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On the Tryptic Peptides from Hemoglobin Chains of Six Carnivores

Bernadine Brimhall,[1] * Peter Stenzel,[1] Steven L. Dresler,[1] Mark Hermodson,[2] Kenneth Stangland,[1] James Joyce[1] and Richard T. Jones[1]

[1] Department of Biochemistry, School of Medicine, University of Oregon Health Sciences Center, Portland, Oregon 97201, USA
[2] Division of Medical Genetics, University of Washington Medical School, Seattle, Washington, USA

Summary. The amino acid compositions of the tryptic peptides of the following carnivore hemoglobin chains have been determined: gray fox (*Urocyon cinero-argenteus*); raccoon (*Procyon lotor*); polar bear (*Thalarctos maritimus*); coati mundi (*Nasua nasua*) β chain; coati mundi (*Nasua narica*) two β chains; cat (*Felis catus*) α chain; and lion (*Panthera leo*) β chain. These provide a basis for future sequencing of these hemoglobins and construction of an evolutionary tree. The specific results are summarized in the following article (Stenzel and Brimhall, 1977).

Key words: Carnivore hemoglobins – Tryptic peptide compositions – Gray fox – Polar bear – Coatimundi – Domestic cat – Lion – Raccoon

Introduction

The family tree of carnivores deduced from fossil evidence and comparative morphology has been summarized in diagrams by Romer (1947) and by Young (1962). They estimate that the cat (Feloidae) and dog (Canoidae) families separated from one another in the late Eocene, approximately 40 million years ago. Then during the Miocene, which began 20–25 million years ago, the bear (Ursidae) and raccoon (Procyonidae) families branched off from the dog family. This paper describes a study of the tryptic peptide compositions of the hemoglobins of some of these carnivores (arctoid fissipeds) as an indication of the diversity to be found and as a basis for sequencing techniques which will be necessary for construction of a meaningful evolutionary tree on a biochemical basis. A knowledge of tryptic peptide compositions is an important adjunct to sequencing operations, particularly with solid state automatic sequenators, and provides useful information on the degree of relatedness of these animals.

The amino acid sequence of dog hemoglobin has been reported in the foregoing article (Brimhall et al., 1977). It was found to have two α-chains differing only at

* To whom offprint requests should be sent.

Residue 130. The tryptic peptide composition of coyote hemoglobin showed it to be identical to that of dog except that it has only one α chain sequence (Runkel et al., 1974). In addition to these, Hombrados et al. (1976) have reported the β chain sequence of the badger, and Taketa et al. (1977) in a preceding article have presented the tryptic peptide compositions of the two β-chains of cat hemoglobin.

Methods

Through the courtesy of Dr. Michael Schmidt of the Washington Park Zoo, Portland, blood was obtained from a 9-year-old polar bear, four coatimundis (two *Nasua narica* and two *Nasua nasua*), and two female lions. About 15 ml blood from each was drawn under anesthesia and preserved in EDTA. The gray fox, an adult female, and the raccoon were unfortunate enough to be dispatched by a chicken farmer near Portland, Oregon. A composite blood sample was obtained from three domestic cats, presumably unrelated but of unknown parentage.

Cellulose acetate electrophoresis was run on the hemolysates at pH 8.4 (Schneider, 1973). Chain electrophoresis was carried out on *N. narica* and lion in urea-mercaptoethanol according to the method of Schneider (1974).

The hemolysate of lion hemoglobin was applied to a column of Amberlite IRC as described by Taketa et al. (1973), with the following modifications. The column was 3.5 x 30 cm, although 20 cm would have been satisfactory. One buffer approximately 0.05 M in phosphate at pH 6.4 was used throughout: 60 g $NaH_2PO_4 \cdot H_2O$, 19 g Na_2HPO_4 anhy., 650 mg KCN, diluted to 12 l.

The column was loaded and run until the first band was eluted with the jacket temperature at 10°. The temperature was allowed to come to room temperature in order to elute the second band.

The two hemoglobins of *N. narica* were separated on DEAE-Sephadex with Tris-HCl buffers from pH 8.1 to 7.3 (Huisman and Dozy, 1965). From these fractions and from hemolysates of the other bloods, globin was prepared by the acid-acetone procedure (Schroeder et al., 1963). Globin chains were separated by Dintzis' (1961) method, using a pyridine formate concentration gradient on a column of carboxymethyl-cellulose.

The chains were aminoethylated (Jones, 1964) and glycinamidated (Carraway and Koshland, 1972) before hydrolysis with trypsin. As a result of glycinamidation of the chain, each free carboxyl group (side groups of Asp and Glu residues and the C-terminus) is converted to the glycinamide derivative. The C-terminal glycinamide is removed from arginine by tryptic hydrolysis. On acid hydrolysis of the tryptic peptides, a glycine residue and a molecule of ammonia are liberated for each derivatized Asp and/or Glu residue present. This extra glycine is detected by amino acid analysis, along with the glycine which results from any glycyl bonds present in the original peptide.

Hydrolysis with TPCK-treated trypsin (Worthington) was carried out in a solution containing 50 mg of chain and 1 mg trypsin adjusted to pH 8.5 with trimethylamine at 37° for 3 h for α-chains and room temperature for 2 h for β-chains (Baglioni, 1965). The solution was then acidified to pH 3 and the peptides separated by chromatography. This results in the cleavage of the chains on the C-terminal side of lysyl, arginyl and aminoethylcysteinyl residues.

The tryptic peptides from 50 mg of hydrolysate were separated according to the method of Jones (1970) on a 0.9 x 16 cm column of Aminex A-5 (Bio-Rad) cation-exchange resin using a linear gradient of 2 pyridine-acetate buffers (250 ml each) from pH 3.1 to 5.0 at a flow rate of 30 ml/h. Improved resolution of the tryptic peptides from 90 mg of cat α-chain was obtained on a 0.9 x 30 cm column of A-5 resin using a linear gradient of 2 buffers (375 ml each), one consisting of 0.2 M pyridine-acetic acid, pH 3.1, and the other a mixture of 90 ml of pH 3.1 buffer plus 285 ml of pH 5.0, 2.0 M pyridine-acetic acid. After a total of 560 ml of developer had been passed through the column at 30 ml/h, pH 5.0 buffer alone was used to elute the remaining peptides. The peaks from the A-5 chromatograms were purified by rechromatography on a 0.9 x 16 cm column of Aminex 50W-X4 (Bio-Rad) with a linear gradient of 250 ml each of pH 3.1 and 5.0 pyridine-acetate buffers. Occasionally, e.g., peptide αT-12C of fox, an Aminex 1 x 2 anion-exchange column was used with equal volumes of two buffers – 1 % collidine and 0.2 M acetic acid (Guest et al., 1967).

The methionyl residues in selected cases were located by cleavage of the tryptic peptide with cyanogen bromide in 0.1 N HCl (Gross, 1967) followed by chromatography on Aminex 50W-X4 as described above. Chymotryptic cleavage was carried out at room temperature overnight after adjustment of the pH to 8.5 with Tris buffer (Smyth, 1967). For cleavage at aspartic acid residues the peptide was exposed to a solution of 0.5 M acetic acid in an evacuated, sealed ampoule at 110° overnight (Schultz, 1967). For this procedure it was obviously necessary to use tryptic peptides from the non-glycinamidated chains. Carboxypeptidases A and B were used according to Ambler (1967). Aminopeptidase M was used to confirm the presence of tryptophane residues by digestion of the peptide at 37° overnight in a solution adjusted to pH 8–9 with Tris buffer (Light, 1972).

The subtractive Edman degradation method (Konigsberg, 1972) was used in the manual determination of amino acid sequences. For automatic sequencing of the 20 residues at the N-terminus of the slow *N. narica* β-chain, the Beckman Sequencer Model 890C was employed according to the method of Hermodson et al. (1972).

Amino acid analysis were run on a Beckman Spinco 120 amino acid analyzer after 22 h hydrolysis (or longer for Val-Val linkages) at 110° in 6 N HCl containing 9 mg % of phenol in an evacuated ampoule. Aminex A-5 resin (Bio-Rad) was used in the short column for basic amino acids and A-6 resin in the long columns for neutral and acidic amino acids. When aminoethyl cysteine was present, a 12-cm rather than a 5-cm A-5 column was used for the basic amino acids, the aminoethyl cysteine emerging between lysine and histidine.

Results and Discussion

This section has been divided into 6 parts dealing with the hemoglobin of each animal: I Fox, II Polar Bear, III Raccoon, IV β-Coatimundi, V α-Cat, and VI β-Lion. Each is discussed with reference to the sequence of dog hemoglobin which is given in the preceding article (Brimhall et al., 1977). The differences in composition of these carnivores and the probable locations of the substituents in the chains are compared in Table 2 of the following article (Stenzel and Brimhall, 1977).

Table 1. Tryptic peptides from the aminoethyl, glycinamidated α-chain of fox hemoglobin

Residue No.	αT-1 1-7	αT-2 8-11	αT-3 12-16	αT-4 17-31	αT-5 32-40	αT-6 41-56	αT-7 57-60	αT-8 61	αT-9 62-90	αT-10 91-2	αT-11 93-9	αT-12A 100-4	αT-12B 105-11	αT-12C 112-27	αT-13 128-39	αT-14 140-1	Total Residues
Lysine	1.0	1.0	1.0		1.0	1.2	1.1	1.0	1.3		1.1			1.3	1.0		11 Lys
Histidine				1.0		0.8	1.0		1.7			1.1		2.7			9 His
S-Aminoethyl Cysteine												0.9	1.0				2 Cys
Tryptophan			0.1														1 Trp
Arginine				0.8						1.0						1.0	3 Arg
Aspartic Acid + Asparagine	1.0	1.0	1.0	1.8		1.0			4.2		1.8			2.0			14 Asx
Glutamic Acid + Glutamine				1.0	1.2	1.0								1.1			4 Glx
Threonine		1.1	0.9		2.7	0.9			2.0				0.90.9	0.9	2.7		13 Thr
Serine	1.0		1.0		1.1	1.9			2.0			1.0		0.9	1.9		11 Ser
Proline	1.0				1.0	2.0			1.1		1.0			1.9			8 Pro
Glycine				5.1		1.0	1.0		1.1								8 Gly
Alanine	1.0			2.1		1.1	1.1		6.8				1.0	2.1	1.1		16 Ala
Valine	0.9					0.9			1.8		2.0		0.9	1.2	2.0		10 Val
Isoleucine		0.9		0.9													2 Ile
Leucine	1.1			1.1		1.1			6.0	1.0		2.0	3.1	1.3	1.1		17 Leu
Tyrosine				1.0		1.0			1.0							1.0	4 Tyr
Phenylalanine					2.1	2.0					1.0			1.0	2.0		8 Phe
Glycine = Asp + Glu	1.0		1.1	3.0		1.1			4.2		1.0			2.5			

Values are molar ratios of amino acids produced by hydrolysis with hydrochloric acid.

Tryptophan residues are partly destroyed by HCl hydrolysis but small amounts remain. Therefore, in tryptophan containing peptides, the total residues will add up to somewhat less than expected.

Single impurities of less than 0.3 residue have been omitted from the tables.

The extra glycine recovered beyond that normally found in a peptide is given last; it represents the glycinamide attached to Glu and Asp residues. The glycine found in the βT-15 peptide comes from the glycinamide attached to the carboxyl group on the C-terminus of the chain.

Table 2. Tryptic peptides from the aminoethyl, glycinamide β-chain of fox hemoglobin

Residue No.	βT-1 1-8	βT-2 9-17	βT-3 18-30	βT-4 31-40	βT-5 41-59	βT-6 60-1	βT-7 62-5	βT-8 66	βT-9A 67-76	βT-9B 77-82	βT-10A 83-87	$\beta T\text{-}10B_1$ 88-93	$\beta T\text{-}10B_2$ 94-5	βT-11 96-104	βT-12A 105-12	βT-12B 113-20	βT-13 121-32	βT-14 133-44	βT-15 145-6	Total Residues
Lysine	1.3	1.1			1.0	1.0	1.2	1.0	1.0	1.0	1.0		1.0	1.2		1.4	1.0	1.1		14 Lys
Histidine	0.7						0.8					1.0		0.8		1.7		1.0	1.0	8 His
S-Aminoethyl Cysteine												1.0			1.0					2 Cys
Tryptophan		0.3		0.2																2 Trp
Arginine			1.0	1.0																2 Arg
Aspartic Acid + Asparagine			2.1		3.6				2.0	2.9			0.9	1.9	1.0			1.0		16 Asx
Glutamic Acid + Glutamine	2.0		2.0	1.1								1.1		1.0			4.0			11 Glx
Threonine	1.0	1.0		0.9	1.0						1.0						1.0			6 Thr
Serine		1.0			2.0				1.9			0.9								6 Ser
Proline				1.0	1.1									1.0			1.2			4 Pro
Glycine		2.0	2.9		2.2		0.9		1.0		1.0				1.2	1.1		1.2	(1.0)	13 Gly
Alanine	1.1		1.1		2.1		1.1				1.1					1.0	2.2	3.9		13 Ala
Valine	1.0	1.0	3.0	1.0	1.1	1.0			1.0					1.0	1.7	0.9	0.9	2.7		17 Val
Methionine					1.0															1 Met
Isoleucine				0.9																1 Ile
Leucine	1.0	1.8	1.0	2.1	1.0				2.1	2.0		1.8		1.1	2.7	1.0		1.1		19 Leu
Tyrosine				1.0													1.0		0.9	3 Tyr
Phenylalanine					2.8				0.9		0.9			1.0		1.0	1.1			8 Phe
Glycine = Asp + Glu	2.0		2.9		3.0				1.1	1.1		1.1	1.1	2.1			1.2			

For explanation see footnote of Table 1

A. Gray Fox (Urocyon cineroargenteus)

The red fox (Vulpes) is said to have evolved in North America and reached Europe in the early Pleistocene, about 3 million years ago (Kurten, 1971). Whether or not this applies to the gray fox is uncertain.

Seal (1969) examined the blood of 3 gray foxes by electrophoresis and found only one hemoglobin band moving like the single band observed for dog and coyote. According to Hombrados et al., (1976), the α and β chains of fox (species?) have been partially sequenced by Sukhomlinov and Konoshenko.

The hemolysate from erythrocytes of the fox showed no tendency to crystallize, in contrast to the behavior of dog, wolf, and coyote hemoglobins. However, a comparative study of their crystallizing tendency was not made under controlled conditions.

Tables 1 and 2 show the amino acid compositions of the tryptic peptides of the α and β chains of fox hemoglobin. Three differences in composition were found between the tryptic peptides of hemoglobin from the fox and the (α 130-Thr) hemoglobin of the dog. The sequence location of each difference was assigned as follows:

a. Threonine for serine in βT-2. A one-step Edman degradation removed 0.5 residue of serine from the N-terminus of βT-2 in chain position #9; therefore, it is probably the serine in position #12 which has been replaced by threonine.

b. Glycine for serine in βT-5. There are three serines in the dog and coyote βT-5 peptides at #44, 49 and 56. Since a number of mammalian hemoglobins have glycine at Res. 56, including raccoon and badger, this was selected as the most probable site for the substitution in fox.

c. Asparagine for threonine in αT-12. After rechromatography of the αT-12C peptide on Aminex X4 resin, there were 3 residues of glycine and 2.5 of alanine. It was then rechromatographed on an anion column, Dowex 1 x 2, with a collidine-acetic acid gradient. This reduced the glycine to 2.5 and the alanine to 2 residues. Since extra glycine might indicate an Asp rather than Asn as the substituent, the peptide was treated with 0.5 M acetic acid after which only three products were obtained on chromatography: free Lys (Res. 127), free Asp (Res. 126) and a peptide corresponding to Res 112–125. Therefore the substituted residue is probably asparagine, not aspartic acid, since the peptide was not cleaved at Res. 115. Coyote and dog αT-12C peptides have threonines at Res. 115 and Res. 118. When the fox αT-12C peptide was split with chymotrypsin, three chymotryptic peptides were found: (H, H, P, N, E, F), (T, P, A, V, H), and (A, S, L, D, K). The isolation of H, H, P, N, E, F, Res. 112–117, shows that asparagine has replaced the threonine at Res. 115.

B. Polar Bear (Thalarctos maritimus)

The polar bear is a late offshoot of the Alaskan brown bear. In the 20–25 million years since the bear and dog families separated, at least 20 differences in amino acid sequence have developed between dog and polar bear hemoglobins, as revealed in the compositions of tryptic peptides shown in Tables 3 and 4. Their α chains differ by at least 14 residues, but only 6 differences were found between their β chains, suggesting that the α and β chains may have evolved at different rates.

It is of interest that the α chain of polar bear probably contains serine at Res. 111 instead of the cysteine residue which is found in the other carnivores examined with

Table 3. Tryptic peptides from the aminoethyl, glycinamide α-chain of polar bear hemoglobin

Residue No.	αT-1 1-7	αT-2 8-11	αT-3 12-16	αT-4 17-31	αT-5 32-40	αT-6 41-56	αT-7 57-60	αT-8 61	αT-9 62-90	αT-10 91-2	αT-11 93-9	αT-12A 100-4	αT-12B 105-27	αT-13 128-39	αT-14 140-1	Total Residues
Lysine	0.9	1.0	1.0		1.1	1.0	1.1	1.0	1.0		1.1		1.0	1.0		11 Lys
Histidine				1.0		1.0	1.0		2.0			1.0	2.9			9 His
S-Aminoethyl Cysteine												0.8				1 Cys
Tryptophan			0.5													1 Trp
Arginine				0.8						0.9					1.0	3 Arg
Aspartic Acid + Asparagine	1.0	1.0	1.0			1.0			4.0		2.1		1.0			11 Asx
Glutamic Acid + Glutamine				2.9		1.0							1.0			5 Glx
Threonine			0.9		2.6	0.9			1.8				1.7	2.1		11 Thr
Serine	1.0	1.0		1.0	1.0	1.9			2.0			1.0	2.0	2.9		14 Ser
Proline	1.1				1.0	1.8					1.0		1.9			7 Pro
Glycine				3.9		1.0	0.9		2.0							8 Gly
Alanine	1.1		1.1	2.0	0.9	1.2	0.9		8.3				4.1	1.2		20 Ala
Valine	0.9	0.9				0.9			1.0		2.0		1.7	2.0		10 Val
Isoleucine				0.9												1 Ile
Leucine	1.0			1.1		1.1			7.0	1.1		1.0	4.0	1.0		17 Leu
Tyrosine				0.9		1.0									1.0	3 Tyr
Phenylalanine					2.0	1.8					1.0	1.0	1.0	2.0		9 Phe
Glycine = Asp + Glu	1.0		1.1	2.8		1.0			4.1		1.3		1.9			

For explanation see footnote of Table 1

Table 4. Tryptic peptides from the aminoethyl, glycinamide β-chain of polar bear hemoglobin

Residue No.	βT-1 1-8	βT-2 9-17	βT-3 18-30	βT-4 31-40	βT-5 41-59	βT-6 60-1	βT-7 62-5	βT-8 66	βT-9A 67-76	βT-9B 77-82	βT-10A 83-87	βT-10B$_1$ 88-93	βT-10B$_2$ 94-5	βT-11 96-104	βT-12A 105-12	βT-12B 113-20	βT-13 121-32	βT-14 133-44	βT-15 145-6	Total Residues
Lysine	1.0	1.0			1.0	1.0	1.1	1.0	1.0	1.1	1.1		1.0	1.0		1.1	1.0	1.0		14 Lys
Histidine	1.0						0.9					1.1		0.9		1.8		1.0	1.0	8 His
S-Aminoethyl Cysteine												0.9			0.9					2 Cys
Tryptophan		0.2		tr																2 Trp
Arginine			0.9	1.0																2 Arg
Aspartic Acid + Asparagine			2.2		4.9				2.0	3.0			1.0	1.9	1.1			1.0		17 Asx
Glutamic Acid + Glutamine	2.1		2.0	1.0								1.1		0.9			3.9			11 Glx
Threonine	0.9	1.0		1.0							0.9						0.9			5 Thr
Serine		1.0			2.9				1.8			0.9								7 Ser
Proline				0.9	0.8									0.9			0.9			4 Pro
Glycine	1.1	2.1	3.0		1.0		1.0		1.0		1.0				1.1	1.1		1.1	(1.2)	13 Gly
Alanine			1.2		2.0		1.0				1.0					1.1	2.1	3.7		12 Ala
Valine	0.9	1.0	2.9	1.9					1.1					1.0	1.8	0.9	1.1	2.7		16 Val
Methionine					1.0															1 Met
Isoleucine					1.0															1 Ile
Leucine	1.0	2.1	1.1	2.1	1.1	1.0			1.8	2.1		2.1		1.0	2.7	0.9		1.1		20 Leu
Tyrosine				1.0													1.0		1.0	3 Tyr
Phenylalanine					3.1				1.1		1.0			1.0		1.0	1.0			8 Phe
Glycine = Asp + Glu	2.1		3.0		3.1				0.9	1.0		1.1	1.0	1.9			1.3			

For explanation see footnote of Table 1

Table 5. Tryptic peptides from the aminoethyl, glycinamide α-chain of raccoon hemoglobin

Residue No.	αT-1 1-7	αT-2 8-11	αT-3 12-16	αT-4 17-31	αT-5 32-40	αT-6 41-56	αT-7 57-60	αT-8 61	αT-9 62-90	αT-10 91-2	αT-11 93-9	αT-12A 100-4	αT-12B 105-11	αT-12C 112-27	αT-13 128-39	αT-14 140-1	Total Residues
Lysine	1.1	1.0	1.1		1.1	1.0	1.0	1.0	1.0		1.0			1.1	1.0		11 Lys
Histidine				1.0		0.9	1.0		2.1			1.0		2.8			9 His
S-Aminoethyl Cysteine												0.9	1.1				2 Cys
Tryptophan			0.2														1 Trp
Arginine				0.9						1.0						1.1	3 Arg
Aspartic Acid + Asparagine	1.0	1.0	1.0			1.1			4.0		2.0			1.0			11 Asx
Glutamic Acid + Glutamine				3.0		1.0								1.1			5 Glx
Threonine			0.9		2.9	0.9			1.0				1.0	1.0	3.0		11 Thr
Serine	0.9				1.1	1.9			1.9			1.1		0.9	2.7		11 Ser
Proline	0.9				1.1	1.8			1.0		1.0			1.8			8 Pro
Glycine		0.3		4.9		1.1	1.0		1.9								9 Gly
Alanine	1.1	1.1	1.1	2.2	1.0	1.1	0.9		6.0				1.0	3.1			18 Ala
Valine	1.0					0.9			2.1		2.0		0.9	0.9	2.1		10 Val
Isoleucine		0.9		0.8													2 Ile
Leucine	1.0			1.1		1.2			7.0	1.0		2.0	3.0	1.1	1.1		18 Leu
Tyrosine				1.0		1.0			1.0							0.9	4 Tyr
Phenylalanine					1.9	1.9					1.1			1.1	2.1		8 Phe
Glycine = Asp + Glu	1.0		1.0	3.0		1.1			4.0		1.1			2.0			

For explanation see footnote of Table 1

Table 6. Tryptic peptides from the aminoethyl, glycinamide β-chain of raccoon hemoglobin

Residue No.	βT-1 1-8	βT-2 9-17	βT-3 18-30	βT-4 21-40	βT-5 41-59	βT-6 60-1	βT-7 62-5	βT-8 66	βT-9A 67-76	βT-9B 77-82	βT-10A 83-87	βT-10B$_1$ 88-93	βT-10B$_2$ 94-5	βT-11 96-104	βT-12A 105-12	βT-12B 113-20	βT-13 121-32	βT-14 133-44	βT-15 145-6	Total Residues
Lysine	1.2	1.0			1.1	1.1	1.1	1.0	1.1	1.1	1.1		1.0			1.2	1.1	1.1		13 Lys
Histidine	1.0						1.0					1.0		1.0		2.0		0.9	1.0	8 His
S-Aminoethyl Cysteine												0.9			1.0					2 Cys
Tryptophan		0.1		0.3																2 Trp
Arginine			1.0	1.0										1.0						3 Arg
Aspartic Acid + Asparagine	1.0		1.0		3.0				1.1	2.9			1.0	1.9	1.0			1.0		14 Asx
Glutamic Acid + Glutamine	1.0		2.9	1.0	1.0				1.1			1.0		1.0			3.0			12 Glx
Threonine	0.9	2.8		0.9							0.9						1.0			7 Thr
Serine					2.7				1.7			0.9								6 Ser
Proline				1.0	1.0									1.0			1.8			5 Pro
Glycine		1.1	2.8		1.8		0.9		0.9		1.0				0.9	1.0		1.0	(1.0)	12 Gly
Alanine	1.1	1.1	1.2		2.1		1.0				1.0					1.1	2.1	4.0		14 Ala
Valine	0.9	1.0	2.8	2.0		0.9			0.9					1.0	1.8	0.9	1.0	2.7		17 Val
Methionine					0.8															1 Met
Isoleucine					1.0															1 Ile
Leucine	1.1	1.0	1.0	2.2	1.1				1.9	2.1		2.0		1.1	3.0	1.1		1.0		18 Leu
Tyrosine				1.0													1.0		0.9	3 Tyr
Phenylalanine					3.1				1.0		1.0			1.0		1.0	1.0			8 Phe
Glycine = Asp + Glu	2.0		3.0		3.0				1.0	1.0		1.1	1.0	2.1			1.1			

For explanation see footnote of Table 1

Table 7. Amino acid differences among raccon, polar bear and dog hemoglobins

Peptide	Polar Bear	Raccoon	Dog	Probable Residue
α Chain				
T-2	Ser	Ala	Thr	8
	Val	Ile	Ile	10
T-3	Ala	Ala[a]	Ser	12
T-4	Ser	Gly	Gly	19
	Glu	Glu	Asp	23
	Glu	Glu	Asp	30
T-5	Ala	Ala	Gln	34
T-9	Gly	Val	Val	70
	Ala	Pro	Pro	77
	Leu	Tyr	Tyr	89
	Thr, Ala	Leu,Gly	Thr, Ala	?
T-12A	Phe	Leu	Leu	100
T-12B,C	Ser	Cys	Cys	111
	Ala	Ala	Thr	115
T-13	Ala	Ser	Thr[b]	130
β Chain				
T-1	Gly	Ala	Ala	5
	Glu	Asp	Glu	6 or 7
T-2	Thr	Thr	Ser	9
	Leu	Ala	Leu	10
	Ser	Thr	Ser	11
	Gly	Thr	Gly	12
T-3	Asp	Glu	Asp	21
T-4	Val	Val	Ile	33
T-5[c]	Asp	Glu	Asp	43
	Ser	Ser	Thr	50
	Pro	Ala	Pro	51
	Ile	Ile	Val	54
	Asn	Gly	Ser	56
	Ala[d]	Pro	Ala	58
T-9A	Asp	Glu	Asp	73
T-11	Lys	Arg	Lys	104
T-13	Gln	Pro	Gln	125
Differences	←——— 24 ———→			
	←——— 25 ———→			
	←——— 20 ———→			

a Placed by Edman Degradation
b The dog chain with Thr at Res. 130 is used here
c Placed in the raccoon by acetic acid cleavage of the tryptic peptide
d Placed by carboxypeptidases A+B

the possible exception of coatimundi. This produces a change in the peptide chromatograph since peptides αT-12B and C come out as one peptide instead of the usual two. Table 7 shows the differences in amino acid composition of tryptic peptides among dog, bear and raccoon hemoglobins. An attempt has been made to predict the position in the chain occupied in each instance by analogy with the dog and other published sequences (Dayhoff, 1972).

Residue 58 of the βT-5 peptide of polar bear was found to be Ala by treatment of the peptide with carboxypeptidases A and B, which removed lysine, alanine and asparagine from its C-terminus.

C. Raccoon (Procyon lotor)

Tables 5 and 6 show the amino acid compositions of the tryptic peptides from raccoon hemoglobin. These results show that since bear and raccoon and dog families diverged, at least 25 differences in sequence have developed between dog and raccoon hemoglobins and 24 between polar bear and raccoon, in contrast to Seal's suggestion (1969) that these animals all have the same hemoglobin which has remained unchanged for approximately 40 million years. Dog and raccoon β chains differ by 16 residues; there are nine differences between their α chains. Table 7 shows the differences in amino acid composition of tryptic peptides among dog, bear, and raccoon hemoglobins.

The αT-3 peptide of raccoon with the amino acid composition Ala, Thr, Trp, Asp, Lys was subjected to a one-step Edman degradation. Because 0.6 residue of alanine was removed, alanine can be placed at the N-terminus of the peptide, Res. 12.

The βT-5 peptide of raccoon was treated with 0.5 M acetic acid which splits out aspartyl residues to give smaller peptides as shown below in comparison with the dog βT-5 peptide (see Table 8 for amino acid compositions). This procedure showed that there are at least six amino acid differences in the sequences of the dog and raccoon βT-5 peptides instead of only three as found by their total amino acid compositions:

Res. #	41		43							50	51		
Dog βT-5	Phe	Phe	Asp	Ser	Phe	Gly	Asp	Leu	Ser	Thr	Pro	Asp	- - -
Raccoon βT-5 + Acetic Acid	(Phe,	Phe,	*Glu,*	Ser,	Phe,	Gly)	Asp	(Leu,	Ser,	*Ser, Ala*)		Asp	- - -

Res. #	54		56		59		
Dog βT-5 (contd)	Ala	Val	Met	Ser	Asn	Ala	Lys
Raccoon βT-5 + Acetic Acid	(Ala,	*Ile,*	Met,	*Gly*)			
	(Ala,	*Ile,*	Met,	*Gly,*	Asn,	*Pro,*	Lys)

Table 8. Amino acid composition of acetic acid cleavage products of βT-5 raccoon tryptic peptide[a]

Res. in Chain	41–46	48–51	53–56	53–59
Lysine				1.0
Asparagine				1.2
Serine	1.0	1.8		
Glutamic Acid	1.0			
Proline				0.9
Glycine	1.0		0.9	1.0
Alanine		1.0	1.1	1.0
Methionine			0.9	0.5
Isoleucine			1.1	1.0
Leucine		1.0		
Phenylalanine	3.0			

a Obtained from chain which was not glycinamidated

Table 9. Tryptic peptides from the aminoethyl, glycinamide β-chain of coatimundi (Nasua nasua) hemoglobin

Residue No.	βT-1 1-8	βT-2 9-17	βT-3 18-30	βT-4 21-40	βT-5 41–59	βT-6 60-1	βT-7 62-5	βT-8 66	βT-9A 67-76	βT-9B 77-82	βT-10A 83-87	$\beta T\text{-}10B_1$ 88-93	$\beta T\text{-}10B_2$ 94-5	βT-11 96-104	βT-12A 105-12	βT-12B 113-20	βT-13 121-32	βT-14 133-44	βT-15 145-6	Total Residues
Lysine	1.0	1.0			1.1	1.1	1.0	1.0	1.1	1.0	1.1		1.1			1.1	1.0	1.1		13 Lys
Histidine	1.0						0.9					1.0		0.9		1.8		0.9	1.0	8 His
S-Aminoethyl Cysteine												0.9			1.0					2 Cys
Tryptophan		0.2		0.4																2 Trp
Arginine			0.9	0.9										1.0						3 Arg
Aspartic Acid + Asparagine		1.0	2.0		3.1				1.0	3.0			0.9	1.9	1.1			1.0		15 Asx
Glutamic Acid + Glutamine	2.0		2.0	1.2	1.0				1.1			1.0		1.0			3.8			13 Glx
Threonine	0.9	1.7		1.0							0.9						0.9			6 Thr
Serine					2.8				1.7			0.9								6 Ser
Proline				1.0	2.0									0.9			0.8			5 Pro
Glycine	0.9		2.8	0.3	2.0		1.0		1.0		0.9				1.1	1.0		1.1	(1.2)	12 Gly
Alanine		2.1	1.1		1.1		1.1				1.1					1.1	2.0	4.1		13 Ala
Valine	0.9	1.1	3.0	1.9		1.0			0.9					1.0	2.0	0.9	1.0	2.7		17 Val
Methionine					1.0															1 Met
Isoleucine					0.9															1 Ile
Leucine	1.1	1.1	1.1	1.9	1.2				2.1	2.0		2.1		0.9	2.9	1.1		1.1		18 Leu
Tyrosine				1.0													1.1		0.9	3 Tyr
Phenylalanine					3.0				1.0		1.0			1.0		0.9	1.2			8 Phe
Glycine = Asp + Glu	2.0		2.9		3.0				1.0	1.1		1.1	1.1	2.1			1.2			

For explanation see footnote of Table 1

Table 10. Composition of tryptic peptides of *N. narica* which differ from those of *N. nasua*

	From slow Hb			From fast Hb	
	βT-2A	βT-2B	βT-10	βT-2	βT-10
Lys	1.0	1.0		1.0	
His			1.0		1.0
AE Cys			0.9		0.7
Trp		0.2		0.2	
Thr		1.0	1.0	1.9	1.0
Ser		1.0	1.1	1.0	1.0
Glu			1.0		1.0
Gln			1.0		1.1
Gly		1.1	1.0		1.0
Ala		1.0	1.0	2.0	1.0
Val		1.0		0.9	
Leu		1.0	2.1	1.1	2.2
Phe			0.9		1.0
Gly			(1.0)		(1.0)

Table 11. Differences in tryptic peptide composition among 3 coatimundi β-chains and those of Raccoon

Tryptic Peptide	Raccoon	Coatimundi			Probable Residue[a]
		N. nasua	*N. narica*		
	Ala	Gly	Slow	Fast	5
βT-1	Asp	Glu	Glu	Glu	6 or 7
βT-2	Thr	Thr	Lys	Thr	9
	Thr	Ala	Ser	Ser	13
	Gly	Asn	Gly	Ala	16
βT-3	Glu	Asp	Asp	Asp	21
βT-5	Ala	Pro	Pro	Pro	51
βT-10	Lys	Lys	Gln	Gln	87
βT-13	Pro	Gln	Gln	Gln	125

No. of Differences: ---4---; ----7----; ---2---; ----3-------; ---8----------; ---8----------

a Deduced by comparison with the sequence of dog β-chain and from the sequences of the *N. narica* βT-2 peptides

D. Coatimundi (Nasua nasua and Nasua narica)

The coatimundi is a close relative of the raccoon. One species, *Nasua nasua,* is found throughout most of South America. A second species, *Nasua narica,* inhabits southwestern United States to South America (Walker, 1964). Seal (1969) found that blood from nine *N. nasua* individuals showed only one band on electrophoresis, while 6 *N. narica* individuals had two bands – a fast one moving in the same position as human Hb A and a slow one with about 0.85 % the mobility of Hb A.

The two hemoglobins of *N. narica* were easily separable on DEAE Sephadex. Chain separation by globin electrophoresis showed that the α-chains of these two hemoglobins and of dog hemoglobin all had the same mobility. However the fast *N. narica* β-chain moved faster than the β-chains of dog and the slow *N. narica* fraction. Therefore it is the β-chains of *N. narica* rather than the α-chains which contain amino acid substitutions with charge differences.

The amino acid compositions of the β-chain tryptic peptides from the hemoglobin of *N. nasua* are given in Table 9. For the other two β-chains, only those peptides which are different have been included in Table 10. From these results it is obvious that the major difference between the β-chains of the two species is at Res. 87 where *N. nasua* has lysine and *N. narica* has glutamine. Aside from this, the remaining differences lie in the βT-2 peptides. Table 11 lists the differences between raccoon and the three coatimundi β-chains.

Manual sequencing was done on βT-2 from the fast *N. narica* β-chain. Chymotryptic digestion gave the two smaller peptides shown below in parentheses. Subtractive Edman degradations (arrows) showed that Thr was the N-terminal residue of the tryptic peptide and that Ser was the N-terminal residue of the Ser-Leu-Trp chymotryptic peptide. Tryptophan can be placed at the C-terminus of the latter peptide because of chymotryptic specificity (Res. 9 through 17):

Thr-Ala, Val, Thr (Ser-Leu-Trp) (Ala-Lys)
$\longrightarrow$ $\longrightarrow$

On the slow *N. narica* β-chain, the automatic sequenator gave the following sequence beginning at the N-terminus of the chain (Res. 1 through 20):

Val-His-Leu-Thr-Gly-Glu-Glu-Lys-Lys-Ala-Val-Thr-Ser-Leu-Trp-Gly-Lys-Val-Asn-Val

The presence of lysine at Res. 9 provides the charge difference between the slow and fast *N. narica* β-chains, the latter having threonine in this position. The lysine at Res. 9 is hard to detect manually because on tryptic hydrolysis it splits out and is added to the lysine already present as peptide βT-8. The remaining portion of the slow βT-2 peptide was found to be one residue shorter than the corresponding peptide from the fast β-chain and sequencing was necessary to determine whether this was due to a deletion or to the presence of lysine. Lysine in the #9 position has also been found in the β-chain of newt hemoglobin (Sullivan, 1974).

The tryptic peptides of the coatimundi α-chain have all been analyzed except for Res. 105–127. They show at least six differences from raccoon. This makes a total of at least 13 differences between the hemoglobins of the raccoon and coatimundi, both of which are members of the Procyonidae family.

E. Cat (Felix catus) α-chain

Taketa and coworkers (1974) and Lessard (1970) have separated the α and β chains of the 2 hemoglobins of the domestic cat. Amino acid analyses, fingerprints of tryptic peptides and hybridization experiments indicated that the α chains were the same but that the β chains were different. From hybridization experiments and PMB titrations, they concluded that there are three active cysteine residues per α chain. In addition the N-terminus of the α chain was found to be Val-Leu.

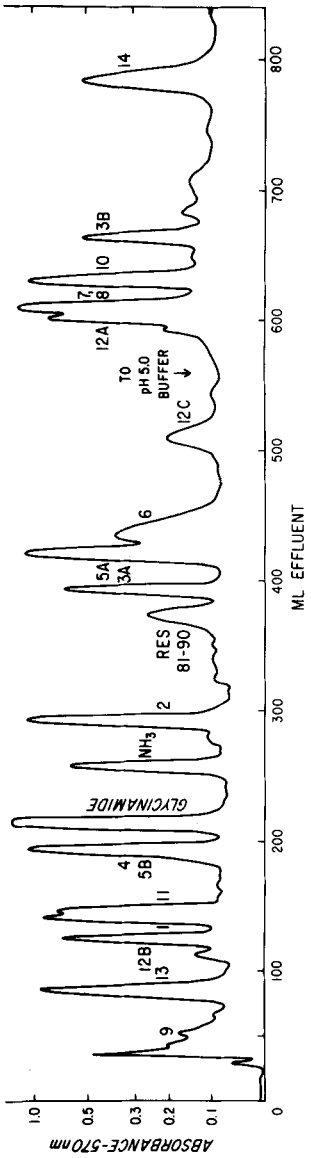

Fig. 1. Peptide pattern of the tryptic hydrolysate of 90 mg. of aminoethylated, glycinamidated α-chain from cat hemoglobin. Separation was obtained on a column of Aminex A-5 (0.9x30 cm) developed with a gradient of pyridine-acetic acid buffers. One-tenthof the effluent was reacted continuously with ninhydrin and the absorbance of the reaction products recorded automatically. The numbers above the zones identify the tryptic peptides found therein

Table 12. Tryptic peptides from the aminoethyl, glycinamidated α-chain of cat hemoglobin

Residue No.	αT-1 1-7	αT-2 8-11	αT-3A 12-13	αT-3B 14-16	αT-4 17-31	αT-5A 32-34	αT-5B 35-40	αT-6 41-56	αT-7,8 57-61	αT-9 62-90	αT-10 91-92	αT-11 93-99	αT-12A 100-4	αT-12B 105-11	αT-12C 112-27	αT-13 128-39	αT-14 140-1	Total Residues
Lysine	1.0	0.9		1.0			1.0	1.1	1.1	1.1		1.1			1.1	1.1		10 Lys
Histidine					1.0			2.0	0.9	2.0			0.9		3.0			10 His
S-Aminoethyl Cysteine			0.9			0.9							1.0	0.8				4 Cys
Tryptophan				0.8														1 Trp
Arginine					1.0						1.0						1.0	3 Arg
Aspartic Acid + Asparagine	1.1	1.1						1.0		3.7		1.9			1.0			10 Asx
Glutamic Acid + Glutamine					3.0			1.0	1.1	1.0					1.1			7 Glx
Threonine						1.0	2.0	0.9		1.8				0.9	1.0	2.0		10 Thr
Serine	1.0	1.0			1.0		0.9	2.0		2.2			0.9		1.1	2.9		13 Ser
Proline							1.1	1.0		0.9		1.1			2.1			6 Pro
Glycine		0.3		1.0	3.0			1.1	1.1									6 Gly
Alanine	2.0		1.0		3.1			1.0	1.0	6.8				1.0	3.0	1.0		20 Ala
Valine	1.0	0.9						1.0		2.0		1.9		1.0	0.9	2.0		11 Val
Methionine										2.0								2 Met
Isoleucine					1.0													1 Ile
Leucine	1.1				1.1			1.1		4.0	1.0		1.1	3.1	1.0	1.0		14 Leu
Tyrosine					1.1			1.0		1.0							1.0	4 Tyr
Phenylalanine						1.1	1.0	2.0				1.1	1.0		0.9	2.1		9 Phe
Glycine = Asp + Glu	1.0				3.0			1.1		4.0		1.0			2.0			

For explanation see footnote of Table 1

Figure 1 shows the peptide pattern from the tryptic hydrolysis of the aminoethylated, glycinamidated cat α chain. After rechromatography of each peak, amino acid analyses were run on the purified peptides. Table 12 gives the amino acid composition of the 17 tryptic peptides comprising the α chain. The peptides are numbered to conform with the tryptic peptides of human hemoglobin.

Four aminoethyl cysteine peptides were found. Two of the cysteines at Res. 13 and 34 have not previously been found in these positions in other animals. The cysteines were assigned to these positions because neither αT-3 nor αT-5 was found as one peptide.

	αT-3
Dog	Ser Thr Trp Asp Lys
Cat	(Ala,Cys)(Trp,Gly,Lys)
Human	Ala Ala Trp Gly Lys
	αT-5
Dog	Thr Phe Gln Ser Phe Pro Thr Thr Lys
Cat	(Thr,Phe,Cys)(Ser,Phe,Pro,Thr,Thr,Lys)
Human	Met Phe Leu Ser Phe Pro Thr Thr Lys

The tryptophan in peptide αT-3B was confirmed by digestion of the peptide with Aminopeptidase M followed by amino acid analysis. The other two cysteines at Res. 104 (αT-12A) and Res. 111 (αT-12B) have been found in these same positions in the dog, fox, coyote and raccoon. The αT-12A peptide differs in having phenylalanine at its N-terminus (Res. 100) rather than leucine. The position was determined by one Edman degradation which removed 0.8 residue of phenylalanine. The opossum α chain also has phenylalanine at this position (Stenzel, 1974).

There are 4 compositional differences between the cat αT-4 peptide and its dog homologue. In order to narrow down the possibilities, the peptide was cleaved by chymotrypsin into three smaller peptides:

Res. #	17	19				23	24	25	26			29	30	31
Dog αT-4	Ile	Gly	Gly	His	Ala	Gly	Asp	Tyr	Gly	Gly	Glu	Ala	Leu	Asp	Arg
Cat αT-4 + Chymotrypsin	(Ile	Gly	Ser,	His,	Ala,	Gly,	Glu,	Tyr)(	Gly	Ala,	Glu,	Ala,	Leu)(	Glu	Arg)

The peptide containing Res. 17–24 was subjected to three Edman degradations. The results are represented by arrows under the residues involved, which showed that serine in the cat replaced glycine in the dog at Res. 19. Glutamic acid probably replaces aspartic acid at Res. 23. The chymotryptic peptide containing Res. 25–29 lost its one glycine residue after one Edman degradation, so that alanine probably replaces the other glycine, Res. 26. The third chymotryptic peptide, Res. 30–1, showed that glutamic acid replaces aspartic acid at Res. 30. Table 13 contains the amino acid compositions of these and subsequent peptides.

Similarly, the cat αT-9 peptide, with *three* compositional differences from dog, was hydrolyzed into smaller peptides with the result that *four* sequence differences were found. For purposes of this discussion, only Res. 62 to 80 of T-9 are shown in the diagram below because the remaining residues 81–90 are the same in cat and dog.

Table 13. Amino acid composition of cyanogen bromide and chymotryptic peptides from αT-4 and αT-9 of cat hemoglobin

	αT-4		αT-4 + Chymotrypsin					αT-9 + CNBr		αT-9 + Chymotrypsin				
	Ed II 19-31[a]	Ed III 20-31	25-29	Ed I 26-29	17-24	Ed I 18-24	30-1	62-73	81-90	62-66	74-80	81-86	87-9	90
Lysine	not run								1.2					1.0
Histidine	not run				1.0	1.0		1.0	1.0				1.0	
Homoserine								0.7						
Arginine							0.9							
Aspartic acid								1.1	1.1	1.0[c]	2.2[c]	1.0		
Threonine								1.0			1.0			
Serine	0.8	0.4			0.9	1.0			1.6			1.9		
Glutamic acid	3.1	3.0	1.0	1.1	1.0	1.1	1.1	0.9						
Proline											0.8			
Glycine	2.1[c]	2.1	1.0	0.3	1.8	1.8								
Alanine	3.1	3.0	2.0	1.8	1.0	1.1		4.0	2.2	2.0	1.0	1.1	1.0	
Valine								1.8		1.0				
Methionine											0.7			
Isoleucine	0	0			1.0	0								
Leucine	1.0	1.0	1.0	1.1				1.0	1.9	1.1	1.0	2.1	1.0	
Tyrosine	0.9	1.0			1.0	0.8			0.9					
Glycine[b]			1.0	1.0	1.1	1.0	1.0	1.1	1.2					

a Residue numbers in chain
b From glycinamidated Asp and Glu residues
c From unglycinamidated chain
Underlined residues are those removed by Edman degradation

	Part of αT-9 peptide																		
Res. #	62					68					73								80
Dog	Val	Ala	Asp	Ala	Leu	Thr	Thr	Ala	Val	Ala	His	Leu	Asp	Asp	Leu	Pro	Gly	Ala	Leu
Cat																			
+ CNBr	(Val,Ala,Asp,Ala,Leu,Thr,Gln,Ala,Val,Ala,His,Met)																		
Cat																			
+ Chymo-																			
trypsin	(Val,Ala,Asp,Ala,Leu)																		
																			(Asp,Asp,Leu,Pro,Thr,Ala,Met)

After treatment with cyanogen bromide, only two of the three possible peptides were recovered. One contained Res. 81–90 and did not differ from the corresponding residues of the dog chain. A second peptide contained Res. 62–73 which corresponded to the terminal portion of αT-9 and had a homoserine which is formed where methionine is cleaved. This places a methionyl residue at Res. 73 where dog has leucine. This cyanogen bromide peptide also contained glutamine, presumably replacing threonine #67 or 68 in the dog.

The αT-9 peptide was then treated with chymotrypsin. Four of the resulting chymotryptic peptides were the same as in dog. A fifth chymotryptic peptide, Res. 74–80, con-

Table 14. Amino acid differences among cat, dog and human α-chains

Peptide	Cat	Dog[a]	Human[a]	Probable residue in α-chain
T-1	Ala	Pro	Pro	4
T-2	Ser	Thr	Thr	8
	Val	Ile	Val	10
T-3	Ala	Ser	Ala	12
	Cys	Thr	Ala	13
	Gly	Asp	Gly	15
T-4	Ile[b]	Ile	Val	17
	Ser[b]	Gly	Ala	19
	Glu	Asp	Glu	23
	Ala	Gly	Ala	26
	Glu	Asp	Glu	30
T-5	Thr	Thr	Met	32
	Cys	Gln	Leu	34
T-6	His	Pro	His	50
T-7,8	Ala	Ala	Gly	57
	Gln	Lys	Lys	60
T-9	Gln	Thr	Asn or Thr	67 or 68
	Met	Leu	Val	73
	Leu	Leu	Met	76
	Thr	Gly	Asn	78
	Met	Leu	Leu	80
	Tyr	Tyr	His	89
T-12A	Phe[b]	Leu	Leu	100
T-12B	Cys	Cys	Ala	111
T-12C	His	His	Leu	113
	Ala	Thr	Ala	115
T-13	Phe	Phe	Leu	129
	Ser	Ala	Ala	131

a Human and dog have been sequenced
b Located by Edman degradation

Table 15. Tryptic peptides from the aminoethyl, glycinamide β-chain of lion hemoglobin

Residue No.	βT-1 1-8	βT-2 9-17	βT-3 18-30	βT-4 31-40	βT-5 41-59	βT-6 60-1	βT-7 62-5	βT-8 66	βT-9A 67-76	βT-9B 77-82	βT-10A 83-87	βT-10B$_1$ 88-93	βT-10B$_2$ 94-5	βT-11 96-104	βT-12A 105-12	βT-12B,13 113-32	βT-14 133-44	βT-15 145-6	Total Residues
Lysine	1.1	1.0			1.1	1.1	1.1	1.0	1.0	1.2	1.0		1.1			1.2			11 Lys
Histidine							1.0					1.1		1.0		2.8	1.0	1.1	8 His
S-Aminoethyl Cysteine												1.0			1.0				2 Cys
Tryptophan		0.1		0.2															2 Trp
Arginine			1.0	0.9										0.9			0.9		4 Arg
Aspartic Acid + Asparagine		1.0	2.0		2.8				2.0	2.9			1.0	1.7	1.1	1.0			16 Asx
Glutamic Acid + Glutamine	1.9		1.9	1.0	1.0							0.9		1.0		4.0			12 Glx
Threonine				1.0															1 Thr
Serine	1.9	1.0			4.0				2.1			0.9					1.0		11 Ser
Proline				1.0										0.9		1.0			3 Pro
Glycine		2.0	2.9		1.1		1.0		1.1		1.0				1.0	1.0	1.2	(1.1)	12 Gly
Alanine	1.0		1.1		3.0		1.0				1.9					3.1	3.7		15 Ala
Valine		1.0	2.7	2.0		0.9			0.8					1.0	1.9	1.9	2.9		16 Val
Methionine					1.0														1 Met
Isoleucine					1.0					1.0									2 Ile
Leucine	1.1	2.2	1.0	2.2	1.0				2.0	1.1		1.9		1.2	3.0	1.1	1.3		18 Leu
Tyrosine				1.0														0.9	2 Tyr
Phenylalanine	1.0				3.0				1.2		1.1			1.2		3.0			10 Phe
Glycine = Asp + Glu	2.0		2.8		2.1				1.2	2.0		1.0	1.1	2.0		1.0			

For explanation see footnote of Table 1

tains threonine rather than glycine which occupies Res. 78 in dog. It also has methionine in place of one of the two leucines (Res. 76 or 80) in dog. The methionine was placed at Res. 80 by treating the chymotryptic peptide with carboxypeptidase A which released methionine. Thus αT-9 cat contains two methionines for two leucines, a threonine for glycine, and a glutamine for threonine as compared to αT-9 dog.

An attempt has been made in Table 14 to predict the residues at which the cat α chain differs from the known sequences of dog and human α chains.

It cannot be assumed that the α chains of the two cat hemoglobins are identical merely because they show the same electrophoretic behavior. However, if there were any differences in amino acid residues not involving charge differences, the 2 tryptic peptides involved would be expected to elute together from the chromatographic column. Amino acid analysis of the zone would then show fractional residues of the amino acids which were different. No such fractional residues were found, at least above the 0.2 residue level.

The 21 differences between cat and dog α chains plus 18–20 differences between their β chains represents a total of approximately 40 differences which probably have developed since the Feloidae and Canoidae branched off from one another in the late Eocene.

F. Lion (Panthera leo) β chain

Taketa et al. (1973) have separated the two major hemoglobins of lion and other felines by chromatography. Both lion hemoglobins, which Taketa has named Hb B′ (slower) and C′ (faster), have acetylated N-termini on their β chains. Their hybridization experiments showed that the α chains were responsible for the difference in electrophoretic mobility between the 2 lion hemoglobins; it is the β chains which provide the electrophoretic difference in the cat, but only because one β chain has an acetylated N-terminus.

Electrophoresis of the original hemolysate in pH 8.4 buffer on cellulose acetate showed two bands, both faster than either hemoglobin of domestic cat. Electrophoresis of the two hemoglobin fractions from IRC chromatography showed a good separation. Chain separation by globin electrophoresis in urea-mercaptoethanol confirmed the presence of two different alpha-chain bands, but only one beta-chain band.

Table 15 shows the amino acid compositions of the tryptic peptides of the lion β chain. The β chains of both fast and slow hemoglobins were analyzed in this manner but no difference was found between them. The tryptic peptide compositions of fast and slow α chains, except for the two αT-9 peptides, have also been determined. Although neither αT-9 peptide could be isolated, duplicate amino acid analyses of the two α chains show that the fast chain has one more Asx and Gly (a glycinamidated Asp residue) and one less Ala than the slow chain. The differences between the two α chains appear to be located in the αT-9 peptide, since all the other peptides have been isolated and show no differences.

Both lion hemoglobins are electrophoretically faster than either of the cat hemoglobins because lion has Gln for Lys at Res. α56 and Glu for Ala at Res. α120. The fast lion hemoglobin differs from the fast cat hemoglobin by at least three residues in the β chain and 4 or more residues in the α chain.

Wurster (1969), on the basis of karyotypic similarities, believes that modern species of Felids are derived from a common ancestor as recently as five million years ago.

Further comparisons of the presumed sequences of these carnivore hemoglobins and a discussion of their significance is presented in the next article (Stenzel and Brimhall, 1977).

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