Anthropoid Evolution: The Molecular Evidence

John E. Cronin

It has become apparent, in recent years, that macromolecules can provide phylogenetic information elucidating the branching order of lineages (cladistics), the amount of evolution occurring along lineages (patristics), and the approximate times of divergence of these lineages (chronistics). Within the primates, particularly with respect to the Anthropoidea, a number of different cladograms have been constructed using macromolecular comparisons including DNA (Kohne et al. 1972; Hoyer et al. 1972), hemoglobin (Wilson and Sarich 1969; Goodman and Moore 1973), fibrinopeptides (Doolittle et al. 1971; Wooding et al. 1972), myoglobin (Romero-Herrera et al. 1973), mixed immunology (Goodman and Moore 1971), albumin (Sarich and Wilson 1967; Wilson and Sarich 1969; Sarich 1970; Cronin and Sarich 1975; Sarich and Cronin 1977), transferrin (Cronin and Sarich 1975; Sarich and Cronin 1977).

The serum protein albumin has been studied in a number of vertebrate groups, other than primates, including carnivores (Sarich 1969a, 1969b, 1973), ranid frogs (Wallace, King and Wilson 1973) and hylid frogs (Maxson and Wilson 1975).

In this paper we construct two independent phylogenies of the Anthropoidea using the two serum proteins, albumin and transferrin. The groups examined consist taxonomically of three superfamilies, Hominoidea, Cercopithecoidea and Ceboidea (Napier and Napier 1967). We find that the two molecular cladograms are in excellent agreement with each other and furthermore, that both are in accord with other molecular data and macromolecular cladograms. Finally, the total macromolecular evidence supports the use of "molecular clocks."

Experimental Procedures

Serum albumin and serum transferrin were purified for immunological comparisons from those species listed in Tables 1, 2 and 3. In every case except that of *Tupaia* the proteins were purified from a single individual.

Transferrin purification

Transferrin was purified by a modification of the Stratil and Spooner (1971) procedure (Sarich 1974; Cronin and Sarich 1975). To a given serum was added $FeCl_3$ (0.1 mg/ml of serum). Rivanol (2-ethoxy 6-9 diaminoacridine lactate; 1 mg/ml serum) was dissolved in 0.25 M tris (pH 8.8; 0.25 mg/ml of serum) and then diluted 4 to 5 fold with distilled water. The serum was then added to the rivanol solution and cooled to 5°C. The solution was centrifuged (12,000g) and $(NH_4)_2\ SO_4$ was added to 40% saturations, then subsequently to 70% saturation. The final solution was centrifuged at 12,000g and the precipitate, usually a salmon pink, redissolved into a minimal volume of isotris buffer dialyzed against isotris containing citric acid and ferrous ammonium sulfate to promote saturation.

The solution was then subjected to polyacrylamide disc gel electrophoresis (Wallace 1971; Maxson and Wilson 1975), alternatively. An 8% cyanogun (95% acrylamide, 5% bis) solution was used in the gel mixture with a tris-glycine buffer (0.1M tris, 0.05M glycine pH 8.8) as both well and gel buffer. In this case, equilibration was prolonged to 1 hour at 5 ma/tube (Palmour and Sutton, 1971).

Albumin purification

Albumin was usually purified from serum by polyacrylamide disc gel electrophoresis (Wallace and Wilson 1972). In the single case of *Cacajao,* albumin was purified by the heat caprylate method of Hoch and Chanutin (Sarich 1967).

Antisera Production

Antisera were prepared in rabbits according to the method of Sarich (1967). Individual antisera were pooled in inverse proportion to their microcomplement fixation (MC'F). Titres and all reported results are from pooled antisera (Prager and Wilson 1971a and b). Purity tests were conducted according to published techniques (Wallace, King and Wilson 1971). In some cases, minor components were observed in the gels, indicating that antibodies had been induced to proteins other than albumin or transferrin. However, these minor components do not interfere with microcomplement fixation experiment results as identical results are obtained whether one uses the purified protein or unmodified primate serum as the antigen source (Sarich 1966; Cronin 1975).

Microcomplement Fixation

Quantitative microcomplement fixation experiments as devised by Wasserman and Levine (1961) were modified by Sarich and Wilson (1966) and extensively detailed by Champion et al. (1974). Immunological distance (y) is generally related to the percent

difference in amino acid sequence (x) between two molecules by the equation y=5x (Prager and Wilson 1971a and b; Champion et al. 1974). Evidence suggests that one unit of immunological distance is approximately equivalent to one amino acid difference between two species albumin or transferrin (Maxson and Wilson 1974; Sarich 1974).

Transferrin Saturation

The possibility exists that the binding of iron to transferrin alters the configuration of the molecule in such a way as to affect immunological recognition in microcomplement fixation experiments. Normally, transferrin is about one third saturated with apo transferrin (Tf), monoferro, and diferro molecules present in plasma (Palmour and Sutton 1971). Presumably upon injection into the rabbit, molecules of all three types would be present.

To test the effect of saturation on immunological recognition human transferrin was deprived of iron, resaturated and tested immunologically with purified human transferrin and serum as controls. Microcomplement fixation tests were performed using anti-human transferrin (genotype CC) with apo Tf, purified Tf, serum and 80% saturated Tf. No difference was detected in any of these duplicated experiments. In addition similar procedures were carried out on chicken ovo transferrin and identical results obtained (Prager et al. 1974).

Results

Construction of Phylogenetic Trees

The phylogenetic trees were constructed using the method of Sarich (Sarich 1969; Cronin and Sarich 1975). The immunological distance values used were the average of the values determined by reciprocal tests. The phylogenetic trees presented have a stated percent deviation (Fitch and Margoliash 1967) as a measure of "goodness of fit" where input data is compared with the output data. The latter is calculated by adding up the immunological distance units separating any pair of species in the cladogram.

The method of tree construction is similar to that of Fitch and Margoliash (1967). The difference lies in the fact that to apportion lineage lengths between two species A and B, Fitch and Margoliash average the distances from A to every other species and from B to every other species. The method as described in Cronin and Sarich (1975) does not use every other species as an outside reference point but uses the next closest group to align branch lengths in the group under study. For example, anti-cercopithecoid antisera would serve as outside reference points for the Hominoidea and similarly the anti-ceboid antisera would serve as outside reference points for aligning the cercopithecoid lineages. The different methods of construction yield similar although not identical results (Farris 1972; Wallace et al. 1973; Maxson and Wilson 1975).

Antisera to the purified transferrins from 5 hominoid species, 5 cercopithecoid species, and 5 ceboid species were used to investigate the phyletic relationships of the suborder Anthropoidea. Using the microcomplement fixation technique each antiserum was tested with transferrin of every species listed in Table 1. Table 1 gives all immunological distances between all 105 possible pairs of species. Thus, between every pair of species 2 immunological distances were obtained.

Similarly antisera to the purified albumins from 5 hominoid, 5 cercopithecoid and 10 ceboid species were used to investigate the phyletic relationships within the Anthropoidea. Table 2 gives the matrix of immunological distance units for all 45 pairs of catarrhine species. Table 3 gives the matrix of immunological distance units for all 45 pairs of platyrrhine species.

The trees constructed are shown in Figures 1 and 2. The percent standard deviation of the transferrin cladogram is 8.9%. The lowest tree for 20 eukaryotic cytochrome c sequences constructed by Fitch and Margoliash (1967) was 8.7%. No tree this large has been published previously using immunological data. For a tree of 12 hylid frog species Maxson and Wilson (1975) report 10% standard deviation. For a tree of 15 cercopithecoid species (Cronin and Sarich in prep.) a 12.5% standard deviation was obtained. The anthropoid albumin tree has a 8.0% standard deviation.

Correlation of Albumin and Transferrin Distances

Albumin immunological distances are correlated with transferrin immunological distances. To illustrate this one may consider all the pairs of catarrhine species that have been subjected to both albumin (Table 2) and transferrin (Table 1) comparison. For these 45 pairs a product moment correlation coefficient of 0.86 was observed ($p \ll 0.01$). Similarly when the average albumin distances given in Table 4 for primates as a whole are compared with the corresponding transferrin distances $r = 0.83$ ($p \ll 0.01$).

The equation for the regression lines were as follows:

Catarrhine $A = mT + b$

Primates $A = mT + b$

Where A is the albumin immunological distance and T is the transferrin immunological distance. Transferrin immunological distances are on the average 1.5 fold greater than albumin distances between the same species. Similar results have been found in comparison of carnivore, rodent and chiroptera albumins and transferrins (Sarich 1973). In birds transferrin distances average 2.2 times the albumin distances. (Prager et al. 1974).

TABLE 1
Immunological Distances Among Anthropoid Transferrins

	ANTISERA															
ANTIGEN	HOMO	PAN	GORILLA	PONGO	HYLOBATES	PRESBYTIS	THEROPITHECUS	PAPIO	CYNOPITHECUS	ERYTHROCEBUS	CERCOCEBUS	ATELES	AOTUS	CEBUS	CALLITHRIX	SAGUINUS
Homo	-	13	5	33	27	64	55	61	49	52	42	73	73	75	70	86
Pan	7	-	5	36	27	54	52	56	46	46	41	71	73	74	70	74
Gorilla	8	10	-	37	26	59	50	52	45	49	39	75	73	62	68	76
Pongo	16	18	24	-	28	52	50	50	42	47	38	85	83	71	74	88
Hylobates	20	22	23	35	-	55	47	45	44	45	58	78	78	82	74	85
Presbytis	34	33	37	43	43	-	20	15	19	21	21	79	89	96	90	104
Theropithecus	41	41	51	66	63	43	-	3	20	24	24	100	100	112	103	113
Papio	37	36	37	61	45	34	5	-	18	21	23	98	100	109	101	110
Cynopithecus	34	33	31	44	39	34	13	16	-	12	12	86	89	100	85	101
Erythrocebus	34	33	31	40	37	34	16	20	19	-	23	86	86	91	81	94
Cercocebus	35	28	36	39	42	31	13	20	7	9	-	82	94	89	75	92
Ateles	64	78	61	83	84	95	85	68	82	75	79	-	28	30	26	25
Aotus	67	71	60	82	80	92	89	110	77	79	81	31	-	32	35	28
Cebus	66	83	70	101	86	90	95	108	72	81	79	32	30	-	36	32
Callithrix	67	82	70	92	89	97	85	99	71	76	81	28	30	29	-	8
Saguinus	64	80	68	95	87	97	92	106	78	75	81	28	30	35	6	-

TABLE 2
Immunological Distances Among Catarrhine Albumins.

	ANTISERA									
ANTIGEN	HOMO	PAN	GORILLA	PONGO	HYLOBATES	M. IRUS	C. AETHIOPS	PRESBYTIS	C. GALERITUS	P. PAPIO
Homo	-	4	4	11	10	37	49	42	37	34
Pan	7	-	9	11	14	37	49	46	38	31
Gorilla	5	7	-	11	10	32	62	51	37	36
Pongo	12	8	10	-	11	42	57	48	38	40
Hylobates	13	13	12	11	-	36	49	44	35	43
M. irus	37	30	21	38	36	-	12	24	6	0
C. aethiops	44	42	21	33	37	10	-	22	9	4
Presbytis	42	40	22	38	38	24	34	-	28	16
C. galeritus	39	32	20	33	33	7	0	19	-	4
P. papio	40	30	18	34	36	2	7	25	8	-

TABLE 3
Intraplatyrrhine Albumin Immunological Distances

	ANTISERUM											
ANTIGEN	AOTUS	ATELES	CEBUS	CALLICEBUS	ALOUATTA	PITHECIA	SAIMIRI	CACAJAO	SAGUINUS	CALLITHRIX	RELATIVE	R[a]
Aotus trivirgatus	0	31	29	26	28	18	32	22	32	27	-12	
Ateles geoffroyi	33	0	31	19	21	27	29	17	43	42	0	
Cebus capucinus	37	43	0	30	36	25	38	37	59	56	+12	
Callicebus cupreus	36	33	29	0	36	28	48	24	50	41	-2	
Alouatta palliata	38	12	30	22	0	31	42	31	56	55	+6	
Pithecia monachus	29	29	18	12	34	0	24	4	50	30	-4	
Saimiri scuirea	40	42	31	34	36	39	0	43	60	46	+8	
Cacajao rubicundus	33	36	29	24	33	9	45	0	41	34	+2	
Saguinus leucopus	36	27	17	16	25	26	27	14	0	29	+3	
Callithrix jaccus	29	39	37	22	45	37	40	22	29	0	+2	

[a]These numbers indicate the mean amount by which the given albumin species is more distant (+) or less distant (-) than *Ateles* from a series of nonplatyrrhine albumins *(Homo, Pan, Macaca, Cercopithecus, Presbytis, Nycticebus)* from Sarich and Wilson 1967; Sarich 1970.

TABLE 4
Immunological Distances Among Primate Albumins and Transferrins[a]

TRANSFERRIN	H	C	P	*Ta*	*Tu*	*Cy*	Le	Lo	Non-primates[b]	
Hominoidea	0	33	58	123	98	117	112	94	+19	
Cercopithecoidea	45	0	56	128	102	114	110	107	+19	A
Platyrrhini	76	91	0	109	89	98	112	107	+12	L
Tarsius	172	164	156	0	93	109	122	95	+5	B
Tupaia	170	190	180	159	0	103	117	108	-5	U
Cynocephalus	175	156	177	161	183	0	109	113	+5	M
Lemuriformes	164	171	164	156	178	162	0	88	0	I
Lorisiformes	196	195	193	178	183	185	118	0	-4	N

[a]These data have been gathered using antisera to the albumins and transferrins of *Homo, Pan, Gorilla, Pongo, Hylobates, Papio, Cercocebus, Cercopithecus, Presbytis, Aotus, Ateles, Cebus, Callithrix, Saguinus, Lemur, Avahi, Lepilemur, Propithecus, Phaner, Daubentonia, Nycticebus, Galago, Tupaia,* and *Cynocephalus;* to the transferrin of *Macaca nigra;* and to the albumins of *Ursus, Genetta, Hyaena, Felis, Hipposideros, Pteropus, Tadarida, Antrozous, Bradypus, Cabassous, Tamandua, Scapanus,* and *Solenodon.*

[b]These numbers represent the amount by which those albumins are more (+) or less (-) distant from the various nonprimates than the lemuriform mean. A similar analysis is not possible for the transferrins as antisera to nonprimate transferrins do not generally react with those of primates.

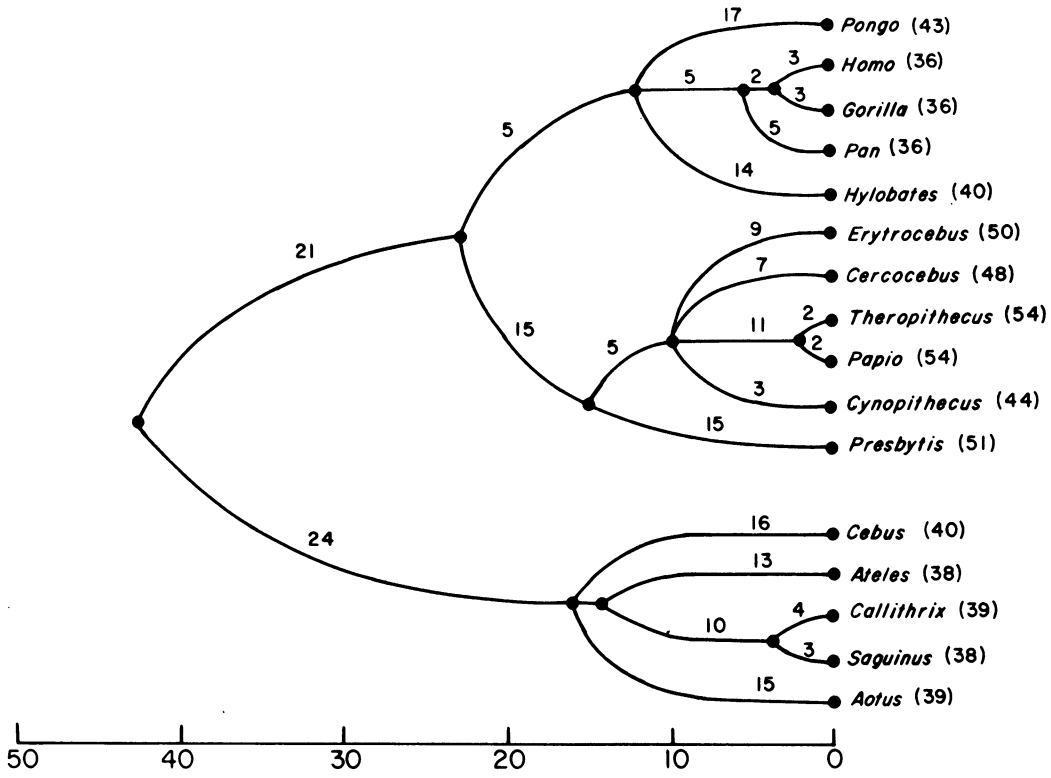

Figure 1. A transferrin cladogram of the Anthropoidea constructed according to the method of Sarich (1969). The immunological distances on which this cladogram is based are those of Table 1. The percent standard deviation is 8.9%. The number in parentheses following the species names represent the amount of transferrin immunological distance accumulated on that species lineage from the node separating the catarrhines and platyrrhines.

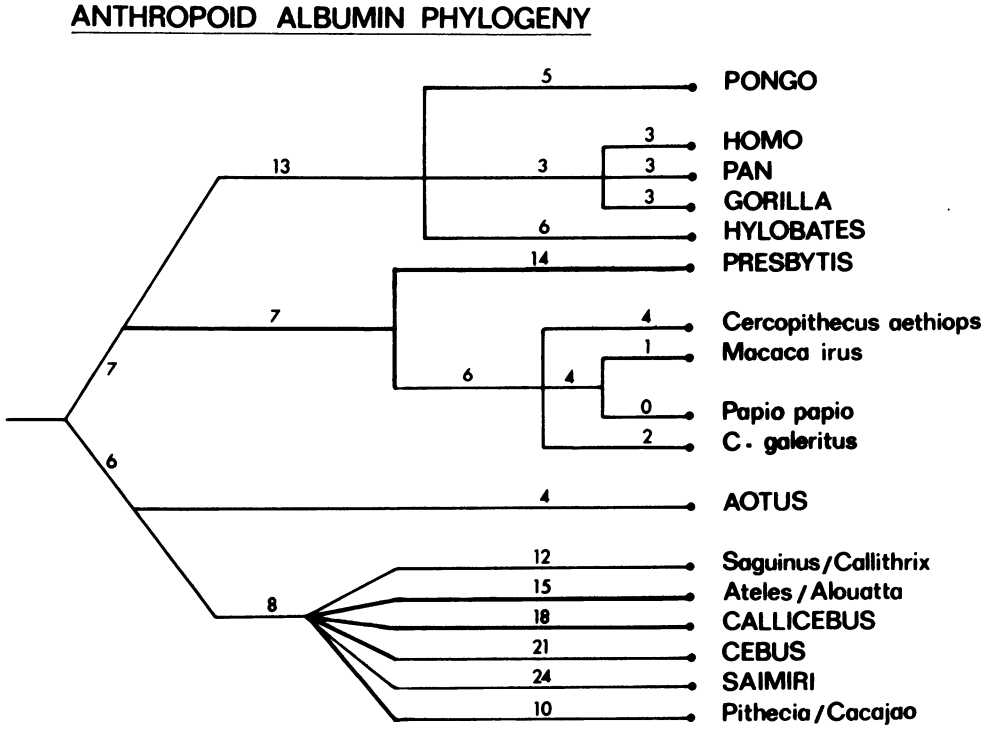

Figure 2. An albumin cladogram of the Anthropoidea constructed as in Figure 1. The immunological distances on which this cladogram is based are those of Tables 2 and 3. The percent standard deviation is 8.0%.

Anthropoid Evolution

The agreement, in terms of cladistics, between the albumin and transferrin cladograms is evident. No major differences are readily apparent. There is one difference and that is in terms of the placement of the ceboid lineage *Aotus*. Previous albumin studies placed it diverging before the major ceboid radiation (Sarich 1967). The transferrin data, however, suggest that it is one of the 7 major ceboid lineages which radiate from a common node (Cronin and Sarich 1975) as do the hemoglobin data (Boyer et al. 1971).

Beyond that specific discrepancy (discussed in depth in Cronin and Sarich 1975) no other specific disagreements are noted. It is clear that the species within Anthropoidea comprise a group with a shared lineage of some considerable time depth. The initial divergence is between the Old and New World forms, the Catarrhini and Platyrrhini. The former group subsequently diverges into the two constituent subunits, the Hominoidea and the Cercopithecoidea. Thus, the major groupings apparent at the molecular level are in good agreement with the classification of the Anthropoidea (Napier 1967).

The fact that the two molecular cladograms are in such close agreement supports the using of immunological data in order to elucidate the phylogenetic relationship of taxa.

Given the data, it is readily apparent that the Anthropoidea is composed of three clusters of species, including the Hominoidea, the Cercopithecoidea and the Ceboidea. The former are closer to each other from almost every molecular measure, except for the primate lysozyme data, than they are to the latter group the Ceboidea. Reciprocity problems are inherent in the lysozyme — anti lysozyme system as has been pointed out (Prager et al. 1974). The overwhelming evidence is that the Catarrhini are a unit relative to the Platyrrhini.

All the molecular data support the conclusion that the Anthropoidea share a common lineage subsequent to the divergence of any prosimian stock. Along this lineage accumulated some 70 units of combined albumin and transferrin units of change, three myoglobin sequence changes, seven fibrinopeptide changes, five beta hemoglobin changes and two units of antigenic distance, before the divergence of the New World monkeys.

The Ceboidea form a fairly tight unit (evolutionary radiation) with a division into units, the Callithricidae plus *Callimico,* and a number of other fairly independent ceboid lineages (Cronin and Sarich 1975; Baba et al. 1975; Boyer et al. 1971).

The Catarrhini form a group relative to the Platyrrhini, with respect to serum proteins, albumin and transferrin (Sarich 1970; Sarich and Cronin 1975), prealbumin, albumin, alpha 2 macroglobulin, thryglobulin, transferrin, ceruloplasmin and gamma globulin (Goodman et al. 1971), DNA (Kohne 1972), myoglobin (Romero-Herrera 1973), hemoglobin (Goodman et al. 1973, 1974), fibrinopeptides (Wooding et al. 1972) and carbonic anhydrase (Nonno et al. 1969).

The Catarrhini are traditionally broken into two taxonomic units, the Hominoidea and the Cercopithecoidea on morphological grounds. This is also true with respect to the molecular division (all references above and figures this paper). The Cercopithecoidea have had little detailed genetic work. Yet it is apparent that the basic division into leaf eaters (Colobinae) and non leaf eaters (Cercopithicine) is recognized at the molecular level. (Sarich 1970; Barnicot and Wade 1970; Goodman and Moore 1971). A detailed presentation of the evolution of the Cercopithecoidea as determined by the microcomplement fixation technique is in preparation (Cronin and Sarich) and a brief preliminary version is in press (Sarich and Cronin 1977).

Albumin and Transferrin Molecular Clocks

It appears that albumin accumulates approximately 50 units of change per 60 million years per lineage or 100 units per 60 million years of separation. These results are found for a large variety of vertebrates besides primates (Sarich 1970) including carnivores (Sarich 1969), ranid frogs (Wallace, et al. 1973), hylid frogs (Maxson and Wilson 1975) and iguanid lizards (Gorman et al. 1971).

Transferrin accumulates about 150 units per 60 m.y. of separation in mammalian and snake lineages (Mao et al. 1971; Cronin and Sarich 1975; Sarich 1974). In birds, the rate of transferrin evolution is ¼ to ½ that found in mammals and snakes. Similar results are observed in serum albumin, with a rate of evolution ⅓ that found in mammals (Prager et al. 1974). This phenomen of an evolutionary slowdown is not unique to these two molecules. Lysozyme and cytochrome c seem to have evolved about twice as slowly in birds as in mammals (Prager et al. 1974). This problem points to the fact that rates of evolution *cannot be assumed;* they *must be calculated* for every group under study.

In the equation: Immunological Distance = K X Time (10^6 year), K approximates 1.67 for albumin and 2.5 for transferrin per million years for mammalian lineages. Thus, 250 units of combined albumin plus transferrin immunological distance calibrates to approximately 60 million years. Using this calibration point for albumin and transferrin it can be seen in Table 5 what the approximate times of separation of various taxa are for the two molecules independently and averaged.

The dates of divergence calculated from the immunological data presented in this study are in good agreement with dates proposed by Sarich (1970); Sarich and Wilson (1967a and b); and Wilson and Sarich (1969). Certain differences are, however, appa-

TABLE 5

Times of Divergence Among Various Primate Taxa Predicted by Albumin and Transferrin Immunological Distances

Taxa	Time of Divergence (10^6 yrs.)[a]		
	Albumin	Transferrin	Average
Homo vs *Pan* vs *Gorilla*[b]	3.6	4	3.8
Homo vs *Pongo* vs *Hylobates*[b]	7.2	9.6	8.4
Colobinae vs Cercopithecinae	14.4	10.8	12.6
Hominoidea vs Cercopithecoidea	22.2	18	20.1
Platyrrhini vs Catarrhini	34.2	33.6	33.9
Anthropoidea vs Prosimii	70.2	64	67.1

[a] Times of divergence are calculated on the basis that approximately 100 ablumin and 150 transferrin units accumulate between two taxa over sixty million years.

[b] The taxa listed are cladistically equally divergent from a common node so that it is a three way comparison. Since all three lineages separated cladistically from the same node, they have existed for equal evolutionary time.

rent. It was postulated that prosimians and anthropoids differed by about 100 albumin units. This figure has had to be adjusted upward by about 10 units given the additon of five new lemurid albumin antisera.

A second point of difference is the date of 30 million years for the hominoid-cercopithecoid divergence used to calculate the man-chimpanzee divergence time of approximately 5 m.y. BP (Sarich and Wilson 1967b). In subsequent publications a divergence time of approximately 22 million years has been estimated for the hominoid-cercopithecoid separation (Sarich 1970, 1971). The date estimated from the transferrin data of 18 m.y. BP is in close agreement with this latter figure. The initial publication (Sarich and Wilson 1967b) *assumed* a hominoid-cercopithecoid divergence time of approximately 30 m.y. BP. From this date, given the ratio of the man-chimpanzee albumin distance of 6 units versus the hominoid-cercopithecoid distance of 35 immunological distance (I.D.) units; and given the hypothesis of the regularity of protein evolution a time of divergence of 5 m.y. BP was calculated for man and chimpanzee. Additional albumin data have now been gathered. Given the data from Table 3, a mean hominoid-cercopithecoid distance of 37 I.D. units is obtained. A cladistic analysis of the hominoids indicates that man, chimpanzee and gorilla diverged from a common node into three separate lineages. Each lineage is a sample of the amount of change given the time of divergence for those lineages. A mean distance using all descendent lineages from a node may give a better representation of the actual distance between species descending from that node than only one measurement. This consideration gives a mean man-chimpanzee-gorilla albumin distance of 6 units. The same analysis on the transferrin data reveal a mean distance of 8 units. The hominoid-cercopithecoid mean is 45 transferrin units. The ratio is 1:6.1 for albumin and 1:5.6 for tranferrin. Given the small distances involved between the great apes and man, the two ratios are consistent and predict that the human-chimpanzee divergence is approximately 5 m.y. BP.

Agreement Among Genetic Distances

Fortunately, with Anthropoidea we do not merely need to rely upon only a comparison of albumin and transferrin genetic distances. The reliability of these phylogenies may be tested with other molecules and there is good agreement with the cladograms produced by studies of hemoglobin (Wilson and Sarich 1969; Goodman and Moore 1973) fibrinopeptides (Doolittle et al. 1971; Wooding et al. 1972), DNA (Kohne et al. 1972; Hoyer et al. 1972) myoglobin (Romero-Herrera et al. 1973) transferrin (Cronin and Sarich 1975) and mixed immunology (Goodman and Moore 1971).

Table 6 lists the relative genetic distances between 5 primate taxa. The molecular data when considered as a whole support the hypothesis that the immunological data are not unique or unrepresentative of such macromolecular evidence. The correlation coefficient for the nine molecular systems among the 5 taxa compared is 0.96 ($p \ll .01$). The rank correlation coefficient is 1.0 (Cronin, Sarich and Dempster in prep.). It appears that data on any one of the molecules yields substantially the same conclusions as data from any other molecular system.

TABLE 6
Relative Molecular Differences Among Various Primate Groups Referred to a Catarrhine-Platyrrhine Distance of 1.0

Groups Compared	Albumin[a]	Transferrin[b]	DNA[c]	Mixed Immunology[d]	Myoglobin[e]	Fibrinopeptides[f]	Beta Haemoglobin[h]	Carbonic Anhydrase[i]	Lysozyme[j]
Homo vs *Pan*	0.12	0.13	0.15	0.12	0.007	0	0	.05	0
Homo vs *Pongo* vs *Hylobates*[g]	0.25	0.30	0.33	0.29	.143	0.3	.43	NA	NA
Hominoidea vs Cercopithecoidea	0.58	0.53	0.61	0.53	0.47	0.5	.85	.65	.90
Catarrhini vs Platyrrhini	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Anthropoidea vs Prosimii	2.1	1.8	2.6	1.67	1.5	1.8	1.57	1.73	1.45

[a] From Sarich (1970) and subsequent work in this laboratory. The number is immunological distance between the groups divided by the catarrhine-platyrrhine albumin immunological distance.

[b] Data from this laboratory and expressed as in *a*.

[c] Calculated from the data in Hoyer et al. (1972) and Kohne, Chiscon, and Hoyer (1972) by dividing the ΔT_m for the groups by the corresponding catarrhine-platyrrhine value.

[d] From Goodman and Lasker (1973).

[e] From Romero Herrera and Lehmann (1973). The calculations are based on amino acid differences.

[f] From Wooding and Doolittle (1972); Tables 1 and 2). The calculations are based on amino acid differences.

[g] The taxa listed are cladistically equally divergent from a common node so that it is a three-way comparison.

[h] From Goodman and Lasker (1973). The data presented is based on amino acid differences found between the taxa.

[i] From Nonno et al. (1969).

[j] From Hanke et al. (1973; Table 11). Only the data gathered with the anti human lysozyme is included.

NA: Data Not Available

Implications for Primate Evolution

The albumin-transferrin "molecular clock" suggests that the Anthropoidea diverged from other primate lineages approximately 70 m.y. BP. This date is very close to the date postulated for the beginning of the Tertiary. Furthermore, if correct, the separation of various major lineages of the primates predates the postulated first known primate *Purgatorius* (Van Valen and Sloan 1965; Clemens 1974). Anthropoids may have arisen in North America in the late Cretaceous. Subsequently, the ancestral anthropoids migrated to the Old World along with other ancestral primate forms before the North American-European land connection was broken in the Eocene (Walker 1972).

The initial divergence among the Anthropoidea, that between the Old World forms (man, apes and Old World Monkeys) and the New World forms (New World monkeys) occurred about 35 m.y. BP in the Old World, according to "molecular clock" considerations. This argues that the introduction of anthropoids into South America was via Africa. Anthropoid grade forms are found in deposits in Africa at a slightly younger date (Simons 1965, 1967, 1972). Similar conclusions have been reached from interpretations of morphological and fossil data. (Hershkovitz 1972; Hoffstetter 1972, 1974). There are however, objections to this interpretation (Simons 1972).

The next major division within the Anthropoidea, the separation of the lineages leading to the Hominoidea and the Cercopithecoidea occurred approximately 20 m.y. BP and was most likely African in location.

Alternative interpretations exist as to the origin and divergence of the major lineages of the Catarrhini (Delson 1974; Simons 1969, 1972); a more detailed picture of within-hominoid and within-cercopithecoid relationships will be presented elsewhere. Yet, it is clear that the basic picture of anthropoid cladistics has been documented through the use of macromolecular comparisons and is in substantial agreement with interpretations based on anatomical data. However, the problem as to the time of the origin of these major

anthropoid lineages remains controversial (Simons 1972; Delson 1974; Read 1975; Delson and Andrews 1976).

In conclusion the fact that the macromolecules are in agreement as to the relationships among the Anthropoidea is an important observation. Macromolecules may be as unambiguous a source of phylogenetic information as *any* body of data. It is interesting that within each life form a history of that form is encoded. We need only to know how to read the enclosed message.

Acknowledgement

The author wishes to thank V.M. Sarich for help in both the research and in the writing of this article. Dr. A. Wilson generously provided laboratory space. Thanks go to A. Hill and P. Hornbeck for technical assistance. L. Brunker generously aided in the preparation of the technical illustrations. Thanks also go to E. Meikle, N. Boaz and L. Brunker in editing and commenting on the drafts of the manuscripts. T. Cord generously provided her time in typing the manuscript.

REFERENCES CITED

Barnicot, N.A., and P.T. Wade 1970 Protein Structure and the Systematics of Old World Monkeys. *In* Old World Monkeys: Evolution, Systematics and Behavior, J.R. Napier and P.H. Napier, eds. New York: Academic Press.

Boyer, S.H. et al. 1971 Primate Haemoglobins: Some Sequence and Some Proposals Concerning the Character of Evolution and Mutation. Biochem. Genetics 5:405-448.

Champion, A.C., E.M. Prager, D. Wachter, and A.C. Wilson 1974 Microcomplement Fixation. *In* Biochemical and Immunological Taxonomy of Animals, C.A. Wright, ed. London: Academic Press.

Clemens, W.A. 1974 *Purgatorius,* An Early Paromomyid Primate (Mammalia). Science 184:903-905.

Cronin, J.E. 1975 Molecular Systematics of the Order Primates. Ph.D. Dissertation, University of California, Berkeley.

Cronin, J.E., and V.M. Sarich 1975 Molecular Systematics of the New World Monkeys. J. Human Evol. 4:357-375.

Cronin, J.E., V.M. Sarich and E. Dempster 1977 Rates of Molecular Evolution in the Primates. Unpublished Manuscript.

Delson, E. 1975 Evolutionary History of the Cercopithecidae. *In* Approaches to Primate Paleobiology, F. Szalay, ed. Contrib. Primate. 5:167-217.

Delson, E., and P. Andrews 1975 Evolution and Interrelationships of the Catarrhine Primates. *In* Phylogeny of the Primates, W.P. Luckett and F.S. Szalay, eds. New York: Plenum.

Doolittle, R.F., G.C. Wooding, Y. Sen, and H. Riley 1971 Hominoid Evolution as Judged by Fibrinopeptide Structures. J. Molec. Evol. 1:74-83.

Farris, J.S. 1970 Methods for Computing Wagner Trees. Syst. Zool. 19:83-92.

Fitch, W.H. and E. Margoliash 1967 Construction of Phylogenetic Trees. Science 155:279-284.

Goodman, M. and W. Moore 1971 Immunodiffusion Systematics of the Primates. I. The Catarrhines. Syst. Zool. 20:19-62.

Goodman, M., and G.W. Moore 1974 Phylogeny of Haemoglobin. Syst. Zool. 32:508-532.

Gorman, G.C., A.C. Wilson and M. Nakanishi 1971 A Biochemical Approach the Study of Reptilian Phylogeny: Evolution of Serum Albumin and Lactic Dehydrogenase. Syst. Zool. 20:167-185.

Hershkovitz, P. 1970 Notes on Tertiary Platyrrhine Monkeys and Description of a New Genus from the Late Miocene of Columbia. Folia Primat. 12:1-37. 1972 The Recent Mammals of the Neotropical Region: A Zoogeographic and Ecological Review. *In* Evolution, Mammals and Southern Continents, A. Keast, F.C. Erk and B. Glass, eds. Albany: State University of New York Press.

Hoffstetter, R. 1972 Relationship, Origins and History of the Ceboid Monkeys and Caviomorph Rodents: A Modern Reintrepretation. *In* Evolutionary Biology, Vol. 6, T. Dobzhansky, M.K. Hecht and W.C. Steere, eds. New York: Appleton, Century Crofts.

Hoyer, B.H., N.W. van de Velde and M. Goodman 1972 Examination of Hominoid Evolution by DNA Sequence Homology. J. Human Evol. 1:645-649.

Kohne, D.E., J.A. Chiscon, and B.H. Hoyer 1972 Evolution of Primate DNA Sequences. J. Human Evol. 1:627-644.

Mao, Shao-hsien, and H.C. Dessauer 1971 Selectively Neutral Mutations, Transferrins and the Evolution of Natricine Snakes. Comp. Biochem. Phys. 40:669-680.

Maxson, L., and A.C. Wilson 1974 Convergent Morphological Evolution Detected by Studying Proteins of Tree Frogs in the *Hyla exemia* Species Group. Science 185:66-68.

1975 Albumin Evolution and Organismal Evolution in Tree Frogs (Hylidae). Syst. Zool. 24:1-15.

Napier, J.R., and P.H. Napier 1967 A Handbook of Living Primates. New York: Academic Press.

Palmour, R.M., and H.E. Sutton 1971 Vertebrate Transferrins, Molecular Weights, Chemical Composition, and Iron Binding Studies. Biochem. 10:4026-4032.

Prager, E.M., and A.C. Wilson 1971a The Dependence of Immunological Cross-Reactivity Upon Sequence Resemblances Among Lysozymes. J. Biol. Chem. 246:5978-5989.

1971b The Dependence of Immunological Cross-Reactivity Upon Sequence Resemblance Among Lysozymes. II. Comparison of Precipitin and Microcomplement Fixation Results. J. Biol. Chem. 246:7010-7017.

Prager, E.M., A.H. Brush, R.A. Nolan, M. Nakanishi and A.C. Wilson 1974 Slow Evolution of Transferrin and Albumin in Birds According to Microcomplement Fixation Analysis. J. Mol. Biol. 3:243-262.

Read, D. 1975 Primate Phylogeny, Neutral Mutations and Molecular Clocks. Syst. Zool. 24:209-221.

Romero-Herrera, A.E., H. Lehmann, K.A. Joysey and A.E. Friday 1973 Molecular Evolution of Myoglobin and the Fossil Record: A Phylogenetic Synthesis. Nature 246:389-395.

Sarich, V.M. 1967 A Quantitative Immunological Study of Evolution of Primate Albumins. Ph.D. Dissertation. University of California, Berkeley.

1968 Human Origins: An Immunological View. *In* Perspectives on Human Evolution, S.L. Washburn and P.C. Jay, eds. New York: Holt, Rinehart and Winston.

1969a Pinneped Origins and the Rate of Evolution of Carnivore Albumins. Syst. Zool. 18:286-295.

1969b Pinneped Phylogeny. Syst. Zool. 18:416-422.

1970 Primate Systematics with Special Reference to Old World Monkeys: A Protein Perspective. *In* Old World Monkeys: Evolution, Systematics and Behavior, J.R. Napier and P.H. Napier eds. New York: Academic Press.

1973 The Grand Panda is a Bear. Nature 245:218-219.

1974 Just How Old is the Hominid Line? Yearbook of Physical Anthropology 17:98-112.

Sarich, V.M., and A.C. Wilson 1966 Quantitative Immunochemistry and the Evolution of Primate Albumins. Microcomplement Fixation. Science 154:1563-1566.

1967a Rates of Albumin Evolution in Primates. Proc. Nat. Acad. Sci. 58:142-148.

1967b Immunological Time Scale for Hominid Evolution. Science 158: 1200-1203.

Sarich, V.M., and J.E. Cronin 1977 Molecular Systematics of the Primates. *In* Molecular Anthropology, M. Goodman and R. Tashian, eds. New York: Plenum (In Press.)

Simons, E.L. 1965 New Fossil Apes from Egypt and the Initial Differentiation of Hominoidea. Nature 205: 135-139.

1967 The Earliest Apes. Sci. Amer. 217:28-35.

1972 Primate Evolution. New York: Macmillan Co.

Stratil, A., and R.L. Spooner 1971 Isolation and Properties of Individual Components of Cattle Transferrin: The Role of Sialic Acid. Biochem. Gen. 5:347-365.

Walker, A. 1972 The Dissemination and Segregation of Early Primates in Relation to Continental Configurations. *In* Calibration of Hominoid Evolution, W.W. Bishop and J.A. Miller, eds. New York: Scottish Academic Press.

Wallace, D.G. 1971 A Comparison of Amphibian Albumins. Taxonomic and Evolutionary Significance. Ph.D. Dissertation, University of California, Berkeley.

Wallace, D.G., M.C. King and A.C. Wilson 1972 Albumin Differences Among Ranid Frogs: Taxonomic and Phylogenetic Implications. Syst. Zool. 22:1-14.

Wallace, D.G., and A.C. Wilson 1972 Comparison of Frog Albumins with That of Other Vertebrates. J. Mol. Evol. 2:72-86.

Wooding, G., and R. Doolittle 1972 Primate Fibrinopeptides: Evolutionary Significance. J. Hum. Evol. 1:553-563.

Van Valen, L., and R.E. Sloan 1965 The Earliest Primates. Science 150:743-745.

Wasserman, E. and L. Levine 1961 Quantitative Microcomplement Fixation and Its Use in the Study of Antigenic Structure by Specific Antigen-Antibody Inhibition. J. Immunology 87:290-295.

Wilson, A.C., and V.M. Sarich 1969 A Molecular Time Scale for Human Evolution. Proc. Nat. Acad. Sci. 63:1088-1093.