rials, is that a shortening of the carbon-silicon bond results from the inductive effects. This may also explain their general physiological inertness and also the fact that the living tissue shows no foreign-body reaction toward them. This inertness has increased the use of the silicone materials, in many physical forms, in medicine and medical research (*18*). Indeed, medical-grade silicone compositions range from use in non-irritating catheters and drainage tubes to use in heart valves and the encapsulation of implanted heart-regulating devices. The ready diffusion of oxygen and carbon dioxide through thin films of the silicone rubber, along with their inertness to blood, makes possible their use as membranes in heart-lung machines (*19*).

Summary

The silicones are in most cases relatively complex mixtures rather than pure molecular species. Their structures and chemical properties can be controlled, however, with sufficient reproducibility to give compositions with unique characteristics. The organic groups serve not only as a device for controlling the siloxane structures, but offer an important means of modifying the physical and chemical properties. Studies of the inductive effects in the organosilicon compounds enhance our understanding of the nature of chemical bonds. In each field of application an extensive technology has developed for the adaptation of silicones to special needs. The possibility of new compositions to serve science and technology seems to be unlimited.

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Molecules and Monkeys

Study of primary structure of primate hemoglobin sheds light on organismal and molecular evolution.

John Buettner-Janusch and Robert L. Hill

The era of the molecule, the protein molecule, is upon us. Anthropology, as it attempts to reconstruct the phylogeny of man and his fellow members of the order Primates, must take cognizance of molecules. It is unlikely that significant quantities of proteins will ever be extractable from fossil primates. But we can study the differences in many proteins of the living primates, a group of mammals that exhibit a remarkable degree of evolutionary stratification.

The respiratory protein hemoglobin is an excellent subject for a study of molecular evolution. The protein is easily obtainable in good yield. It has been extensively studied for many years, and much is known about it. The hemoglobin of one primate, *Homo sapiens*, has been intensively studied from genetic, biochemical, and molecular points of view. The formal genetics of many forms of human hemoglobin has been carefully worked out. Normal human hemoglobin A, designated Hb $\alpha_2^A \beta_2^A$, is a tetramer made up of two pairs of polypeptide chains called α and β (Figs. 1 and 2). The synthesis of the two chains is controlled by nonallelic genes. Various abnormal and variant human hemoglobins have been identified. The specific molecular differences among them have been characterized, and population frequencies have been determined for several of them. Thus an excellent model exists for research on hemoglobins of other primates (*1*).

Primate Phylogeny

We can place the living members of the order Primates in eight separate monophyletic taxa at various levels of the Linnaean hierarchy (Table 1). We assign these eight taxa to four infraorders, two superfamilies, and two families. There are a number of ways in which the phylogeny of the Primates may be interpreted in the classification

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(2, 3). The phylogeny and classification we use are derived from Simpson's classic work on mammals and his subsequent discussions of primate systematics (4).

The Tupaiiformes, tree shrews of southeast Asia, are best placed with the Primates (5), even though they have many features which are intermediate between primate and insectivore. Often, in the past, they have been assigned to the Insectivora. The Tupaiiformes are of interest just because they appear to have characteristics intermediate between insectivores and primates.

The Lorisiformes of Asia and Africa and the Lemuriformes, unique to Madagascar, probably are the living representatives of an adaptive radiation that began in the late Paleocene or early Eocene. Though lemurs and lorises are often considered closely related, there are many good reasons for separating them at a fairly high level (6).

The Tarsiiformes are prosimians whose lineage is an ancient one, although one prominent monographer of the Primates wishes to classify them with the higher primates (7). They became a distinct phyletic group in the late Paleocene or very early Eocene.

The Cercopithecoidea are a major adaptive radiation that consists, today, of the monkeys of Asia and Africa. They are assumed to have a phyletic position intermediate between the prosimians and the apes. It is not unlikely that most modern cercopithecines are part of a relatively recent adaptive radiation, one that may just now be coming to an end. Some identifiable Cercopithecoidea are found in deposits of Oligocene age.

The Ceboidea developed in isolation in the New World with many structural and adaptive parallels to the primates of the Old World. Like the Malagasy lemurs, they are an example of the extent to which a primate stock may radiate and differentiate in isolation. They are a kind of evolutionary experiment and a side issue. They appear as a distinct group in Miocene deposits in South America.

The apes of Asia and Africa constitute the Pongidae—*Pan* (gorilla and chimpanzee), *Pongo* (orangutan), and *Hylobates* (gibbon and siamang) (3). Fossils of distinctly pongid aspect have been found in the late Oligocene deposits of Egypt (8).

The Hominidae became a separate phyletic line sometime in the Miocene (9). *Homo*, the single contemporary genus in this family, was fully differentiated in the early Pleistocene, and by that time the structural modification which led to the adaptive radiation of *Homo* was already being perfected.

Living primates constitute, in a crude way, a series of successively more advanced forms (10). They are an *échelle des êtres* in brief, and for this reason they are of great value for students of mammalian evolution. The evolutionary stratification of the living primates is the foundation for our study of hemoglobin. Hemoglobin for the studies reported here was obtained from representatives of the most primitive to representatives of the most advanced members of the order (Table 1). Unfortunately, we have not yet been able to beg, borrow, or steal a sample of *Tarsius* hemoglobin.

It must be noted that there is an implicit assumption here. We assume that *Tupaia glis* has a more primitive hemoglobin that *Lemur fulvus* and that hemoglobin of *Hylobates lar* is more advanced than either. Though the various living primates *represent* Paleocene, Eo-

(αT-1) 5 (αT-2) 10 (αT-3) (αT-4) 20
Val-Leu-Ser-Pro-Ala-Asp-Lys-Thr-Asn-Val-Lys-Ala-Ala-Try-Gly-Lys-Val-Gly-Ala-His-Ala-Gly-

30 (αT-5) 40 (αT-6)
Glu-Tyr-Gly-Ala-Glu-Ala-Leu-Glu-Arg-Met-Phe-Leu-Ser-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-

50 (αT-7) 60 (αT-9)
His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val-Lys-Gly-His-Gly-Lys-Lys-Val-Ala-Asp-Ala-Leu-

70 80
Thr-Asn-Ala-Val-Ala-His-Val-Asp-Asp-Met-Pro-Asn-Ala-Leu-Ser-Ala-Leu-Ser-Asp-Leu-His-Ala-

90 (αT-10) (αT-11) (αT-12) 110
His-Lys-Leu-Arg-Val-Asp-Pro-Val-Asn-Phe-Lys-Leu-Leu-Ser-His-Cys-Leu-Leu-Val-Thr-Leu-Ala-

120 (αT-13) 130
Ala-His-Leu-Pro-Ala-Glu-Phe-Thr-Pro-Ala-Val-His-Ala-Ser-Leu-Asp-Lys-Phe-Leu-Ala-Ser-Val-

(αT-14)
Ser-Thr-Val-Leu-Thr-Ser-Lys-Tyr-Arg

Fig. 1. The α chain of human hemoglobin A. The peptides obtained by digestion with trypsin are numbered αT-1, αT-2, and so on, according to nomenclature proposed by Gerald and Ingram (28).

(βT-1) (βT-2) (βT-3) 20
β chain: Val-His-Leu-Thr-Pro-Glu-Glu-Lys-Ser-Ala-Val- Thr-Ala-Leu-Try-Gly-Lys-Val-Asn-Val-
γ chain: Gly-His-Phe-Thr-Glu-Glu-Asp-Lys-Ala-Thr-Ileu-Thr-Ser-Leu-Try-Gly-Lys-Val-Asn-Val-
Lemur: Thr-Leu-Leu-Ser-Ala-Glu-Glu-Asp-Ala-His-Val- Thr-Ser-Leu-Try-Gly-Lys-Val-Asn-Val-

30 (βT-4) 40
β chain: Asp-Glu-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-Leu-Val-Val- Tyr-Pro-Try-Thr-Gln-Arg-
γ chain: Glu-Asp-Ala-Gly-Gly-Glu-Thr-Leu-Gly-Arg-Leu-Leu-Val-Val- Tyr-Pro-Try-Thr-Gln-Arg-
Lemur: Glu-Lys-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-Leu-Val-Val (Tyr,Pro,Try,Thr,Glu,Arg,

(βT-5) 50 (βT-6)
β chain: Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys-Val-
γ chain: Phe-Phe-Asp-Ser-Phe-Gly-Asn-Leu-Ser-Ser-Ala-Ser-Ala-Ileu- Met-Gly-Asn-Pro-Lys-Val-
Lemur: Phe,Phe,Glu,Ser,Phe,Gly,Asp)(Leu,Ser,Ser,Pro,Ser,Ala,Val, Met,Gly,Asp,Pro,Lys,Val,

(βT-7) (βT-9) 70 80
β chain: Lys-Ala-His-Gly-Lys-Lys-Val-Leu-Gly-Ala-Phe-Ser-Asp-Gly-Leu-Ala-His-Leu-Asp-Asn-
γ chain: Lys-Ala-His-Gly-Lys-Lys-Val-Leu-Thr-Ser-Leu-Gly-Asp-Ala-Ileu-Lys-His-Leu-Asp-Asp-
Lemur: Lys,Ala,His,Gly,Lys,Lys,Val,Leu,Ser,Ala, Phe,Ser, Glu,Gly)(Leu,His, His,Leu,Asp,Asp,

(βT-10) 90 (βT-11) 100
β chain: Leu-Lys-Gly-Thr-Phe-Ala-Thr-Leu-Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-Asp-Pro-
γ chain: Leu-Lys-Gly-Thr-Phe-Ala-Gln-Leu-Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-Asp-Pro-
Lemur: Leu,Lys,Gly, Thr,Phe,Ala, Ala, Leu,Ser,Glu, Leu,His, Cys, Val, Ala, Leu,His,Val,Asp, Pro,

(βT-12) 110 120
β chain: Glu-Asn-Phe-Arg-Leu-Leu-Gly-Asn-Val-Leu-Val-Cys-Val-Leu-Ala-His-His-Phe-Gly-Lys-
γ chain: Glu-Asn-Phe-Lys-Leu-Leu-Gly-Asn-Val-Leu-Val-Thr-Val-Leu-Ala-Ileu-His-Phe-Gly-Lys-
Lemur: Glu,Asp,Phe, Lys, Leu, Leu,Gly,Asp, Ser, Leu,Ser, Asp,Val,Leu,Ala,Asp, His,Phe,Gly,Lys)...

(βT-13) 130 (βT-14) 140
β chain: Glu-Phe-Thr-Pro-Pro-Val-Gln-Ala-Ala-Tyr-Gln-Lys-Val-Val-Ala-Gly-Val-Ala-Asn-Ala-
γ chain: Glu-Phe-Thr-Pro-Glu-Val-Gln-Ala-Ser-Try-Gln-Lys-Met-Val-Thr-Gly-Val-Ala-Ser-Ala-
Lemur: ... Val-Val-Ala-Gly-Val(Ala,Asp)Ala,

(βT-15)
β chain: Leu-Ala-His-Lys-Tyr-His
γ chain: Leu-Ser-Ser-Arg-Tyr-His
Lemur: Leu,Ala,His,Lys,Tyr,His

Fig. 2. The β chain of human hemoglobin A, the γ chain of human hemoglobin F, and the partial sequence of β-like chain of *Lemur fulvus* hemoglobin. The tryptic peptides are indicated for the human β chain.

cene, Oligocene, Miocene, Pliocene, and Pleistocene evolutionary developments, they are contemporary organisms. Each monophyletic lineage is the product of many genetic, adaptive, selective, and mutational events which have occurred since differentiation from the common stock. When we compare lemur to man we are comparing the contemporary products of about 55 million years of evolutionary divergence.

Materials and Methods

Hemoglobins from a number of nonhuman primates have been examined by various electrophoretic methods. Similarities to and differences from human hemoglobin have been reported by several investigators (*11*). We screened red cell hemolysates from a number of individuals of many primate species by the method of vertical starch-gel electrophoresis. A variety of patterns of migration of hemoglobin in such gels resulted (*12*). This screening enabled us to determine the so-called normal hemoglobin of each species. We were also able to demonstrate that hemoglobin polymorphism occurs in certain species (*12*).

We tested hemolysates from many species for the presence of alkali-resistant hemoglobin (*13*). The hemoglobin of all adult prosimian primates proved to have a large proportion of alkali-resistant pigment, as did the hemoglobin of the African elephant and the elephant shrews (Macroscelididae) of East Africa (*14*).

The normal hemoglobin of a species was further characterized by the technique of "fingerprinting" (*15*). Hemoglobin, globin, α chain, or β chain was digested with trypsin, and a "fingerprint" of the resultant digest was made on paper (*16*) or by means of column chromatography (*17*). Globins and α and β chains were prepared by methods previously described (*18*). Peptides were isolated after column chromatography of tryptic digests (*19*). The amino acid compositions of chains and peptides were determined by a method described elsewhere (*20*). Methods for determining NH_2-terminal amino acids and sequences of amino acids have also been described (*21*).

The α, β, and γ chains of Hb $\alpha_2^A \beta_2^A$ and Hb $\alpha_2^A \gamma_2^F$ are used as standards. A tryptic peptide from hemoglobin of a nonhuman primate is considered homologous to a human peptide if it migrates in the same way that the human peptide does on a paper fingerprint, if it is eluted with the same volume of buffer from ion-exchange columns, and if its amino acid composition is identical to that of the human peptide. Specific reactions with spray reagents that detect histidine, tyrosine, tryptophan, and arginine on paper fingerprints provide additional information.

Detailed studies of sequences have been made only for the hemoglobin of *Lemur fulvus*. The sequences of amino acids in other hemoglobins have been deduced, as described elsewhere (*16*). The final story of the evolution of primate hemoglobin must await complete elucidation of sequences of the polypeptide chains of hemoglobin from many species. However, the first chapter may now be written, based on less rigorous, less time-consuming, yet valid, methods.

Table 1. Classification of the order Primates, showing the eight major taxa and the representative genera used for studies on hemoglobin.

Classification	Genus	Common name
Suborder: Prosimii		
Infraorder:		
Tupaiiformes (I)	*Tupaia*	Tree shrew
Tarsiiformes (II)		
Lorisiformes (III)	*Galago*	Bush baby
Lorisiformes (III)	*Perodicticus*	Potto
Lemuriformes (IV)	*Lemur*	Lemur
Lemuriformes (IV)	*Propithecus*	Sifaka
Suborder: Anthropoidea		
Superfamily:		
Ceboidea (V)	*Saimiri*	Squirrel monkey
Ceboidea (V)	*Cacajao*	Uakari
Cercopithecoidea (VI)	*Papio*	Baboon
Cercopithecoidea (VI)	*Cercopithecus*	Guenon
Hominoidea		
Family:		
Pongidae (VII)	*Pongo*	Orangutan
Pongidae (VII)	*Hylobates*	Gibbon
Hominidae (VIII)	*Homo*	Man

Table 2. Differences (in terms of amino acid replacements) between the α chains of nonhuman primate hemoglobin and of human hemoglobin.

Primate	Number of peptides examined	Number of amino acids	Probable minimum number of replacements
Lemur fulvus	10	101	6
Propithecus	4	21	4
Lemur variegatus	4	24	3
Lemur catta	2	33	0
Galago	2	33	1
Perodicticus	3	37	0
Hylobates	5	53	0

Alpha- and Beta-Chain Evolution

The synthesis of the α and β chains of Hb $\alpha_2^A \beta_2^A$ are controlled by nonallelic genes. We assume that the homologous chains of hemoglobin of the other primates are similarly controlled. The α chains of primates seem to have evolved rather little, if we consider the small number of amino acid replacements found when these chains are compared with human α chains. The number of differences between the human α chain and α chains of various primates are listed in Table 2. The conservatism or the relative stability of the α chains is striking by comparison with the variability exhibited by the β-like chains of nonhuman primates (Table 3). The α chains may have constraints placed on them. If a functioning hemoglobin is to be synthesized, then one of the two chains may have to remain stable. To put this another way, the β-like chain may be able to form functional hemoglobin in a variety of forms, but the α chain cannot do so. Our data suggest that the number of effective mutations in the β (or β-like) locus limits the number of effective mutations in the α locus. An effective mutation is one that has persisted and become the only form of the gene at the locus in question in a population of animals.

The α chain of Hb $\alpha_2^A \beta^A$ must be able to form functional hemoglobin with β, γ, and δ chains. Now we also know, from the hemoglobins of nonhuman primates, that there are many other β-like or non-α chains synthesized. These must form functional proteins with the α-like chains.

The variability in the primate β-like chains is much more extensive than that of the α chains. We compared the probable sequences of non-α chains of various nonhuman primates with the β and γ chains of Hb $\alpha_2^A \beta_2^A$ and Hb $\alpha_2^A \gamma_2^F$ (Table 3). The comparison with γ chains was suggested by our earlier observation that hemoglobin from adult prosimian primates is resistant to alkaline denaturation (*13*), as is the fetal hemoglobin of man, Hb $\alpha_2^A \gamma_2^F$. Comparison of the total numbers of replacements is worth while, but evaluation of the significance of similarities and differences will have to wait until sequences of amino acids in various primate hemoglobins have been determined.

Partial sequences of β-like chains in hemoglobin of *Lemur fulvus* have been

determined (*21*), and we can compare these sequences with homologous sequences of human β and γ chains. We find a high degree of homology among the three chains. The positions in the sequence at which β and γ chains differ from each other are also most frequently the positions at which the *L. fulvus* β-like chain differs from one or the other (Fig. 2).

Invariant Sequences

Large segments of primate hemoglobin molecules appear to be invariant. Sequences which appear to be invariant are listed in Tables 4 and 5. These apparently immutable segments of the molecule suggest that replacements here disrupt the synthesis or function of hemoglobin. The obvious conclusion, to which many workers have jumped, is that the invariant segments are the functionally important part of the molecule. The segments in which effective mutations have occurred are sometimes believed to be less important functionally simply because of the mutability exhibited. However, an effective mutation, wherever it occurs, is preserved only because it plays some functional role in the life of an organism. Mutations in the invariant segments were not preserved by natural selection. But whether this is due to the disrupting character of *any* mutation in these segments is yet to be demonstrated.

The numerous identities in the primary structures of primate hemoglobins are every bit as important to an understanding of molecular evolution as are the differences. Since millions of mutations undoubtedly have occurred in the millions of animals that have made up any one of the evolutionary lineages we are studying, it is remarkable how few mutations have been passed by the censor of natural selection. The invariant sequences of hemoglobin are not the only such sequences that are known to exist in proteins of animals of various evolutionary grades. Cytochrome *c*, ACTH, and insulin are well-known examples (*22*). As we have pointed out, natural selection is the process by which mutations become effective in populations.

Resolution of primate hemoglobins by electrophoresis on starch gels revealed that several of the species which we are studying are apparently polymorphic with respect to hemoglobin. Several of these polymorphisms are being investigated in detail, particularly those found among gibbons and orangutans. Earlier work showed that one of the orangutan hemoglobins is apparently quite similar to the Norfolk type of human hemoglobin (*23*); the other seems very similar to human Hb $\alpha_2^A \beta_2^A$. No obvious differences were found among fingerprints of the two gibbon hemoglobins which proved to have different electrophoretic mobilities in starch gels.

Rates of Evolution

The calculation of evolutionary rates is a favorite pastime of evolutionary biologists, and we, too, can indulge ourselves. We shall use as data the number of replacements in the α- and β-like chains of *Lemur fulvus*. The standards are the α, β, and γ chains of Hb $\alpha_2^A \beta_2^A$ and Hb $\alpha_2^A \gamma_2^F$. Lemuriformes were a distinct evolutionary lineage sometime in the Eocene, therefore the maximum time which separates lemurs from men, with reference to their common ancestor, is about 55×10^6 years. When the α chain of *L. fulvus* is compared with the α chain of man, six replacements are found. Thus we obtain an average value of 9.1×10^6 years for the time it takes to fix an effective mutation in the α chain of the normal hemoglobin of a population of lemurs. The results are very different if we base our calculations on the non-α chains. Comparison of the β-like chain of *L. fulvus* with the human β chain shows differences at 23 positions. This gives us 2.4×10^6 years for the average time it takes to fix an effective mutation in the β-like chain of a population of *L. fulvus*. Another rate is obtained if the

Table 3. Differences (in terms of amino acid replacements) between β-like chains of nonhuman primate hemoglobin and β chain or γ chain of human hemoglobin A or F.

Primate	Number of peptides examined	Number of amino acids	Probable minimum number of replacements	
			Relative to β chain	Relative to γ chain
Lemur fulvus	12	134	23	36
Lemur variegatus	11	96	23	25
Galago	10	87	9	21
Perodicticus	5	49	8	9
Propithecus	3	30	4	10
Papio	6	64	3	18
Hylobates	7	65	0	18
Homo (γ chain)	15	146	39	0
Homo (β chain)	15	146	0	39

Table 4. Tryptic peptide sequences which are identical in α chains of primate hemoglobins.

Peptide*	Primates in which sequences are probably identical
αT-1	*Homo, Hylobates, Perodicticus, Lemur fulvus, L. variegatus*
αT-2	*Homo, Hylobates, Perodicticus, Lemur fulvus, L. catta*
αT-5	*Homo, Lemur fulvus, Propithecus*
αT-6	*Homo, Hylobates, Lemur fulvus*
αT-7	*Homo, Hylobates*
αT-9	*Homo, Hylobates, Perodicticus, Lemur catta, Galago*

* Peptides obtained by digestion with trypsin are numbered according to the nomenclature proposed by Gerald and Ingram (*28*).

Table 5. Tryptic peptide sequences which are identical in β chains of primate hemoglobins.

Peptide*	Primates in which sequences are probably identical
βT-1	*Homo, Hylobates*
βT-2	*Homo, Hylobates*
βT-3	*Homo, Hylobates, Papio, Perodicticus*
βT-4	*Homo, Homo* γT-4, *Hylobates, Papio, Perodicticus, Galago, Propithecus, Lemur fulvus, L. variegatus, L. catta*
βT-5	*Homo, Hylobates*
βT-6	*Homo, Homo* γT-6, *Hylobates, Papio, Perodicticus, Galago, Propithecus, Lemur fulvus, L. variegatus, L. catta*
βT-7	*Homo, Homo* γT-7, *Hylobates, Papio, Galago, Propithecus, Lemur fulvus, L. variegatus, L. catta*
βT-14	*Homo, Galago, Lemur fulvus*
βT-15	*Homo, Homo* γT-15, *Galago, Lemur fulvus, L. variegatus*

* Peptides obtained by digestion with trypsin are numbered according to the nomenclature proposed by Gerald and Ingram (*28*).

γ chain of Hb $\alpha_2^A \gamma_2^F$ is used as the referent. The β-like chain of *L. fulvus* differs from the human γ chain at 36 positions. If the γ chain represents the common ancestor, the average value for the number of years it takes to fix an effective mutation is 1.5×10^6. Therefore, if we assume that the α- and β-like chains of *L. fulvus* and, respectively, human α and human β or γ chains have a common ancestor, it is clear that the rate of point mutation is neither constant nor linear, at least for primate hemoglobins.

These calculations suggest that the one genetic locus evolves at a rate four times that at which the other evolves. It is reasonable to suggest that the two rates are not independent, for the synthesis of a functional hemoglobin molecule requires that the product of each locus, an α chain and a β chain, polymerize successfully. The calculations indicate that rates of evolution estimated from the number of changes in amino acid residues in the primary structure of a protein are meaningless. The rate at which hemoglobin changes —that is, the rate at which amino acid residues are replaced—is a function of the pressure of natural selection as well as of the rate at which random changes (mutations) in the genetic material occur. We do not have, at this time, sufficient information to put rates such as we have just calculated into a meaningful context.

Meaning of Amino Acid Substitutions

The demonstrated amino acid substitutions present us with a fascinating research problem. Just what are the advantages conferred on the animal by substituting, for example, threonine in lemurs for valine in humans as the NH_2-terminus of the β-like chain (*21*)? The suggestion that there are many neutral changes may make some sense chemically. The chemical role of some amino acids in polypeptide chains is probably not fully understood. Many of the amino acids are replaced by residues which seem to be their functional equivalents. A residue with a hydrophobic side chain, for example, may be replaced by another with a hydrophobic side chain, or an acidic residue may be replaced by another acidic residue, with no detectable change in function or activity of the protein. But whether these are chemically equivalent is really not the point at issue. The issue is, are they biologically equivalent or neutral? If they are, then we have indeed neutral traits and neutral genes—neutral from the standpoint of natural selection, that is. But if such a replacement is neutral, how has it become characteristic of the hemoglobin of the species?

The only mechanism by which a random mutation—and the original mutations are still assumed to be random—may become an effective mutation is through the action of natural selection. The animals that possess the mutated locus must have a reproductive advantage over the animals in the population that do not have the mutated locus. Eventually, if this advantage exists, the mutated locus will become the more frequent in a population. The only way a selectively neutral trait might become fixed would be through accidents of sampling from one generation to the next. This process is sometimes called genetic drift. Since there are relatively large numbers of effective mutations involved in the β-like chain alone, the probability of the repetition of fortuitous sampling accidents becomes incredibly small, and we must reject this hypothesis.

Measurement of Phylogenetic Distance

The finding of only a few differences in the amino acids of the hemoglobins of two primates should not be welcomed with too much enthusiasm by taxonomists. The fact that hemoglobin of gorilla (*Pan gorilla*) and human hemoglobin differ by only one amino acid residue does not change the systematics of the Primates (*24*). The gorilla still belongs to the family Pongidae, and man, to the family Hominidae. Hominidae is a taxon that is defined by its adaptive relationships and ecological situation, as reflected in the total morphology of its members. It is clear to us that phylogenetic distance is a concept that is confusingly applied.

The phylogenetic distance between two taxa is a function of two sets of events. First, phyletic branching is the basic event. When two taxa—say, the Pongidae and the Hominidae—have become distinct from each other, we say they have branched phyletically from a common stem. Demonstration of such phyletic branching appears in the fossil record. Second, all the genetic, adaptive, biological, and evolutionary events that occurred after branching are also part of whatever it is we mean by phylogenetic distance (*25*). Unfortunately, time is not a particularly useful parameter in measuring phylogenetic relationships. The Hominidae and the Pongidae became distinct from each other relatively recently, from the standpoint of evolutionary time spans. But the adaptive events that occurred in the hominid evolutionary lineage have been so many and of such a distinctive character that the phylogenetic distance between the living Hominidae and the living Pongidae is much greater than some might expect solely on the basis of the time that has elapsed since the two lineages diverged. Similarity in the primary structure of the hemoglobin of a species from each of the two lineages is not particularly significant as a means of distinguishing the lineages. It is, nonetheless, a most important datum in understanding the evolution of hemoglobin, and using it primarily for purposes of classification obscures this importance.

Phylogeny and Hemoglobins

The similarities and differences we have found among primate hemoglobins correspond in large part to the phylogeny which we presented earlier. The hemoglobins of members of the two suborders, the Anthropoidea and the Prosimii, differ from each other more than the hemoglobins of primates within each of these groups differ from each other. The evidence suggests that the hemoglobins of all the Anthropoidea are quite similar, including those of *Homo sapiens*. Hemoglobins of the Prosimii appear to vary among themselves far more than do hemoglobins of the Anthropoidea.

Hemoglobin of the baboon, *Papio,* is an exception. Clearly, this anthropoid hemoglobin differs from human hemoglobin a great deal more than does the hemoglobin of any other of the Anthropoidea examined to date. Peptide fingerprint patterns for *Papio* alone among the Anthropoidea show as many differences from human hemoglobin as patterns for some prosimians do (Fig. 3).

It appears from the fingerprint patterns that the hemoglobin of *Tupaia glis* differs considerably from human hemoglobin. It differs more than the hemoglobin of most of the other nonhuman primates studied does, but probably not much more than that of some of the Lemuriformes. Fingerprints of

Tupaia hemoglobin also differ greatly from the hemoglobin peptide patterns of certain Insectivora—*Rhynchocyon* and *Petrodromus*, elephant shrews of East Africa.

Hemoglobins of various Lemuriformes are more like each other than they are like human hemoglobin or hemoglobins of most other primates. There are many more similarities between the hemoglobins of Lorisiformes and Lemuriformes than there are between the hemoglobins of either of these Prosimii and hemoglobins of the other primates. These resemblances and differences are suggested by studies of amino acid composition, by end-group analysis, and by results of grosser methods, such as starch-gel electrophoresis and peptide mapping.

Hemoglobins of Ceboidea appear to resemble human hemoglobin rather closely. In this respect the Ceboidea are most interesting, for they are not

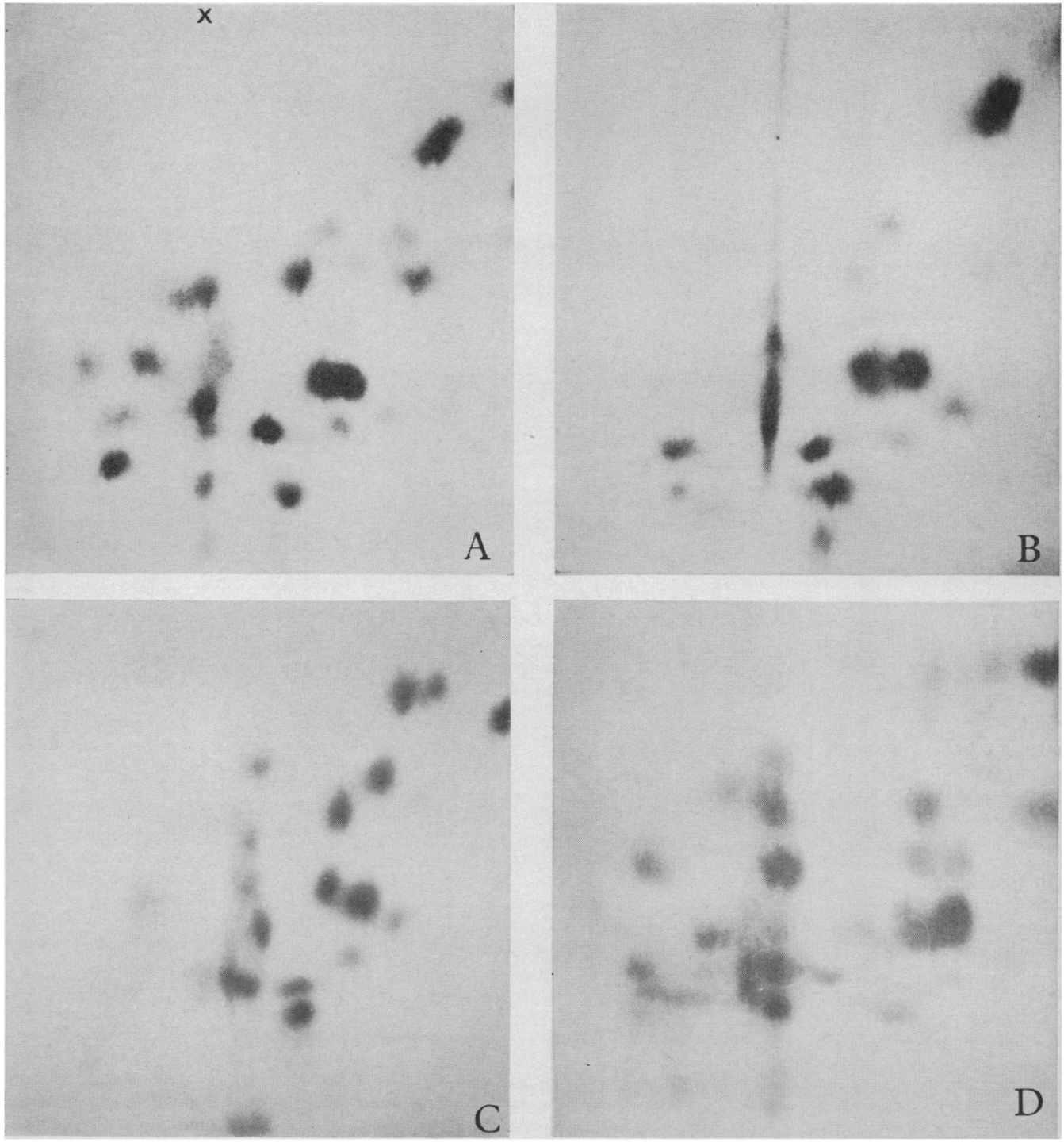

Fig. 3. Paper peptide patterns of (*A*) *Homo*, (*B*) *Hylobates*, (*C*) *Papio*, and (*D*) *Tupaia*. The tryptic digest was applied at top center (X). Electrophoresis was performed horizontally, with the anode (+) to the left. Chromatography was performed in the vertical direction.

closely related to man. They appear as a completely distinct lineage in Miocene deposits of South America. Their dentition separates them from all other living primates. They may be related to an Eocene fossil group, the Omomyidae, which is found in many parts of the world but not in South America. The data from peptide patterns and starch gels suggest that the Ceboidea have developed a hemoglobin quite similar to that of *Homo sapiens*.

We have relatively few data about hemoglobins of the Cercopithecoidea. Those we have suggest that—with the notable exception of *Papio*—these hemoglobins are quite similar to those of man.

The pongid hemoglobins are very much like those of man. The information available suggests that many pongid hemoglobins, if the sources were not known, might be mistaken for one of the many variant human hemoglobins.

Implications for Systematics

The data we have presented are best viewed from within a valid phylogeny of the Primates. The fact that this phylogeny (Table 1) is neither complete nor unchangeable does not discourage us from using it. We must be careful not to tamper with the classification on the basis of a single trait, such as hemoglobin. We keep our hands clean and our hearts pure and rigorously adhere to this particular phylogeny of the Primates. We have not suggested, or even dared to think of suggesting, that it be revised because primate hemoglobin molecules are difficult to interpret in the light of it. Any revision in the phylogeny of the Primates, and in the classification based on it, that has been suggested by one of us in the past has not been based upon hemoglobin research, nor will we suggest any revision on this basis in future publications. We promise not to become taxonomically unhinged if the hemoglobin of *Tarsius spectrum* eventually proves to be identical in structure with elephant hemoglobin. Nor would we suggest that there is a closer phylogenetic affinity between tarsiers and elephants than there is between tarsiers and man. The phylogeny upon which we base our work illuminates the evolutionary differentiation of hemoglobins among primates. The hemoglobin data, however, at this stage are not crucial for reorganizing phylogeny.

The molecular approach to systematics has been enthusiastically embraced by many investigators (*26*). This wave of enthusiasm has aroused considerable reaction from the evolutionists who use the paleontological and organismal approach to systematics (*27*). The exact determination of the differences in the primary structure of a protein from two organisms brings us closer to the genetic basis for similarities and differences than does the analysis of most morphological characters. We are, potentially, better able to characterize the genome of an organism, and of the population to which it belongs, than ever before. Eventually we should be able to study the specific biological effects of molecular changes such as we have described.

Organismal biologists are used to dealing with the effect of natural selection on a complex phenotype, the expression of most if not all of the genome of a population. They offer a counterargument—that the farther from the genes we go, the closer we get to the actual site of the action of selection (*27*). Thus, by implication, they reject the usefulness of the molecular approach to an understanding of the interaction between genome and environment.

The way in which selection acts upon a population is complex and often difficult to analyze in specific organismal and morphological terms, let alone molecular terms. Contemporary evolutionary theory views selection as a process that acts upon populations of organisms. This does not rule out, we believe, the possibility of viewing selection as operating within the nucleus of the cell as well. The population of organisms must be considered when we wish to explain the presence or absence of a genetic trait, even a single amino acid substitution in a hemoglobin polypeptide. We can view selection at a molecular level and ask how the changed molecule reacts to increase the chances for survival of the organisms in which it appears.

We know that it is the population of organisms which evolves, but do not molecules, metabolic pathways, tissues, and organ systems evolve as well? Certainly such systems evolve, though we should refer to their evolution in an organismal framework. When we study the hemoglobins of the Primates we are studying a molecular system within the context of a group of organisms which exhibit a unique degree of evolutionary stratification. Thus, we believe, we have an unbeatable combination for the study of *molecular* as well as *organismal* evolution.

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