Evolution of Hemoglobin in Primates[1]

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INTRODUCTION

The invariant replication of the genetic material (DNA) and the invariant translation of the genetic information into protein structure are conceived as the molecular basis for the maintenance of a species and its distinctness from other species. The extinction of a species or the transformation of a species into one or more descendant species may be thought of as the result of specific chemical changes in the genetic material. But specific chemical changes, mutations, in the genetic material do not produce a species transformation, let alone a generic, familial, or subordinal one, until sufficient changes have accumulated within a population so that it is genetically isolated from all others. Selection on the *population* of organisms within which these mutational alterations occur preserves some and eliminates others. And this is a continuous process (25, 35, 36). In this paper we discuss the interplay between molecular and organismal aspects of these evolutionary events through study of the hemoglobin of man and his primate relatives.

Organisms, populations of them, evolve. And molecules evolve with them. The evolution of proteins, as we have spoken of it in the past, must be understood in an organismal framework, in our case that of the Primates. Paleontologists insist that we are playing with metaphors when we speak of molecules evolving and that we are constructing taxonomies of molecules, not phylogenies of organisms. Such discussions have a way of becoming academic arguments over semantic niceties (33, 36).

The correct phylogeny of the Order Primates is, for an egocentric species such as our own, a matter of overwhelming fascination. It is unfortunate that investigation of such an inherently interesting problem has not yet produced an acceptable, systematic classification of the Primates based on phylogeny (3, 32). A relatively sound and generally acceptable phylogeny of the higher categories—infraorders and super-

[1] The work reported here was supported in part by research grants from the National Institutes of Health, U.S. Public Health Service.

[2] Research Career Development awardee of the U.S. Public Health Service.

families—exists. A systematic account of the species and genera, extant and extinct, is still not available.

There are two major methodological approaches to the problem of the phylogeny of the Primates: paleontological, the study of fossils, and neontological, the comparative study of living forms. It is unwise to immerse oneself in one method without cognizance of the other (29). One paleontologist says, "Phylogeny of the Primates based on analysis of modern forms alone is at best metaphorical and at worse irrelevant" (30). The neontological approach has been devoted, primarily, to comparative osteology and anatomy. There have been attempts at phylogenetic investigation of other traits of the living forms, but, until recently, the results have not been well integrated with other aspects of primate neontology.

If we are to study the phylogeny of molecules, an obvious question must be asked. Do molecules evolve? The obvious answer is yes, for the organisms chosen have evolved. But some molecules are not good subjects for phylogenetic studies, for they vary little among organisms. Before we can pick a protein with which to study evolution, we must have some inkling that it varies in structure among living organisms which became phylogenetically distinct at succeeding time periods.

Hemoglobin has been modified structurally in the course of evolution. The evidence for this modification has been summarized concisely by Ingram (22). The hemoglobins of the Primates were chosen for study for several reasons. The hemoglobin of one of the Primates, *Homo sapiens*, has been the subject of extensive research (26, 27). The body of data available provides an excellent perspective on the differentiation and the variation of the hemoglobin molecule within a single species. We have shown that the normal hemoglobins of various species of Primates are not identical (2, 4-6, 16, 17). Hemoglobin is an ideal protein for studies of molecular evolution. The living primates are one of the orders of Mammalia that promise most as a subject for the study of organismal evolution. The study of the hemoglobins of the Primates should be an unbeatable combination for evolutionary studies. We have been investigating the differences that exist in the primary structures of the hemoglobins among the Primates. We have, thus, been able to *begin* a molecular study of primate evolution and an organismal study of hemoglobin evolution (2, 5, 16, 17).

Primate Phylogeny

Before we discuss the hemoglobins of primates in some detail, we believe a few words about the molecular approach to phylogeny are

needed. This approach has been pursued with enthusiasm by many (11, 14, 15, 17, 19, 38, 39). Such enthusiasm has not always been moderated by a discriminating appreciation of modern evolutionary theory. Torn out of context, the logical and substantive statements may appear reckless and absurd, though within the specific context of comparisons among molecules they are not. Nevertheless, the pitfalls into which one may tumble are demonstrated when the protein is assumed to be definitive in determining phylogenetic relationships and taxonomic issues. For example, the close similarity in the structure of the hemoglobins of man and gorilla (38, 40) is an important datum; it is simply not a datum that is conclusive in systematics.

The immunochemical similarities between the serum proteins of the African apes and man have been used as an argument to remove the great apes from the Pongidae and to place them in the Hominidae (14, 15). But Hominidae is a taxon whose distinctions from Pongidae are determined by the total adaptive complexes and relationships of each. This total adaptive relationship may be determined by serum proteins, as well as by more classical morphological and ecological analyses, but serum proteins are not the sufficient or the necessary criteria for distinction.

Accusations of circular reasoning are sometimes difficult to refute. If phylogeny is inferred from molecular data, and molecular evolution from the phylogeny, then circular reasoning is evident (36). Some investigators choose protein molecules for study on the basis of an accepted phylogeny of the animals from which the proteins are taken. When interpretation of their data agrees with the phylogeny, then added weight is given the molecular approach. When the phylogeny does not conform to the interpretation of the molecular data, the phylogeny is revised. Since these are phylogenies of organisms, not molecules, the validity of such reasoning is suspect. It *is* possible to construct taxonomies of molecules and even phylogenies of molecules, but they have *no phylogenetic significance* apart from the phylogenies of the organisms from which they were taken.

The particular primate phylogeny that is the basis of our work is presented in Table I. If we consider only the living members, the Order Primates consists of eight taxa, monophyletic in origin, which are assigned to various levels of the Linnaean hierarchy. There are various ways in which the phylogeny of the Primates is interpreted (3, 18, 33). The one used here is generally accepted, and it is derived from Simpson's now classic work on mammalian classification (32).

The Tupaiiformes, the tree shrews of southeast Asia, are living representatives of an ancient mammalian stock, which, if it were alive today, would be placed with the Insectivora. But the living Tupaiiformes,

as LeGros Clark showed, are best placed with the Primates (7, 8). They *represent* the first major adaptive radiation of the Primates which occurred in the early Paleocene. They are often considered a kind of intermediate group between Insectivora and Primates.

The Lorisiformes and Lemuriformes probably represent the next major adaptive radiation which occurred in the late Paleocene or early Eocene. These two groups are the galagos (bush babies), lorises, and pottos of Africa and Asia and the lemurs of Madagascar.

The Tarsiiformes are clearly prosimians, though one prominent monographer of the Primates wishes to place them in the same major group as the monkeys, apes, and man (18). They differentiated in late Paleocene or early Eocene times.

TABLE I

CLASSIFICATION OF THE PRIMATES SHOWING THE EIGHT MAJOR TAXA

Order:	Primates
Suborder:	Prosimii
Infraorder:	Tupaiiformes (I) Tarsiiformes (II) Lorisiformes (III) Lemuriformes (IV)
Suborder:	Anthropoidea
Superfamily:	Ceboidea (V) Cercopithecoidea (VI) Hominoidea
Family:	Pongidae (VII) Hominidae (VIII)

The Cercopithecoidea are representatives of another major adaptive radiation. Though they are usually assumed to be intermediate between prosimians and anthropoids, it is not unlikely that most modern cercopithecines are part of a relatively recent adaptive radiation. Some Cercopithecoidea had differentiated in the Oligocene.

The Pongidae are the apes—*Pan* (gorilla and chimpanzee), *Pongo* (orangutan), and *Hylobates* (gibbon) (34). This group was distinct by the late Oligocene.

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

The Hominidae, with a single living member—*Homo*—became a distinct evolutionary lineage sometime in the Miocene (31). The adaptive radiation, based upon erect posture and bipedal locomotion, was fully underway in the early Pleistocene when the genus *Homo* became distinct.

The living primates have long been recognized as constituting a series of successively more advanced forms (20). They have been called a living family tree in miniature. Therein lies their value for students of mammalian evolution. As LeGros Clark stated (9), ". . . the trees of African and Asiatic forests still retain . . . a stratified population of Primates which represents the successive grades of the evolutionary tree of this order." This evolutionary stratification of the living members of our own mammalian order, based principally upon interpretations of comparative anatomy and fossil records, is the foundation for our study of hemoglobin. We selected hemoglobin from those members of the Order which represent stages in development from the most primitive—tree shrews, lemurs—to the more advanced—baboons, apes—to the most advanced form—man.

But first a word of caution. Implicit in our work is the assumption that the hemoglobin of *Tupaia glis* is a more primitive hemoglobin than that of *Lemur fulvus,* and that of *Lemur fulvus* more primitive than that of *Hylobates lar.* Tree shrews, galagos, pottos, tarsiers, lemurs, monkeys, apes, and men may be taken to represent Paleocene, Eocene, Oligocene, Miocene, Pliocene, and Pleistocene developments within the Order (Table II). But we are not investigating Paleocene, Eocene, Oligocene, Miocene, Pliocene, or Pleistocene hemoglobins. We are investigating hemoglobins of living primates who themselves are the products of change and development since their differentiation during various epochs from a common stock or population.

Hemoglobins and Evolution

Human hemoglobin is the base line against which the other hemoglobins are compared. The α, β, and γ chains of *Homo sapiens* are the referents when we speak of substitutions and relative similarities and differences (23, 24, 28). Amino acid sequences of primate peptides presented here were deduced from comparisons with sequences of homologous peptides from human hemoglobins. The methods used for these studies have been described elsewhere (2, 5, 6, 16, 17, 37).

There are some trends or similarities throughout the Order Primates which are significant. The α chain, or α-like chain, seems to be relatively constant throughout the Order. Few replacements have been found when the α chain of human hemoglobin is compared with the α chains

of primate hemoglobins (Table III). Considerable additional data from peptide fingerprint patterns support this finding (17). There is one apparent exception; the baboon, *Papio,* has an α-like chain that is somewhat different from that of other primates.

The non-α or β-like chains of primate hemoglobins are quite variable when compared with the β chain or the γ chain of human hemoglobin (Tables IV and V). The sequence of most of the non-α chain of *Lemur fulvus* has been demonstrated. When we compare this sequence with the

TABLE II

PRIMATES USED IN CURRENT STUDIES OF HEMOGLOBIN[a]

		Genera	
Epoch	Taxon	Genus	Common name
Pleistocene	Hominidae	*Homo*	Man
Pliocene	Hominidae	—	
Miocene	Ceboidea	*Saimiri*	Squirrel monkey
		Cacajao	Uakari
Oligocene	Pongidae	*Pongo*	Orangutan
		Hylobates	Gibbon
	Cercopithecoidea	*Papio*	Baboon
		Cercopithecus	Guenon
Eocene	Lemuriformes	*Lemur*	Lemur
		Propithecus	Sifaka
	Lorisiformes	*Galago*	Bush baby
		Perodicticus	Potto
	Tarsiiformes	—	
Paleocene	Tupaiiformes	*Tupaia*	Tree shrew

[a] The genera chosen are representatives of most of the major taxa of the Order Primates. They are also representatives of the major geological time periods in which each major taxon differentiated.

TABLE III

AMINO ACID REPLACEMENTS—COMPARISON OF α CHAINS OF NONHUMAN PRIMATES WITH α CHAIN OF HUMAN HEMOGLOBIN[a]

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

[a] Data taken from earlier publications (16, 17).

β and γ chain sequences of human hemoglobin, we see that there is an homology among the three. Those positions in the sequence at which human β and γ chains differ correspond to segments of the *L. fulvus* non-α chain at which there are replacements (Table VI). If we compare *L. fulvus* non-α chain with human β chain there are 6 replacements that are homologous with human γ chain out of a total of 23 replacements.

TABLE IV

AMINO ACID REPLACEMENTS—COMPARISON OF β- OR γ-LIKE CHAINS OF NONHUMAN PRIMATES WITH β CHAIN OF HUMAN HEMOGLOBIN[a]

Primate	Number of peptides examined	Number of amino acids	Probable number of replacements
Hylobates	7	65	0
Papio	6	64	3
Perodicticus	5	49	8
Galago	10	87	9
Lemur fulvus	12	134	23
L. variegatus	11	96	23
Propithecus	3	30	4

[a] Data taken from earlier publications (16, 17).

TABLE V

AMINO ACID REPLACEMENTS—COMPARISON OF β- OR γ-LIKE CHAINS OF NONHUMAN PRIMATES WITH γ CHAIN OF HUMAN HEMOGLOBIN[a]

Primate	Number of peptides examined	Number of amino acids	Probable number of replacements
Hylobates	7	65	18
Papio	6	64	18
Perodicticus	5	49	9
Galago	10	87	21
Lemur fulvus	12	134	36
L. variegatus	11	96	25
Propithecus	3	30	10

[a] Data taken from earlier publications (16, 17).

Comparison of *L. fulvus* non-α chain with human γ chain shows 19 replacements that are homologous with β chain out of a total of 36 replacements. Seventeen replacements are unlike the amino acids at those positions in either β or γ chain. One of the most noteworthy differences between the non-α chain of *L. fulvus* and human β and γ chains is the presence of threonine as the NH_2-terminal amino acid. Threonine is also present in the NH_2-terminal position of the non-α chain of the hemoglobin of *Propithecus, Lemur catta,* and *Lemur variegatus* (2).

TABLE VI

PARTIAL SEQUENCES OF β-LIKE CHAIN OF HEMOGLOBIN OF *Lemur fulvus* AND β AND γ CHAINS OF HUMAN HEMOGLOBIN[a]

Homo β Val-His-Leu-Thr-Pro-Glu-Glu-Lys-Ser-Ala-Val-Thr-Ala-Leu-Try-Gly-Lys-Val-Asn-Val-Asp-Glu-
Lemur *Thr-Leu-Leu-Ser-Ala*-Glu-*Glu-Asp-Ala-His-Val*-Thr-*Ser*-Leu-Try-Gly-Lys-Val-Asn-Val-*Glu-Lys*-
Homo γ Gly-His-Phe-Thr-Glu-Glu-Asp-Lys-Ala-Thr-Ileu-Thr-Ser-Leu-Try-Gly-Lys-Val-Asn-Val-Glu-Asp-

Homo β Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-Leu-Val-Val-Tyr-Pro-Try-Thr-Gln-Arg-Phe-Phe-Glu-Ser-
Lemur *Val*-Gly-Gly-Glu-*Ala*-Leu-Gly-Arg-Leu-Leu-Val-Val(Tyr,Pro,Try,Thr,Gln,Arg,Phe,Phe,*Glu*,Ser,
Homo γ Ala-Gly-Gly-Glu-Thr-Leu-Gly-Arg-Leu-Leu-Val-Val-Tyr-Pro-Try-Thr-Gln-Arg-Phe-Phe-Asp-Ser-

Homo β Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys-Val-Lys-Ala-His-Gly-Lys-Lys-
Lemur Phe,Gly,Asp)(Leu,Ser,*Ser,Pro,Ser*,Ala,*Val*,Met,Gly,Asn,Pro,Lys,Val,Lys,Ala,His,Gly,Lys,Lys,
Homo γ Phe-Gly-Asn-Leu-Ser-Ser-Ala-Ser-Ala-Ileu-Met-Gly-Asn-Pro-Lys-Val-Lys-Ala-His-Gly-Lys-Lys-

Homo β Val-Leu-Gly-Ala-Phe-Ser-Asp-Gly-Leu-Ala-His-Leu-Asp-Asn-Leu-Lys-Gly-Thr-Phe-Ala-Thr-Leu-
Lemur Val,Leu,*Ser,Ala,Phe,Ser,Glu,Gly*)(*Leu,His*,His,Leu,Asp,Asp,Leu,Lys,Gly,Thr,Phe,Ala,*Ala*,Leu,
Homo γ Val-Leu-Thr-Ser-Leu-Gly-Asp-Ala-Ileu-Lys-His-Leu-Asp-Asp-Leu-Lys-Gly-Thr-Phe-Ala-Gln-Leu-

Homo β Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-Asp-Pro-Glu-Asn-Phe-Arg-Leu-Leu-Gly-Asn-Val-Leu-
Lemur Ser,Gln,Leu,His,Cys,*Val,Ala*,Leu,His,Val,Asp,Pro,Glu,Asp,Phe,*Lys*,Leu,Leu,Gly,Asp,*Ser*,Leu,
Homo γ Ser-Glu-Leu-His-Cys-Asp-Lys-Leu-His-Val-Asp-Pro-Glu-Asn-Phe-Lys-Leu-Leu-Gly-Asn-Val-Leu-

Homo β Val-Cys-Val-Leu-Ala-His-His-Phe-Gly-Lys...Val-Val-Ala-Gly-Val-Ala-Asn-Ala-Leu-Ala-His-Lys-Tyr-His
Lemur *Ser,Asp*,Val,Leu,Ala,*Asp*,His,Phe,Gly,Lys)...*Val*-Val-*Ala*-Gly-Val(Ala,*Asp*)Ala-Leu-*Ala-His-Lys*-Tyr-His
Homo γ Val-Thr-Val-Leu-Ala-Ileu-His-Phe-Gly-Lys...Met-Val-Thr-Gly-Val-Ala-Ser-Ala-Leu-Ser-Ser-Arg-Tyr-His

[a] Residues italicized in *Lemur* sequences differ from analogous residues in β or γ chains.

The comparison of adult *Lemur* hemoglobin chains with human fetal chains was suggested by our earlier observation that adult prosimian hemoglobin was resistant to alkaline denaturation, as is human fetal hemoglobin (6). Recently we examined hemoglobin from a premature stillborn lemur and a newborn galago by means of starch-gel electrophoresis and alkaline denaturation. No differences between the fetal hemoglobin and the adult hemoglobin from each of these two species were demonstrated.

At this stage in our work certain interpretations of these data are possible. The α-like chains of primate hemoglobins are apparently subject to some kind of constraint, for they are much less variable than the non-α chains. A functional hemoglobin probably requires that one of the two chains remains stable. We know that human α chains form functional hemoglobins with β, γ, and δ chains (22). Now we also know that many other sequences are presented by the β-like chains of nonhuman primates for combination with α chains. Since the genes controlling synthesis of the α, β, γ, and δ chains of human hemoglobin are nonallelic, we assume that the genes for synthesis of α and non-α chains in other primates are also nonallelic. The data presented here suggest that mutations in one locus put a constraint on mutations in the other locus.

TABLE VII

PROBABLE INVARIANT SEQUENCES OF AMINO ACIDS IN α CHAINS OF PRIMATE HEMOGLOBINS[a]

Tryptic peptide[b]	Primates in which compositions are identical
αT-1	*Homo, Hylobates, Perodicticus, Lemur fulvus, L. variegatus*
αT-2	*Homo, Hylobates, Perodicticus, Lemur fulvus, L. catta*
αT-5	*Homo, Propithecus, Lemur fulvus*
αT-6	*Homo, Hylobates, Lemur fulvus*
αT-7	*Homo, Hylobates*
αT-9	*Homo, Hylobates, Galago, Perodicticus, Lemur catta*

[a] Data taken from earlier publications (16, 17) and unpublished observations.

[b] Peptides numbered according to the nomenclature proposed by Gerald and Ingram (13).

Certain segments of the hemoglobin molecule appear to be invariant throughout the Order—this suggests that the function of the molecule or its synthesis is disrupted by alterations in this area (Tables VII and VIII). Mutations which produce substitutions here are quickly lost if they are not lethal. But there are a large number of differences, nonetheless, among the various hemoglobins, most of them in the non-α chains (Tables III, IV, V). Each species which we have examined has a unique hemoglobin, unique in at least one amino acid (16). The meaning of

this is obvious: there are a large number of distinct primate hemoglobins, and the many alterations in sequence of amino acids apparently do not alter the function, and the efficient function, of the molecule.

Now we must consider some of the more general implications of these data. First, we shall consider the question of calculating rates of effective mutation using the hemoglobin of *L. fulvus* as our example.

There are 6 replacements in the partial sequence of the α chain of *Lemur fulvus,* when this sequence is compared with the analogous sequence of human hemoglobin (Table IX). *Lemur* has been phylogenetically separate from man for a maximum of 55×10^6 years. Thus the average number of years for a single mutation to be fixed in the α chain of the hemoglobin of a population of lemurs is 9.1×10^6 years. But the

TABLE VIII

PROBABLE INVARIANT SEQUENCES OF AMINO ACIDS IN β CHAINS OF PRIMATE HEMOGLOBINS[a]

Tryptic peptide[b]	Primates in which compositions are identical
βT-1	*Homo, Hylobates*
βT-2	*Homo, Hylobates*
βT-3	*Homo, Hylobates, Papio, Perodicticus*
βT-4	*Homo, Homo* γT-4, *Hylobates, Papio, Galago, Perodicticus, Propithecus, Lemur fulvus, L. variegatus, L. catta*
βT-5	*Homo, Hylobates*
βT-6	*Homo, Homo* γT-6, *Hylobates, Papio, Galago, Perodicticus, 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*

[a] Data taken from earlier publication (16, 17).

[b] Peptides numbered according to the nomenclature proposed by Gerald and Ingram (13).

rate is quite different if we compare β-like chains of *Lemur fulvus* with human β and γ chains. There are 23 replacements in *L. fulvus* β-like chain when it is compared with β chain of man. The average number of years for a single mutation to be fixed in the β-like chain of the hemoglobin of a population of lemurs is 2.4×10^6 years. If we use human γ chain for comparison, the average number of years is 1.5×10^6. Thus if we make the assumption that *Lemur fulvus* α chains and β-like chains are derived from a common ancestor with human chains, then the rate at which effective mutations occur is neither constant nor linear. At least for lemur hemoglobins.

Second, we must consider the problem of the meaning of the large

TABLE IX

COMPOSITION OF α CHAIN OF HEMOGLOBIN OF *Lemur fulvus* AND α CHAIN OF HUMAN HEMOGLOBIN[a]

	1							10								20			
Homo	Val-	Leu-	Ser-	Pro-	Ala-	Asp-	Lys-	Thr-	Asn-	Val-	Lys-	Ala-	Ala-	Try-	Gly-	Lys-	Val-	Gly-	Ala-
Lemur	(Val,	Leu,	Ser,	Pro,	Ala,	Asp,	Lys)	(Thr,	Asn,	Val,	Lys)	(Ala,	Ala,	Try,	Gly,	*Asp*,	Val,	Gly,	Ala,

							30										40		
Homo	His-	Ala-	Gly-	Glu-	Tyr-	Gly-	Ala-	Glu-	Ala-	Leu-	Glu-	Arg-	Met-	Phe-	Leu-	Ser-	Phe-	Pro-	Thr-
Lemur	His,	Ala,	Gly,	Glu,	*Thr*,	Gly,	Ala,	Glu,	*Glu*,	Leu,	Glu,	Arg)	(Met,	Phe,	Leu,	Ser,	Phe,	Pro,	Thr,

Homo	Thr-	Lys-	Thr-	Tyr-	Phe-	Pro-	His-	Phe-	Asp-	Leu-	Ser-	His-	Gly-	Ser-	Ala-	Gln-	Val-	Lys-	Gly-
Lemur	Thr,	Lys)	(Thr,	Tyr,	Phe,	Pro,	His,	Phe,	Asp,	Leu,	Ser,	His,	Gly,	Ser,	*Gly*,	Glu,	Val,	Lys)	(*Ala*,

Homo	His-	Gly-	Lys-	Lys-	Val-	Ala-	Asp-	Ala-	Leu-	Thr-	Asn-	Ala-	Val-	Ala-	His-	Val-	Asp-	Asp-	Met-
Lemur	His,	Gly,	Lys)	(Lys)	(Val,	Ala,	Asp,	Ala,	Leu,	Thr,	Asp,	Ala,	Val,	Ala,	His,	*Leu*,	Asp,	Asp,	Met,

Homo	Pro-	Asn-	Ala-	Leu-	Ser-	Ala-	Leu-	Ser-	Asp-	Leu-	His-	Ala-	His-	Lys-	Leu-	Arg-	Val-	Asp-	Pro-
Lemur	Pro,	Asn,	Ala,	Leu,	Ser,	Ala,	Leu,	Ser,	Asp,	Leu,	His,	Ala,	His,	Lys)	(Leu,	Arg)	(Val,	Asp,	Pro,

Homo	Val-	Asn-	Phe-	Lys	.	.	.	Tyr-	Arg										
Lemur	Val,	Asp,	Phe,	Lys)	.	.	.	(Tyr,	Arg)										

[a] Residues italicized in *Lemur* sequences differ from analogous residues in *Homo*.

number of amino acid substitutions found among various primate hemoglobins. When we use the α, β, and γ chains of human hemoglobin as the referents, we find a relatively large number of amino acid substitutions in the hemoglobins of the Primates during their long evolutionary history. This implies a large number of point mutations. The amino acid substitutions at many positions in the sequences are considered chemically equivalent by protein chemists. That is, the substitution of an aspartyl for a glutamyl residue, or of leucyl, isoleucyl, or valyl for each other, is not expected to have any great effect on the activity. It is also known that, in some proteins, certain residues can be extensively altered or eliminated with no significant loss of activity (1, 10). Does this suggest that there are neutral traits and, hence, neutral genes? The evidence that highly modified amino acids can be incorporated into proteins without altering their activity or function is taken from *in vitro* experiments. There is no evidence yet, from complex organisms such as the Primates, that such "neutrally altered" proteins would function and would persist in a population of organisms.

If these replacements are *biologically* equivalent, then we have neutral traits. But what is the evidence that these traits are neutral? The fact that they may seem to have an equivalent role in the molecule does not answer the question. The question really is, how does an effective mutation, which is a relatively rare event, become common or fixed in a population? At present the only mechanism we know by which this occurs is natural selection. The animals that carry the mutation must have a reproductive advantage over others in the population that do not carry it. Unless this is the case, the trait is likely to disappear through accidents of sampling, sometimes called genetic drift, or to remain at a very low frequency. If the known substitutions in lemur hemoglobins are selectively neutral, then it seems we must postulate synchronous mutations throughout the population or species. And this is highly improbable.

We are confronted by a difficulty, for we cannot at the moment demonstrate increased biological advantage for any of these substitutions. On the evidence we have, however, we can reason that neutral substitutions probably do not occur. First, there appears to be an invariant segment of the hemoglobin molecule (Tables VII and VIII). If neutral substitutions are possible, i.e., functionally equivalent amino acid residues, why not here? Second, there is a lesson in the variable hemoglobins of one primate, *Homo sapiens*. At least one case is known in this species where a single substitution has profound physiological effects—the case of hemoglobin S (21). And the only sound explanation for the relative frequency of hemoglobin S in certain populations is positive selection on the heterozygotes, that is, positive differential fertility of the AS

heterozygote individuals over AA and SS homozygotes (12). This is quite independent of whether or not it is indeed *Plasmodium falciparum* which is the *agent* of selection.

Finally, we must consider hemoglobin data in the context of primate phylogeny and systematics. There are some general trends worth pointing out and some things to be said about the use of such data in analyzing primate systematics.

The hemoglobins of the Anthropoidea and the Prosimii differ from each other more than the hemoglobins of primates within each group differ from each other (17). On the basis of our evidence from fingerprints, electrophoresis, and some peptide compositions, the hemoglobins of the Anthropoidea are very similar to those of man. Hemoglobins of the Prosimii vary among themselves much more than do hemoglobins of the Anthropoidea.

One interesting exception to the general rule is the hemoglobin of the baboon, *Papio*. Clearly, this hemoglobin appears to differ a great deal more from human hemoglobin than does that of all the other Anthropoidea examined so far. Fingerprint patterns alone of *Papio* hemoglobin show as many differences from human hemoglobin fingerprint patterns as do those of some prosimian hemoglobins. Nevertheless it is unlikely that we shall wish to switch the phylogenetic position of the baboon solely on the basis of this evidence.

Phylogenetic distance between two taxa is a confusingly applied concept. As Mayr has clearly shown, two things make up phylogenetic relationships. One is the fact that phyletic branching has occurred. The other consists of all the genetic, ecological, and selective events that occurred after branching (25).

The hemoglobin of *Tupaia*, judging from fingerprints alone, differs considerably from human hemoglobin, more than the hemoglobin of most of the other primates studied. But probably no more so than does hemoglobin from some Lemuriformes. It is worth noting here that *Tupaia* hemoglobin differs considerably from hemoglobins of certain Insectivora, namely the Macroscelididae, elephant shrews of East Africa.

The hemoglobins of the Lemuriformes resemble each other more than they resemble human hemoglobin or hemoglobin from most of the other primates. There appear to be many more similarities between hemoglobins of Lemuriformes and Lorisiformes than there are between the hemoglobins of either and those of the other primates. Amino acid composition, end group analysis, and grosser methods such as starch-gel electrophoresis and peptide mapping confirm this (2, 5, 16, 17).

The hemoglobins of the Ceboidea are apparently similar to human hemoglobin. The Ceboidea are most interesting in this respect, for they

are not closely related to man. They are descended from an ancient stock which must have been phylogenetically distinct from other primates well before their first appearance in Miocene deposits of South America. Dentition separates them from all other living primates, and the only presently known fossil group to whom they relate are the Omomyidae, Eocene prosimian fossils found in many parts of the world, but not in South America. The hemoglobin data, based solely on peptide patterns and starch gels, suggest that their hemoglobin has become quite similar to that of *Homo sapiens* (5, 17).

We have relatively little information about the hemoglobins of the Cercopithecoidea. What we have suggests that they are quite similar to human hemoglobin. There is, of course, the one exception, *Papio*.

Pongid hemoglobin is very much like that of man. The information available indicates that several pongid hemoglobins, if the source were not known, might easily be lost among the many variant human hemoglobins (5, 17, 38, 40).

Our notions of primate classification will not be changed by the hemoglobin data we have presented. The differences and similarities among primate hemoglobins reflect, to a large extent, present classifications and notions of phylogeny. The exceptions present us with further research problems, not new classifications.

In this paper we have tried to show how to synthesize an approach to molecular-organismal evolution. We have tried to show where this synthesis should begin, what organisms to use as substrates, what proteins to use as enzymes, and how much classical evolutionary biology to stir in as inhibitors. After a few more years of effort we hope to have an elegant and useful product.

Summary

Hemoglobins from several primates, representative of the various taxa within the Order, have been examined. Determination of partial sequences and peptide patterns indicated that the α chains are conservative, differing relatively little among the various species. The β (or β-like) chains vary considerably. The implications of these data for calculation of effective mutation rates, for molecular evolution, and for phylogeny were discussed.

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