subthreshold concentrations of ozone that caused injury suggested synergistic effect by ozone and sulfur dioxide. The suspicion of synergism is based on the disruption of an indicated balance of oxidized and reduced sulfur in plant

tissues (*6*). We now report the results of tests of the suspected synergism between ozone and sulfur dioxide absorbed by tobacco leaves.

Our experiments during the winter used potted tobacco plants grown in the greenhouse under natural conditions. Cigar-wrapper varieties Bel-W3 and Bel-B, ozone-sensitive and ozone-resistant, respectively, and virescent variety Consolation 402 were exposed to doses of ozone and sulfur dioxide, individually and mixed, in a dynamic-flow, walk-in, fumigation chamber equipped with an activated-charcoal filter to remove phytotoxic air pollutants (*7*). Fumigation was basically with mixed gases to study the suspected synergism and with individual gases to provide controls. All tests were made in the same chamber, 3 days being required for completion of the mixed-gas and control exposures for each trial. Four trials were conducted: one each during January, February, March, and April 1966. Under our conditions, tobacco plants are most sensitive to ozone when fumigated at the 6- to 7-leaf stage, during rapid growth, 3 to 4 weeks after seedlings are transplanted into 3-inch (7.5-cm) pots; the span of each trial was limited to this period.

Plants were selected for uniformity, preconditioned in the chamber for 1.5 hours, and fumigated for 2 or 4 hours. The chamber was operated at 800 to 1100 mphot (cool-white fluorescent), 24° to 26°C, and 80- to 100-percent relative humidity. One plant of each variety was removed for observation of the stomatal condition at the beginning and end of the fumigation period, since at these low concentrations stomata must be open to permit absorption of toxicants (*8*). Stomatal measurements were made from silicone impressions (*9*) taken from predetermined areas on the lower and upper surfaces of mature leaves.

Ozone was produced from tank oxygen by high-voltage discharge (*7*), and sulfur dioxide was infiltrated into the stream of carbon-filtered air from a cylinder of 1 percent sulfur dioxide in air. A Mast 724-1 (*10*) automatic-recording ozone meter was used to monitor ozone concentrations during fumigations as near as possible to 3 pphm; the concentration was critically controlled and varied no more than 25 percent from the 3-pphm average. The Mast instrument was equipped with a U-tube chromium trioxide scrubber (*3*) when doses were of mixed ozone and sulfur dioxide or of ozone alone. Sulfur dioxide was introduced into the air stream with a flowmeter at a constant rate of 250 ml/min; concentrations were determined manually by the West-Gaeke method (*11*). Ozone does not interfere with the West-Gaeke method because of the relative insolubility of ozone in the sampling reagent (*4*). The rate of introduction of sulfur dioxide provided a concentration between 15 and 36 pphm as revealed by 5-minute air samples collected from the chamber four times during each fumigation. Preliminary tests with these facilities showed that administration of between 50 and 100 pphm sulfur dioxide for at least 2 hours was required to cause acute injury to tobacco.

Table 1. Injury to tobacco leaves by fumigation with dilute mixtures of ozone and sulfur dioxide; each value is the average for four experiments (total of 18 plants; seven leaves per plant scored).

Fumigation			Leaf damage to varieties (%)					
Duration (hr)	Toxicant (pphm)		Leaves (No.)			Area		
	O_3	SO_2	Bel-W3	Consolation	Bel-B	Bel-W3	Consolation	Bel-B
2	3.0		0	0	0	0	0	0
2		24.0	0	0	0	0	0	0
2	2.7	24.0	38	37	25	15	12	9
4	3.1		0	0	0	0	0	0
4		26.0	0	0	0	0	0	0
4	2.8	28.0	75	76	48	41	43	23

No symptoms appeared after plants were exposed to either gas alone at concentrations used in the combined-gas experiments. Symptoms in the combined-gas experiments (Table 1) closely resembled typical ozone injury as tiny white flecks scattered randomly over the upper surface of mature leaves; the degree of injury was greatest on mature leaves, and total injury increased when time of exposure was extended from 2 to 4 hours. Examination of silicone impressions showed that lower-surface stomata were open at the beginning of fumigations and remained open throughout (Table 2). Removal of the U-tube scrubber from the Mast instrument caused immediate reduction in indicated concentrations to a constant level near 0 pphm, and demonstrated the interference by sulfur dioxide.

Our results offer a tentative explanation of occasional inconsistencies in the recording of ozone thresholds. We believe that sulfur dioxide interference with analysis of ozone has caused concentrations of ozone to be mistakenly interpreted, and that synergism between the two gases in leaf tissues in fact reduces the threshold necessary for injury. Although the chronic and acute effects of sulfur dioxide on plants have been studied for more than 50 years, a subtle threat to crops is posed when concentrations of ozone and sulfur dioxide may concomitantly increase during episodes of air pollution.

Table 2. Stomatal apertures of three varieties before and after fumigation with ozone and sulfur dioxide mixed and singly; average values for four plants and four experiments with each treatment.

Variety	Width of apertures on surface (μ)			
	Before		After	
	Upper	Lower	Upper	Lower
Ozone and sulfur dioxide				
Bel-W3	1.2	3.9	1.1	1.4
Bel-B	1.8	4.2	1.1	1.4
Consolation	1.6	4.6	1.8	2.6
Ozone				
Bel-W3	1.9	4.0	0.8	1.2
Bel-B	1.6	3.6	.8	1.5
Consolation	1.7	4.1	1.1	2.7
Sulfur dioxide				
Bel-W3	1.1	4.0	0.6	2.3
Bel-B	2.0	4.4	.5	1.7
Consolation	3.5	4.8	.6	3.5

H. A. MENSER
H. E. HEGGESTAD
Crops Research Division, Agricultural Research Service, Beltsville, Maryland

References and Notes

1. F. D. H. Macdowall, E. I. Mukammal, A. F. W. Cole, *Can. J. Plant Sci.* **44**, 410 (1964).
2. H. E. Heggestad and H. A. Menser, *Phytopathology* **52**, 735 (1962).
3. B. E. Saltzman and A. F. Wartburg, Jr., *Anal. Chem.* **37**, 779 (1965).
4. H. L. Helwig and G. L. Gordon, *ibid.* **30**, 1810 (1958).
5. I. A. Leone, E. Brennan, R. H. Daines, *Plant Disease Reptr.* **49**, 911 (1965).
6. M. D. Thomas, *WHO Monogr. 46* (Columbia Univ. Press, New York, 1961), pp. 233–78.
7. H. A. Menser and H. E. Heggestad, *Crop. Sci.* **4**, 103 (1964).
8. F. D. H. Macdowall, *Can. J. Plant Sci.* **45**, 1 (1965).
9. I. Zelitch, *Proc. Nat. Acad. Sci. U.S.* **47**, 1423 (1961).
10. Mention of a trade name is for identification and does not imply endorsement by USDA.
11. P. V. West and D. C. Gaeke, *Anal. Chem.* **28**, 1816 (1956).
12. We thank G. H. Hodges for assistance with fumigations.

19 May 1966