With respect to the size distribution of the volcanic dust, Meinel and Meinel (*1*) reported seeing a silvery disk around the sun while the dust veil was still visible in the daytime. I also noted a wide white disk shortly before sunset when the lower troposphere was shielded from sunlight by dense cirrus clouds at the horizon. Both observations strongly suggest an aerosol scattering function characteristic of power law size distributions.

The twilight data presented here indicate that stratospheric aerosol existed over New Mexico and probably on discrete dates over New England by early November 1974, a few days before the strong sky phenomena were noted by Meinel and Meinel (*1*) over Mexico. The activity of Fuego Volcano continued into November, but the stratospheric dust may have originated from one or more of the strong ash eruptions which took place between 13 and 23 October, especially during the night of 17 October. "Ash has been carried at least 200 km to the north and west" (*7*). The dust obviously rose higher than suspected. A glance at the upper air chart for 50 mbar $\approx$ 20 km clearly shows that dust at this level would first have been carried west, probably to Hawaii, where the northernmost patches could have turned north around a small semipermanent high and entered the westerlies. In fact, a high dust cloud was observed at Mauna Loa Observatory starting on 26 October and measured by lidar at 19.5 km (*6*). Owing to probable spread over a large altitude range, the movement of the ash clouds in this period may have been quite complex. Indeed, a west-southwesterly circulation over the United States by early November makes it possible that the sightings mentioned earlier of weak volcanic twilights during that period were caused by patches of Fuego dust.

During 18 months of lidar observations at Mauna Loa Observatory, no clouds were observed above the tropopause prior to 8 October 1974. Since this was 1 week before the Fuego eruptions became violent, Fegley and Ellis (*6*) discuss the possibility that the dust layer, which was weaker and 2 km lower than the Fuego clouds observed later, may have been caused by high-altitude aircraft. However, I also noted stratospheric dust in New Mexico in mid-October 1974. From jet altitude, a few distant haze streaks at an elevation angle of about 10° were seen in the afternoon of 15 October for 30 minutes until descending to El Paso. I also saw during twilight of the same day faint spotty dust clouds which probably were in the stratosphere. In the dusk of the next day while at Sacramento Peak, I noticed a very distant dust seam appearing at the horizon and turning red; its estimated altitude is 18 km. A relation of these phenomena to Fuego dust is very unlikely, but they indicate that the dust cloud observed over Hawaii by early October may not have been a local event.

It is likely that a substantial amount of Fuego dust will remain in the tropical stratosphere, which for some time—as probably with the Agung dust—may act as an aerosol source for higher latitudes, where residence times are known to be shorter. At any rate, traces of the Fuego dust should still be detectable in 1976 or 1977.

Note added in proof: Continued twilight photometry at Puerto Rico, Sacramento Peak, and Lexington showed color ratio amplitudes of about 6 until February. By May, the amplitudes had decreased to 3 at Puerto Rico and to 2 at the mid-latitude stations and thus were still higher than the spring values during the years before the Fuego eruption. Lidar data from Mauna Loa Observatory, Hawaii (*6, 8*), and Virginia (*9*) indicate that the volcanic dust load in this latitude range generally was constant until at least March and April, respectively. Light-scattering calculations based on the lidar dust layer profiles gave good agreement with the twilight observations. Spreading of the dust to lower altitudes since early spring explains the abatement of twilight colors. Fuego twilights were noticed in Europe during the winter and in Alaska during February.

F. E. VOLZ
Air Force Cambridge Research Laboratories, Hanscom Air Force Base, Massachusetts 01731

References and Notes

1. A. B. Meinel and M. P. Meinel, *Science* **188**, 477 (1975).
2. G. T. Symons, Ed., *The Eruption of Krakatoa and Subsequent Phenomena* (Royal Society, London, 1888).
3. F. E. Volz, *Appl. Opt.* **8**, 2505 (1969).
4. M. T. McCormick and W. M. Fuller, Jr., *ibid.* **14**, 4 (1975).
5. F. Fernald, personal communication.
6. R. Fegley and H. Ellis, *Geophys. Res. Lett.* **2**, 139 (1975).
7. Event 134-74, cards 1967 and 1971, Smithsonian Institution, Center for Short-Lived Phenomena, Cambridge, Mass.; R. E. Stoiber, personal communication.
8. R. W. Fegley and H. T. Ellis, *Appl. Opt.*, in press.
9. E. E. Remsberg and G. B. Northam, paper presented at the Fourth Conference on the Climatic Impact Assessment Program, Cambridge, Mass., 4–7 February 1975; M. T. McCormick, personal communication.
10. I thank Dr. A. Green and R. McPeters at the University of Florida at Gainesville; MSgt D. E. McWilliams at the Solar Observatory, Ramey Air Force Base, Puerto Rico; and Dr. R. D. Dunn and L. B. Gilliam at Sacramento Peak for their kindness in operating the logarithmic photometers. Mr. Gilliam also made the sun photometer measurements. I am also grateful to Dr. A. B. Meinel for the early notification on the twilight glows.

27 December 1974; revised 14 March 1975

Amino Acid Composition of Proteins: Selection against the Genetic Code

Abstract. *Distribution of amino acids in 68 representative proteins is compared with their distribution among 61 codons of the genetic code. Average amounts of lysine, aspartic acid, glutamic acid, and alanine are above the levels anticipated from the genetic code, and arginine, serine, leucine, cysteine, proline, and histidine are below such levels. Arginine plus lysine account for 11.0 percent of codons and aspartic acid plus glutamic acid account for 11.3 percent; thus the average charge is roughly neutral.*

Most proteins are complex sequences of amino acids. The few exceptions are proteins that consist of short repetitions, such as the "antifreeze" proteins (*1*) and the silk fibroins. Leaving these special proteins aside, we have been interested in the frequency of occurrence of amino acids in the genetic code as compared with their average levels in proteins (*2*). The code has 61 codons for amino acids. A protein with one amino acid per codon would have the following composition: $Ala_4Arg_6Asn_2Asp_2Cys_2Gln_2Glu_2Gly_4His_2Ile_3Leu_6Lys_2MetPhe_2Pro_4Ser_6Thr_4Tyr_2TrpVal_4$ (*3*). We find that the average distribution of some, but not all, amino acids in proteins differs in important respects from these proportions.

Large numbers of amino acid replacements accumulate in proteins during evolution. This becomes evident when homologous proteins in different species of living organisms are compared. This process results from point mutations in DNA, and we shall assume for purposes of argument that the process should lead to a tendency for codons for amino acids to approach the proportions found in the genetic code (*2*).

The internal milieu of cells is approximately neutral in terms of *p*H. In accordance with this, the average charge of most proteins is approximately neutral; that is, they are zwitterionic with, on the average, roughly equal numbers of positively and negatively charged side chains at physiological *p*H. The genetic code contains twice as many codons for the basic amino acids arginine plus lysine as for the acidic amino acids aspartic plus glutamic acids.

Of course, some proteins, such as cytochrome c, are basic, and others such as ferredoxin are acidic. The basicity of a pro-

tein such as cytochrome c may functionally attract the opposite charges in the acidic protein, cytochrome c oxidase. The net result should be a trend toward charge neutrality.

Most compilations of the amino acid content of proteins list aspartic and glutamic acids in figures which actually represent Asp plus Asn and Glu plus Gln. This does not allow the necessary calculations to be made. We have summarized the composition of 68 completely sequenced proteins containing 12,170 amino acid residues. The compilation included 47 eukaryotic, 17 prokaryotic, and 4 virus proteins. Only one representative of each "family" of proteins, such as the globins, was included. These proteins contained, per 61 residues:

$$Ala_{5.3}\,Arg_{2.6}\,Asn_{3.0}\,Asp_{3.6}\,Cys_{1.3}$$
$$Gln_{2.4}\,Glu_{3.3}\,Gly_{4.8}\,His_{1.4}\,Ile_{3.1}$$
$$Leu_{4.7}\,Lys_{4.1}\,Met_{1.1}\,Phe_{2.3}\,Pro_{2.5}$$
$$Ser_{4.5}\,Thr_{3.7}\,Trp_{0.8}\,Tyr_{2.3}\,Val_{4.2}$$

Statistical analysis of these data forces the rejection of the null hypothesis that the distribution does not deviate from that expected from proportions of codons in the genetic code. Arginine plus lysine is 11.0 percent of the proteins, very close to 11.3 percent for aspartic plus glutamic acids.

If these amino acids were in proportion to their occurrence in the genetic code, there would be 9.8 percent arginine and 3.3 percent each of lysine, aspartic acid, and glutamic acid, an imbalance of 13.1 to 6.6 for basic to acidic amino acids. Clearly, natural selection counteracts the genetic code to neutralize the charge on proteins.

Furthermore, in the total of 11.0 percent for the basic amino acids (as opposed to the "code percentage" of 13.1), the amount of arginine, 4.3 percent, is maintained far below the level in the code, while the amount of lysine, 6.7 percent, is actually increased in proteins above the code level.

The imbalance between the ratio of arginine to lysine, in terms of the code, led to the suggestion that arginine was an evolutionary intruder that replaced ornithine in protein synthesis (*4*). Notwithstanding this imbalance, the sum of arginine plus lysine is almost identical to the sum of aspartic plus glutamic acids, which strengthens the proposal that evolutionary selection maintains charge neutrality. The net result is that the sum of basic plus acidic amino acids, 22.3 percent, is close to their representation in the genetic code, 19.7 percent.

As an explanation for the excess of "basic codons," there is a possibility that primitive forms of life existed at a higher *p*H than that encountered in present environments. Even today, the *p*H of seawater, 8.0 to 8.5, is higher than that of body fluids ($\sim$ 7.4), and the early oceans may have been even more alkaline. The quantitative relations between the charged amino acids in prokaryotes and eukaryotes and their relation to the structure of the genetic code have been described by one of us (*5*).

As regards the other amino acids, alanine is present at significantly "higher-than-code" levels, while histidine, cysteine, proline, serine, and leucine are significantly lower. Perhaps its small side chain makes alanine useful as a "filler" in protein molecules. Histidine, cysteine, and proline have special functions and properties, and this may diminish their use. The needs for serine and leucine may not be so great as to require six codons apiece. The other amino acids fall within expected ranges. To some extent, they may constitute a "pool" that can be augmented by mutations from arginine, cysteine, leucine, serine, proline, and histidine and diminished by mutations to lysine, aspartic acid, glutamic acid, and alanine. Selection would be responsible for maintaining the disparities noted between the frequencies in the code and in proteins, of certain amino acids. On the other hand, genetic drift would play a part in allowing neutral interchanges between, for example, isoleucine and valine, whose occurrences in the code and in proteins are at equal levels. Such a model combines selective and neutral mutations to give a picture of dynamic equilibrium in protein evolution, illustrated by the changes that take place incessantly in homologous proteins.

The distribution of amino acids in 207 proteins for which the analyses were reported by Reeck and Fisher (*6*) is as follows, per 61 residues: $Ala_{5.2}Arg_{2.7}(Asn + Asp)_{6.5}Cys_{1.4}(Gln + Glu)_{6.5}Gly_{4.9}His_{1.3}Ile_{3.0}$ $Leu_{3.9}\,Lys_{3.9}\,Met_{1.1}\,Phe_{2.3}\,Pro_{2.9}\,Ser_{3.8}\,Thr_{3.5}$ $Trp_{0.8}Tyr_{2.0}Val_{4.1}$. This distribution does not list separate values for asparagine, aspartic acid, glutamine, and glutamic acid. Apart from this, and for the values for proline and serine, the distribution is quite similar to our compilation, which, like that of Reeck and Fisher (*6*), includes only proteins containing 50 or more amino acid residues. Holmquist and Moise found (*7*) that the distribution of amino acids in proteins is not dependent on length or species of origin.

THOMAS H. JUKES
RICHARD HOLMQUIST
HERBERT MOISE

Space Sciences Laboratory, University of California, Berkeley 94720

References and Notes

1. R. E. Feeney and R. Hofmann, *Nature (Lond.)* **243**, 357 (1973).
2. J. L. King and T. H. Jukes, *Science* **164**, 788 (1969).
3. Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Trp, tryptophan; and Val, valine.
4. T. H. Jukes, *Biochem. Biophys. Res. Commun.* **53**, 709 (1973).
5. W. R. Holmquist, *J. Mol. Evol.* **4**, 277 (1975).
6. G. R. Reeck and L. Fisher, *Int. J. Protein Res.* **5**, 109 (1973).
7. W. R. Holmquist and H. Moise, *J. Mol. Evol.*, in press.
8. We thank the National Aeronautics and Space Administration for support of our work through grant NGR 05-003-460.

10 February 1975; revised 10 March 1975

Induced Adhesion in *Crassostrea virginica* Larvae

Abstract. *Normal motile veliger larvae of the oyster,* Crassostrea virginica, *were observed swimming in pairs or trios. Adhesion between animals is firm and has a specific orientation. This adhesion can be induced in low frequencies by culturing larvae at high densities, and in much higher frequencies by inclusion of an antibiotic mix in the seawater culture medium.*

The phenomenon of adhesion or aggregation of cells and tissues is an important area of investigation in plant and animal developmental biology. This report describes a chance observation (repeated many times since) of adhesion in larvae of the oyster, *Crassostrea virginica*, that has not been previously described. The information presented below leaves many unanswered questions, but we believe it should be reported now.

Adult oysters were spawned in the laboratory according to the methods described by Loosanoff and Davis (*1*). Fertilized eggs were screened on filters of graded pore-size (75, 54, 36 μm) nylon monofilament bolting cloth (Nitex) to remove large particles; the egg suspension was then washed and concentrated on a 20-μm screen with sterile (autoclaved) seawater. This fertilized egg suspension was counted in a Sedgwick-Rafter cell, aliquoted at 250 eggs per milliliter into covered Pyrex preparation dishes containing 200 ml of sterile seawater, and incubated at 24°C. About 24 hours later, the samples in each dish were washed with sterile seawater on a 36-μm Nitex screen, suspended in sterile seawater, and counted. At this time most of the eggs had developed to the straight-hinge veliger stage. The veligers were aliquoted into sterile Pyrex preparation dishes, containing 100 ml of sterile seawater, at whatever concentrations the experimental procedure