in increased oxygen consumption and increased cloacal temperature. The mean body temperature at the time of placement in the restraining device was 41.1° ± 1.3°C. The mean oxygen consumption of seven restrained birds at an ambient temperature of 22° to 23°C was 6.2 ml of oxygen per gram per hour. These values were higher than those reported for resting house sparrows in the daytime (*9*).

Thermode cooling or heating produced a change in oxygen consumption and cloacal temperature in nine birds but no response in ten others. Figure 1 shows the position of the thermode in the 19 birds studied. Consistent increases in cloacal temperature or oxygen consumption, or both, in response to thermode cooling and consistent decreases in cloacal temperature or oxygen consumption, or both, in response to thermode heating indicate a thermally responsive area. The lack of changes in these parameters indicates a nonresponsive area. An area of nonresponsiveness surrounds an area of positive response to thermode stimulation.

The area of localized thermosensitivity is centered in the preoptic area of the sparrows. Cloacal temperature and oxygen consumption change steadily to a maximum response at approximately 15 minutes after initiation of thermal stimulation. Continued heating or cooling resulted in no further thermoregulatory response. Table 1 summarizes the changes in oxygen consumption and cloacal temperature of responsive birds after approximately 15 minutes of thermode heating or cooling. Although the birds are already somewhat hyperactive, cooling the brain elicits further heat production. All birds returned to their normal state within 15 minutes after the cessation of heating or cooling.

In dogs preoptic thermosensitivity is functionally important to temperature regulation (*10*). The body temperature of dogs is not altered by temperature extremes although the central nervous system is thermosensitive. Birds respond to thermal stress without major changes in body temperature (*8*). Since the preoptic region of house sparrows is thermoresponsive, this region is an important center for thermoregulation. These facts indicate that the thermal control system of birds is similar to that of mammals.

Table 1. Effect of 15 minutes of thermal stimulation of the preoptic area in the house sparrow (*Passer domesticus*) on the oxygen consumption and cloacal temperature at an ambient temperature of 22° to 23°C. For comparison, the mean body temperature of the birds at the beginning of the test was 41°C.

Thermode temperature (°C)	Body temperature		Oxygen consumption	
	Maximum change ± range (°C)	*N*	Percentage of initial metabolism ± range	*N*
18°–21°	+0.9° ± 0.4°	8	115 ± 4	2
36°–37°	+0.5	1	113	1
45°	−0.5	2	88	1

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References and Notes

1. H. T. Hammel, *Annu. Rev. Physiol.* **30**, 641 (1968).
2. ———, S. B. Strømme, K. Myhre, *Science* **165**, 83 (1969).
3. H. T. Hammel, F. T. Caldwell, Jr., R. M. Abrams, *ibid.* **156**, 1260 (1967).
4. E. von Saalfeld, *Z. Vergl. Physiol.* **23**, 727 (1936).
5. W. Rautenberg, *ibid.* **62**, 235 (1969).
6. B. Akerman, B. Andersson, E. Fabricus, L. Svensson, *Acta Physiol. Scand.* **50**, 328 (1960); S. E. Feldman, S. Larsson, M. K. Dimick, S. Lepkovsky, *Amer. J. Physiol.* **191**, 259 (1957); F. T. Rogers and R. W. Lackey, *ibid.* **66**, 453 (1923); M. P. Sinha, in *Avian Physiology*, P. D. Sturkie, Ed. (Comstock, Ithaca, N.Y., ed. 2, 1965), p. 228.
7. B. A. Williams and J. E. Heath, *Amer. J. Physiol.*, in press.
8. J. S. Hart, *Physiol. Zool.* **35**, 224 (1962).
9. J. W. Hudson and S. L. Kimsey, *Amer. Zool.* **4**, 294 (1964).
10. H. T. Hammel, J. D. Hardy, M. M. Fusco, *Amer. J. Physiol.* **198**, 481 (1960).
11. H. J. Karten and W. Hodos, *A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)* (Johns Hopkins Press, Baltimore, 1967), p. 177.
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Darwinian Evolution of Proteins

Recently King and Jukes (*1*) have argued that most evolutionary changes in DNA and proteins are primarily due to neutral mutations and random genetic drift. They imply that "classical evolutionists" have given insufficient thought to the problems of macromolecular change. It therefore seems appropriate for a "classical evolutionist" to point out a number of weaknesses in their argument.

Distribution of amino acid changes. King and Jukes have tabulated the distribution of numbers of amino-acid changes in variants of globins, cytochromes c, and the variable (S-) regions of immunoglobulins. In plotting the number of changes per site against the number of sites having the specified number of changes, they claim that the results follow the Poisson distribution. They regard this apparent correspondence as evidence that most of the substitutions occur at random, but they have obtained it by statistically illegitimate methods. Before their figures will fit the Poisson, they are obliged to remove an arbitrary number of sites from the zero ("invariant") category. Manipulations of this sort would allow them to make the figures fit almost any distribution.

The Poisson distribution is based on the assumption of homogeneous probabilities. Disregarding the invariant sites (already known to be aberrant) it is easy to show that the remainder do not satisfy this assumption. If we consider the distribution of individual amino acids at sites where one substitution has been observed and compare it with the distribution at sites where several substitutions have been observed, we find that the distributions are significantly different. With the use of data given by Dayhoff (*2*) for the hemoglobins, the sites with single substitutions show highly significant relative excesses of valine, leucine, isoleucine, proline, and phenylalanine and relative deficiencies of serine, glycine, asparagine, and glutamine ($P < .001$). This observation shows that King and Jukes's "Poisson" distribution for the hemoglobins is in fact a compound of several different distributions, and it invalidates their conclusions. It also casts doubt upon the calculations of Fitch and Margoliash (*3*), which are also based on the Poisson distribution.

Even if the figures were to fit the Poisson, this would constitute no strong argument in favor of the "neutral" hypothesis. The dangers of reasoning of this kind are well known to population geneticists. The distribution of gene-frequencies in the land snail *Cepaea*, for example, was originally (and legitimately) fitted to a distribution based on the assumption of randomness (*4*), but was later demonstrated to have a strong selective component (*5*).

King and Jukes were unfortunate in choosing the S-regions of immunoglobulins as one of their examples. Many workers believe that the variability of the S-regions is due to a mechanism very different from that involved in normal allelic substitutions (*6*, *7*). No

mention of this possibility is made in their paper.

Fibrinopeptide A. King and Jukes make the following statement: "The relative rapidity of evolutionary change in fibrinopeptide A . . . would seem to imply that its primary structure is not very critical. . . ." This is formally equivalent to the statement "The relative rapidity of evolutionary change to melanism in moths would seem to imply that the coloring of these moths is not very critical." The second statement is not only illogical, it is known to be untrue (*8*).

Scurvy. Primates and guinea pigs are unable to convert 2-keto-L-gulonolactone to ascorbic acid and therefore develop scurvy if they are deprived of vitamin C. King and Jukes regard the acquisition of this characteristic as nonadaptive and, therefore, probably the result of selective neutrality. However, the loss of a metabolic pathway can be selectively advantageous if the pathway uses up a precursor that is also needed for some other, more important, pathway. There is no reason to believe that this loss is necessarily nonadaptive.

Mutations to synonymous codons. At first sight, the most convincing of their suggestions is that synonymous mutations may be neutral with respect to natural selection. It is nevertheless possible to suggest four reasons why they may not.

1) It seems likely that the availability of particular nucleotides may vary in different organisms according to the patterns of their metabolism. In this case particular synonymous codons might be favored by natural selection.

2) Despite "wobble," a particular transfer RNA may have different degrees of attachment to different synonymous codons. Since the tRNAs for one amino acid are known to be heterogeneous (*9*), a balance between the number of various types of synonymous codons and the concentrations of various tRNAs could exercise a strong effect on the rate of synthesis of particular proteins. This would be subject to natural selection. It has also been suggested that differentiation of tissues involves the use of particular synonymous codons (*10*).

3) The order and types of nucleotides in a codon determine the spectrum of single-step changes that can occur at the associated site on the polypeptide chain. In proteins that have a long evolutionary history one would expect natural selection to favor particular synonymous codons at critical sites on the chain. Codons would be favored if they minimized mutational damage. This is an extension of the argument put forward by Sonneborn (*11*).

4) The secondary structure of the messenger RNA may be important, and particular codon sequences may be necessary to regulate it (*12*).

In view of these possibilities it is unwise to assume a priori that synonymous mutations are selectively neutral. This argument applies also to King and Jukes's statements about the Treffers mutator gene in *Escherichia coli.* The *mut T* gene, which tends to increase the guanine-cytosine content of the bacterium, apparently fills the third positions of synonymous codons with C and G. It does not markedly impair the viability of mutated strains in culture. However, we cannot therefore maintain that it would survive equally well in natural situations, where it would come into competition not only with normal strains of *E. coli* but also with other microorganisms. Many *Drosophila* mutants survive perfectly well in pure cultures but are rapidly eliminated from natural populations.

The rates of protein evolution. It is suggested by King and Jukes, and others (*13*), that the rates of amino acid substitution for particular proteins have remained roughly constant over long periods of evolutionary time. This apparent constancy is deduced from the fact that the degree of divergence in amino acid substitutions between two forms is approximately proportional to the estimated time of their common ancestry. The data are somewhat sparse, so that it is difficult to state the limits of error involved in the assumption of constancy. They are certainly very wide, and King and Jukes quote one case (guinea pig insulin) where the assumption clearly does not hold. Recent work has suggested other cases (*14*).

Nevertheless, even if we make the unlikely supposition that the phenomenon is general, a rough constancy of rate is easily explained by a selective theory. If we take the view that every single amino acid substitution is the result (at least in part) of natural selection, the correspondence between the phylogenetic trees based on the sequences of particular proteins and those based on conventional techniques leads us to the conclusion that the substitutions destined to succeed are to a considerable degree determined by the biochemical and genetical environment in which they occur. In other words, there is a great deal of selective interaction between sites and between loci. This interaction will result in the selective value of a substitution being determined as much by the internal (genetical and biochemical) environment as by the external environment.

If this argument is correct, the internal "coadaptation" of the genotype will constitute a force resistant to genetic change, as has been suggested by Mayr (*15*). The resulting "evolutionary inertia" will smooth out disturbances produced by environmental fluctuation and lead to a relative constancy of evolutionary rate. Periodically genetic revolutions will occur and lead to inconsistencies, such as the unusually high rate of evolution in guinea pig insulin. There is a weight of evidence for genetic coadaptation to be found in many recent studies of microevolutionary change (*16, 17*).

Amino acid composition. King and Jukes have tabulated the average amino acid frequencies among 53 vertebrate polypeptides and have compared them with the frequencies to be expected from random permutations of nucleic acid bases. The two sets of figures agree remarkably well (apart from an explicable deficiency of arginine). They interpret this agreement as indicating that the average amino acid composition of proteins reflects, more or less passively, the genetic code. If their interpretation of evolution is correct, the amino acid compositions of *individual* proteins should not differ significantly from compositions based on random permutations of bases. It is easy to show that they do. A "representative" series of globular polypeptides (bovine cytochrome c, bovine ribonuclease, bovine trypsinogen, horse hemoglobin, human Bence-Jones, and papain) show statistically highly significant deviations from expectation ($P < .001$), and these deviations are due to different arrays of amino acids in different proteins. Even more striking are the deviations to be found among structural proteins, which were not considered by King and Jukes. Fibroins contain between 25 and 45 percent of glycine and also of alanine. Collagens are high in proline and hydroxyproline; sericins in serine; keratins in cysteine; and myosins in glutamic acid and glutamine (*18*). Despite these wide variations, the *average* proportions for struc-

tural proteins seem to agree reasonably well with those calculated for random permutations of bases, except for excesses of glycine and alanine (*19*).

These observations are most easily explained if we suppose that the genetic code has evolved in such a way that it provides larger numbers of codons for the more common amino acids, and consequently fewer for the rarer, rather than that the proportions of amino acids are passive consequences of random codon assignments. The degree of order in the code, the extent to which chemically related amino acids are coded by mutationally related codons, lends strength to a belief that the code has evolved in relation to the requirements of protein synthesis (*11, 20*).

Contemporary evidence of the selective importance of amino acid substitutions. If the majority of amino acid substitutions that have occurred in the course of evolution have been neutral or near-neutral in selective value we should expect to find evidence of neutrality (or little evidence of selection) in contemporary protein polymorphisms. However, in all the polymorphisms that have so far been studied in detail, the action of natural selection can be inferred.

The only known hemoglobin polymorphisms in man (hemoglobins A, S, and C) have been shown to have a strong selective element (*21*). Some protein polymorphisms show morph-ratio clines that can be related to climatic factors, others show a constancy of morph frequencies over large areas and indicate strong forces maintaining a balance despite great changes in local environments (*22*). Yet others show significant excesses of heterozygotes (*23*) or seasonal changes large enough to exclude random genetic drift (*24*). None seem to show the patterns expected for neutral substitutions, the fixation or near-fixation of different alleles in different isolated populations without relation to their environmental circumstances.

It might be argued that the selective effects mentioned above are the results, not of the protein polymorphisms themselves, but of other closely linked polymorphic loci in linkage disequilibrium with them. If this is so, then the linkage disequilibriums themselves require explanation. In the absence of selection they would not persist for long evolutionary periods, yet for the only two protein polymorphisms that have been studied from this point of view (α-amylase and larval protein 10 in *Drosophila pseudoobscura*) it has been shown that particular alleles have been associated with particular chromosome inversions for a period longer than the history of the species itself (*25*).

The hypothesis of neutrality can be crucially tested by observing natural and artificial populations, but these observations are not considered by King and Jukes.

Conclusion. Few would dispute that both random genetic drift and natural selection have a part to play in the evolution of proteins, as in the evolution of other aspects of the phenotype. It is nevertheless desirable to estimate their relative importance. King and Jukes argue that random genetic drift has been primarily responsible for the majority of amino acid substitutions, but the weight of evidence does not support them. Protein sequences, like other characters, seem to have evolved under the dominating influence of natral selection.

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References and Notes

1. J. L. King and T. H. Jukes, *Science* **164**, 788 (1969).
2. M. O. Dayhoff, *Atlas of Protein Sequence and Structure 1969* (National Biomedical Research Foundation, Silver Spring, Md., 1969).
3. W. M. Fitch and E. Margoliash, *Biochem. Genet.* **1**, 6S (1967).
4. M. Lamotte, *Bull. Biol. Suppl.* **35**, 1 (1951).
5. A. J. Cain and P. M. Sheppard, *Genetics* **39**, 89 (1954).
6. O. Smithies, *Brookhaven Symp. Biol.* **21**, 243 (1969).
7. G. M. Edelman and J. A. Gally, *ibid.*, p. 328.
8. H. B. D. Kettlewell, *Proc. Roy. Soc. London Ser. B* **145**, 297 (1956).
9. H. G. Zachau, D. Dütting, H. Feldmann, F. Melchers, W. Karau, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 417 (1966).
10. B. L. Strehler, D. D. Hendley, G. P. Hirsch, *Proc. Nat. Acad. Sci. U.S.* **57**, 1751 (1967).
11. T. M. Sonneborn, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 377.
12. I am grateful to Professor P. M. B. Walker for drawing my attention to this possibility.
13. 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.
14. E. Margoliash, W. M. Fitch, R. E. Dickerson, *Brookhaven Symp. Biol.* **21**, 259 (1969).
15. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, Mass., 1963).
16. B. Wallace, *Topics in Population Genetics* (Norton, New York, 1968).
17. B. Clarke, in *Evolution and Environment*, E. T. Drake, Ed. (Yale Univ. Press, New Haven, Conn., 1968), p. 351.
18. S. Seifter and P. M. Gallop, in *The Proteins*, H. Neurath, Ed. (Academic Press, New York, 1966), vol. 4, p. 153.
19. T. H. Day, personal communication.
20. C. J. Epstein, *Nature* **210**, 25 (1966).
21. A. C. Allison, *Cold Spring Harbor Symp. Quant. Biol.* **20**, 239 (1955).
22. S. Prakash, *Proc. Nat. Acad. Sci. U.S.* **62**, 778 (1969).
23. M. C. Kelsey, thesis, University of Edinburgh (1969).
24. R. Semeonoff and F. W. Robertson, *Biochem. Genet.* **1**, 205 (1967).
25. S. Prakash and R. C. Lewontin, *Proc. Nat. Acad. Sci. U.S.* **59**, 398 (1968).
26. I am greatly indebted to the following for fruitful discussions, and for criticizing the manuscript; Mr. T. H. Day, Dr. A. H. Maddy, Prof. J. Maynard Smith, Dr. D. T. Parkin, Prof. A. Robertson, Dr. E. M. Southern, and Prof. P. M. B. Walker. The work was supported by the Science Research Council.

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Lightning and the New-Generation Aircraft

In the article by Finger and McInturff (*1*) several meteorological problems associated with flying supersonic aircraft are discussed. Here I would like to bring to the attention of those authors, and of the public as well, a meteorological problem that was not discussed in their article and that should be closely examined by persons involved in operating the SST's and also the "jumbo" jets. This problem is lightning strikes to aircraft.

It has been estimated that there are approximately 500 lightning strikes per year to commercial jet airplanes operating in the United States alone. Most, if not all, of the lightning strikes are triggered by the aircraft, as was very probably true of the lightning flashes that occurred during the launch of Apollo 12. Because of the larger size of the new-generation aircraft (SST's and jumbos), this lightning hazard will increase. If the new aircraft are permitted to fly under the same meteorological conditions that are considered allowable for present aircraft, the probability of the aircraft's being hit by lightning will be considerably increased.

Apollo 12 was launched through a cloud system that was electrically active, as was indicated by potential gradient meters on the ground, although no lightning activity had been observed in the vicinity. The Apollo 12 lightning incident provides a documented example of a large group of electrically active clouds that may not produce natural lightning and may not be considered thunderclouds by the meteorologist but, nonetheless, have the potential for producing triggered lightning and should be avoided by aircraft.

In most cases (there have been