in water but greatly magnified in 0.46*M* D-mannitol. These authors came to the same conclusion that we did, namely, that an increase in "growth potential" of the embryo is the essential factor in germination of dormant seeds. Since Grand Rapids lettuce is a light-promoted seed, the pattern appears to be a general one and to hold for photo-, scoto-, and thermodormancy.

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Hemoglobins in Sheep: Multiple Differences in Amino Acid Sequences of Three Beta-Chains and Possible Origins

Abstract. *Among the three adult sheep hemoglobins (A, B, and C), two (A and B) are reportedly products of alleles. The β-chains of A and B differ by at least seven scattered amino acid residues whereas the β-sequence of C differs from A by at least 16 residues and from B by at least 21 residues. These changes suggest that the origin of C-β antedated the divergence of A and B. Five shared differences between A-β and C-β with respect to B-β can be interpreted as the result of selective advantage in favor of B. A complex of additional mechanisms has possibly been involved in maintaining the A-B-C polymorphism.*

The hemoglobins A and B of domestic sheep, described by Harris and Warren (*1*), form an electrophoretically distinguishable genetic polymorphism. Electrophoretic chromatographic patterns (fingerprints) of tryptic peptides (*2*), molecular dissociation-reassociation experiments (*3*), and amino acid composition of entire polypeptide chains (*4*) establish that relevant variation between the two hemoglobins is limited to the β-chains. Amino acid composition of entire chains indicates that the β-chains from hemoglobins A and B (A-β and B-β), although apparently allelic products (*1, 5*), possess multiple differences (*4*). To this already unusual situation van Vliet and Huisman (*6*) have recently added a third hemoglobin which they term hemoglobin C. An apparently identical component, hemoglobin N, has been described by Braend and Efremov (*7*). Various analyses by van Vliet and Huisman (*6*) and others (*8*) indicate that the characteristic differences between C and the other two hemoglobins are confined to the β-chains. Comparison of amino acid compositions of entire β-chains suggests that there are at least 17 differences between B and C and 12 between A and C (*4*). Two dimensional peptide patterns (fingerprints) (*9*) and partial analysis of tryptic peptides (*8, 10*) clearly indicate that such differences are scattered throughout the chains.

Two features (*11*) influencing the occurrence of hemoglobin C (Fig. 1), are the limitation of hemoglobin C to animals heterozygous or homozygous for the hemoglobin A gene (*6, 7, 8*), and the fact that although traces of hemoglobin C may be present in nonanemic sheep, this component becomes the sole form in animals severely anemic from either blood loss (*6*) or chemically induced hemolysis (Fig. 1). During recovery from anemia, hemoglobin C disappears while hemoglobin A reappears (*6*). These features suggest that the hemoglobin A-B-C system might provide a useful model for examining genetic and cellular factors concerned with regulation of protein synthesis in higher organisms. It seemed desirable first to delineate more exactly the differences between the three known sheep beta chains. The need for such additional information arises from the fact that protein structure and quantity synthesized may, in part, have a common genetic determinant (see *12*).

In this report we describe nearly complete sequences of the three sheep β-chains and discuss the nature and origin of the interchain differences.

Hemoglobins A, B, and C were obtained from a local flock of Dorset sheep. The α- and β-chains were separated by gradient elution from carboxymethylcellulose (*13*). Isolated chains were digested with trypsin, and peptides separated either by high-voltage filter-paper electrophoresis and solvent chromatography (*13*) or by column chromatography. The most successful method consisted of prior separation of peptides into groups by passage through Sephadex G-25 (Pharmacia) columns (1.8 by 240 to 440 cm) (*14*) and subsequent intragroup resolution through elution with a pyridine-acetate gradient from columns of Beckman Spinco 15A resin (*15*). This last combination of methods completely separated all but a few peptides (*16*) and gave higher yields than filter-paper methods.

The amino acid composition of individual peptides was determined (*17*), and the identity of individual tryptic peptides was established both by homology with known sequences of other hemoglobin chains (*18*) and by the radioactive-assembly technique of Dintzis (*13*). The latter method was used to identify B-β and C-β tryptic peptides. The A-β peptides were identified entirely by homology with those from B and C. Sequences of amino acids within peptides were derived by a combination of the fluorescent end-group technique (*19*) and modification of Edman's method of stepwise degradation (*20*). Selective hydrolysis of tryptic peptides by weak acids, chymotrypsin, leucine aminopeptidase, and carboxypeptidase A and B further aided sequence analysis.

Comparison of sheep β-chain sequences is made in Table 1. With one exception, the gross arrangement of tryptic peptides depends upon mutually confirmatory evidence from assembly experiments and from homology with the known sequences of horse and human hemoglobin chains (*18*). The outstanding example of the value of assembly analysis is the exceptional Pro-Asn-Lys (*21*) sequence of C-β-6 to -8, for which there is no obviously homologous peptide in man, horse, or sheep. This sequence is considered NH_2-terminal because its specific activity is the least of any on assembly with radioactive lysine and because we find that proline is the NH_2-terminal amino acid of the intact C-β chain (*22*). The precise placement of the first two resi-

Table 1. Comparison of beta chains of sheep hemoglobins. Sequences joined by hyphens were determined by analysis. Hyphens between adjacent tryptic peptides, which—except for residues 145 to 146—have carboxyl terminal Lys or Arg, have been included only where analysis of overlapping peptides is available. Parenthetical sequences are arranged either by homology with other sheep β-chains or with chains of other species (*18*). The 145 amino acids of sheep A-β- and B-β-chains and the 141 amino acids of sheep C-β-chain are numbered to correspond with numbers assigned to homologous residues among the 146 amino acids of human β- and γ-chain (*18*). No residue is assigned to position 2 in sheep chains and the numbering of the first three C-β residues is arbitrary. Residues showing interchain differences are designated by underscored capital letters.

```
                1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

B-β  NH2-Met- - -Leu-Thr-Ala-GLU-GLU-Lys Ala-ALA-VAL-Thr-Gly-Phe-Trp-GLY-Lys Val-Lys-Val-Asp-Glu-Val-Gly-Ala-Glu-Ala-Leu-Gly-Arg Leu-Leu-Val-Val-Tyr-Pro-
A-β  NH2-Met- - -Leu-Thr-Ala-GLU-GLU-Lys Ala-ALA-VAL-Thr-Gly-Phe-Trp-GLY-Lys Val-Lys-Val-Asp-Glu(Val Gly Ala Glu)Ala-Leu-Gly-Arg Leu-Leu-Val-Val-Tyr-Pro-
C-β                     NH2-PRO-ASN-Lys Ala-LEU-ILE-Thr-Gly-Phe-Trp-SER-Lys Val-Lys-Val-Asp-Glu-Val-Gly(Ala Glu)Ala-Leu-Gly-Arg Leu-Leu-Val-Val-Tyr-Pro-

                37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73

B-β  Trp-Thr-Gln-Arg Phe-Phe-Glx-His-Phe-Gly-Asx(Leu Ser ASX Ala)Asx-Ala-Val-MET-ASN-Asn-PRO-Lys Val Lys Ala-His-Gly-Lys-Lys Val-Leu(Asx Ser Phe Ser Asx
A-β  Trp-Thr-Gln-Arg Phe-Phe-Glx-His Phe(Gly Asx Leu Ser SER Ala Asx Ala Val MET)ASN(Asn ALA)Lys Val-Lys Ala-His-Gly-Lys-Lys Val-Leu-Asx-Ser-Phe(Ser)Asx
C-β  Trp-Thr-Gln-Arg Phe-Phe-Glu-His-Phe-Gly-Asp-Leu-Ser(THR Ala)Asp-Ala-Val-LEU-GLY-Asn-ALA-Lys Val-Lys Ala-His-Gly-Lys-Lys Val-Leu-Asx-Ser-Phe-Ser(Asx

                74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110

B-β  Gly)MET-LYS His-Leu-Asp-Asp-Leu-Lys Gly-Thr-Phe-Ala-Gln-Leu-Ser-Glu-Leu-His-Cys-Asp-Lys Leu-His(Val Asx Pro Glx Asx Phe)Arg Leu-Leu-Gly-Asx(Val Leu
A-β  (Gly VAL GLX His-Leu Asx Asx Leu)Lys Gly-Thr-Phe-Ala(Glx Leu Ser Glx Leu)His-Cys-Asp-Lys Leu-His-Val-Asx-Pro-Glx-Asx-Phe-Arg Leu-Leu-Gly-Asx(Val Leu
C-β  Gly VAL GLX His Leu Asx)Asx-Leu-Lys Gly-Thr-Phe-Ala-Glx(Leu Ser Glx Leu)His-Cys-Asp-Lys Leu-His-Val-Asx-Pro-Glx(Asx Phe)Arg Leu-Leu-Gly-Asx-Val-Leu-

                111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146

B-β  Val Val Val Leu Ala)Arg His HIS-Gly-ASN-Glu-Phe-Thr-Pro-VAL-Leu(Gln Ala ASP)Phe-Gln-Lys Val-Val-Ala-Gly-Val-Ala-ASN-Ala-Leu-Ala-His-LYS-Tyr-His-COOH
A-β  Val Val Val Leu Ala)Arg His-HIS-Gly(SER Glx Phe Thr Pro VAL Leu Glx Ala GLX Phe Glx)Lys Val-Val-Ala-Gly-Val-Ala-ASN-Ala-Leu-Ala-His-ARG-Tyr-His-COOH
C-β  Val-Val-Val-Leu-Ala-Arg His-PHE-Gly-LYS Glx-Phe-Thr-Pro-GLX-Leu-Glx-Ala-GLU-Phe-Gln-Lys Val-Val-Ala-Gly-Val-Ala-SER-Ala-Leu-Ala-His-ARG-Tyr-His-COOH
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dues of C-β compared to A-β and B-β is necessarily arbitrary since it is possible, for example, that a genetic deletion of unknown size and location exists between the codons for C-β-7 and C-β-8. Thus, C-β-6 to -7 may not be genetically homologous with A-β- and B-β-6 to -7, as implied in Table 1, but rather—for instance—homologous with the first two A-β and B-β residues. This argument can be extended to question the homology of lysine residues at β-8. Such possibilities should not, however, be confused with the possible existence of a small but missing tryptic peptide lying between C-β-8 and -9. The existence of such a peptide seems unlikely, since comparison of the sum of amino acids derived from examination of individual peptides with the sum deduced by Huisman *et al.* (*4*), and independently by ourselves, from analysis of acid hydrolyzates of entire sheep β-chains indicates no major discrepancies. We thus presume that the β-chains shown in Table 1 are numerically complete.

A summary of the minimum number of interchain differences is given in Table 2. Differences between sheep β-chains and the β- and γ-chains of human hemoglobin are also compared in this table. The latter comparisons indicate, overall, that each of the three sheep β-chains resembles the human β-chain more closely than the γ-chain. Such resemblance supports the propriety of applying the term β to the chains of adult sheep hemoglobin that are not α-chains. Further inspection indicates that the number of differences between sheep β-chains and human γ-chains approximates that between human β- and γ-chains. These relationships suggest that the genetic duplication which presumably gave rise to the precursors of β and γ (*23*) took place before evolutionary divergence of lines leading to sheep and men.

The amino acid differences (Table 1) can also be examined for the minimum number of nucleotide substitutions, as deduced from the genetic code (*24*), necessary to account for such differences. The comparison of variant proteins with the normal suggests that the number of nucleotide substitutions can be equated with the minimum number of mutations. For example, the amino acid changes present in each of 35 mutant human hemoglobins are explicable by single nucleotide changes within single codons (*25*). Thus, single substitutional mutations usually reflect single nucleotide changes. Consequently, amino acid changes explicable only by double nucleotide changes probably represent at least two separate mutations while those involving triple nucleotide changes probably reflect at least three separate mutations. Two of three interchain comparisons (Table 2) in sheep involve more nucleotide changes, that is, mutations, than immediately are evident from the number of amino acid differences. In some instances, the minimum number of mutations is possibly greater than given and the ratio of nucleotide to amino acid differences is certainly greater. For example, no nucleotide value has been assigned to the presumptive loss of four amino acids from the NH_2-terminus of C-β. Although the mechanism of this loss is unknown (*26*), it probably involves at least one mutation. When the first four amino residues of A-β and B-β are omitted from interchain comparisons, the ratio of nucleotide to amino acid changes becomes more impressive, for example, at least 18 nucleotide changes are necessary to account for the 12 remaining amino acid differences between C-β and A-β.

These nucleotide changes are in part dependent on homology assumed between the NH_2-terminal proline-asparagine (Pro-Asn) sequence of C-β and residues of the other sheep β-chains. The match, given in Table 1, between Pro-Asn of C-β and Glu-Glu (glutamic acid) of the other chains requires a total of four nucleotide changes, whereas a match of Pro-Asn with the Leu-Thr present at A-β- and B-β-3 to -4 requires a total of only two nucleotide changes. However, this latter arrangement necessitates an additional mutation in the form of a genetic deletion between Pro-Asn and Lys. All other possible matches require a total of three to four nucleotide changes plus whatever deletions are necessary. Thus there appears to be a cluster of mutations in the NH_2-terminal region of C-β, to which the two-step mutation required at β-10 can be added. While not unique in interchain comparisons, the clustering at the NH_2-terminal segment of C-β of two-step mutations nonetheless suggested that this segment might be less like human β-chain than the remainder of the chain. This conjecture is not supported by comparisons of the first 12 residues of C-β (C-β-6 to -17) with various other chains (*18*). Changes in these residues between C-β and either human α-chain or human

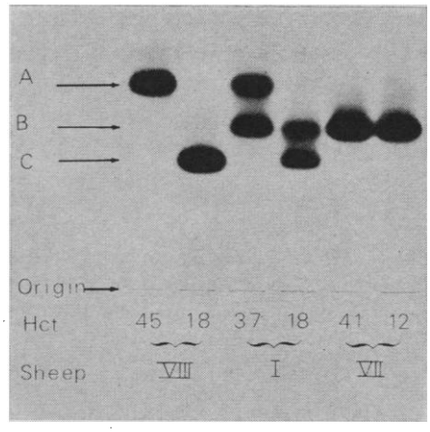

Fig. 1. Starch-gel electrophoresis (*12*) patterns demonstrating the effect of acute hemolytic anemia upon sheep hemoglobins. Gel was stained with amido-black stain. Anode lies at top of figure. Anemia was induced by intramuscular injection of acetylphenylhydrazine, 4 to 7 mg per kilogram of body weight each day for 8 to 12 days. Hematocrits (*Hct*) are those at time of sampling. From left to right are shown paired samples—before and after appearance of anemia—in sheep VIII, homozygous for hemoglobin A; in sheep I, heterozygous for A and B; and in sheep VII, homozygous for B. Results are identical with those in similar sheep after chronic loss of blood (*6*).

Table 2. Sheep hemoglobin β-chain differences. Comparisons are based on sequence and homology as given in Table 1.

Comparison*	Number of minimum differences	
	Amino acids	Nucleotides
A vs. B	7	7– 9†
A vs. C	16	18–19†
B vs. C	21	22–24†
Human β vs. A	27	33
Human β vs. B	25	31
Human β vs. C	32	33
Human γ vs. A	41	50
Human γ vs. B	41	49
Human γ vs. C	40	43
Human γ vs. human	39	48

* Absence of four residues from the NH_2-terminal region of C-β is scored as four amino acid differences in comparisons with A-β and B-β and as five amino acid differences in comparisons with human β and γ. No nucleotide differences are assigned to these particular amino acid changes thereby accounting, for example, for the fact that the total number of amino acid differences between human β and C-β only slightly exceeds the number of nucleotide differences. The minimum amino acid differences between sheep β-chains correspond closely to that described by Wilson *et al.* (*10*). The comparison of human β with sheep A-β approximates the value estimated by Beale *et al.* (*8*). †The range of the minimal nucleotide changes derives from uncertainty concerning distinctions between acid and amide at residues B-β-50, C-β-125, and A-β-129. In addition, certain comparisons involve assumptions of position not specifically established by sequence analysis (Table 1). These occur at A-β-50, -55, -58, -75, -76, -120, -125, and -129; B-β-50 and -129; C-β-50, -75 and -76. When assigning differences between sheep and human chains, Glx residues in sheep are considered equivalent to either Glu or Gln in man and Asx residues equivalent to either Asp or Asn.

β-chain involve a minimum of 11 mutations while those with human γ-chain involve eight mutations. The slightly enhanced correspondence between human C-β and -γ can be discounted by the parallel finding of even greater similarity between homologous portions of human γ and both A-β- and B-β-6 to -17 chains. Accordingly, we find no support for the conjecture that C-β arose by intragenic, nonhomologous crossing over such that an α-like or γ-like gene contributed the NH_2-terminal portion of the chain and a β-like gene the balance of the chain.

An additional feature, based upon nucleotide changes, emerges in comparisons of NH_2-terminal residues of A-β and B-β with the human β- and γ-chains. The NH_2-terminal sequences of man's β- and γ-chains are, respectively, NH_2-Val-His-Leu-Thr-Pro-Glu-Glu-Lys- and NH_2-Gly-His-Phe-Thr-Glu-Glu-Asp-Lys- (*18*). When these sequences are compared with A-β and B-β, questions of homology arise from the fact that one residue is missing from the corresponding tryptic peptide in sheep A-β and B-β chains. Specifically, does the NH_2-terminal Met of A-β and B-β correspond to the second residue of the human chains, or is the fiction of an intrachain gap within the sheep A-β- and B-β-chains necessary to afford maximum homology? No firm solution is provided by simple comparison of amino acids. However, a reasonable decision can be reached on the basis of the number of nucleotide changes required to account for various matches. A match between the second residue (His) of the human β- and γ-chains with the first residue (Met) of A-β and B-β necessitates three nucleotide changes, that is, three mutations. However, if all NH_2-terminals are matched and a gap is allowed such that the third residue of the human chains corresponds to the second residue of A-β and B-β, then—for these two positions—only one nucleotide alteration is required between human β- and sheep β-, and only two between human γ- and sheep β-chains. This type of reasoning, given the fact that triple nucleotide changes are never necessary to account for differences between human β-, γ-, and α-chains, suggests that the latter arrangement is preferable. This preference is reflected in numbering residues (Table 1).

At first sight, the differences between the three sheep β-chains (Table 2) fit easily into an evolutionary scheme whereby the gene duplication giving rise to an ancestral form of β^C occurred before divergence of β^A and β^B. The plausibility of this scheme is supported, first, by the relatively close resemblance of A-β and B-β; and second, by the fact that A-β and B-β each differ from C-β by approximately the same minimum number of nucleotide changes (Table 2). The approximate nature of these numbers does not invalidate this scheme since dispersion equivalent to plus-or-minus the square root of each difference can be expected. It is assumed in this scheme that mutations have randomly affected all three genes and that selection has been equivalent toward animals with these mutations. The possibility that selection might be relaxed against mutations of the essentially inactive β^C gene suggests an alternative evolutionary scheme. If selection had a substantial effect in eliminating animals with mutations of β^A and β^B, then lesser selection against mutations of β^C would result in an apparently greater number of C-β alterations per unit time. It is therefore possible that the origin of C-β is less remote than predicted. If selective disparity is great enough, it is even possible that the duplication producing β^C arose after divergence of β^A and β^B. If β^C arose later as a result of β^A duplication, then the slightly closer similarity between A-β and C-β than between B-β and C-β is explained. Specifically, A-β and C-β share five dissimilarities with respect to B-β (*27*) whereas B-β and C-β bear no changes in common with respect to A-β. These findings are unexpected by the first scheme, in which duplication producing C-β precedes other events, but not by the second scheme in which selection is relaxed against C-β, and β^C arises after divergence of β^A and β^B. Accordingly, these discrepancies invalidate the first scheme. The principal flaw to the second scheme is the substantial selective differential required for its operation. Furthermore, the requirement of a silent β^C gene may not exist in nondomestic populations with parasite-induced anemia. It is also possible that factors which limit the expression of β^C to anemic animals have comparatively recent origin.

A third scheme of A-B-C evolution can be advanced which partially circumvents the flaws inherent in the two just described. In this scheme, supposedly, both the β^A and β^B genes diverged long after the creation of β^C. The gross order of relatedness implicit in Table 2 is thus satisfied as it was in the first scheme. The lack of common differences on the part of B-β and C-β with respect to A-β could derive from vigorous selection in favor of animals with certain B-β mutations. Within a given time, the number of persistent mutations in B-β might thereby exceed those that persist in A-β and C-β. If these mutations involve the five residues wherein β^B differs in the same way from both β^A and β^C (*27*), then the inconsistencies which made flaws in the first scheme are overcome. Once again, an appreciable selective differential is required. However, in this instance only five to six mutations are involved, in contrast to the much larger number demanded by the second scheme. When seeking sources of selective advantage in animals with β^B, it is relevant that the increased oxygen affinity shared by hemoglobins A and C (*6*) in contrast to B probably arises from one or more of the five amino acid differences shared by A-β and C-β with respect to B-β (*27*). A selective differential in some way related to this variation in oxygen affinity is easily imagined but has, at present, no foundation. There is, however, evidence that the presence of hemoglobin B may confer advantage in the form of enhanced reproductive performance (*28*).

If the third scheme for A-B-C evolution is correct (*29*) then the finding of multiple differences between the presumably allelic (*1, 5*) β^A and β^B genes, unexpected from study of human hemoglobin mutants, is potentially explicable as an episode in the selective process whereby one allele replaces another. Simultaneous displacement of both β^C and β^A by β^B could be another feature of this process. Similarities between A-β and C-β suggest that they were produced as duplicates of a pre-existing gene and would thus be, initially at least, linked in coupling. If phase-limited linkage persists, selection against the β^A—β^C loci may operate—where β^C is largely silent—principally through β^A and its product. Coupled linkage between β^A and β^C also provides a mechanism for the limitation of hemoglobin C to animals with the β^A gene. It should be emphasized that these assumptions, although consonant with the origin of interchain differences postulated by the second and third schemes, are unproved. It is still possible, for example, that β^B/β^B animals possess the β^C gene but fail to activate it.

Despite the extent to which selective

advantage in favor of animals with β^B figures in our speculation, it is possible —as Evans (*28*) has suggested—that selective pressures with respect to A and B vary in different environments. Thus, in some circumstances, selection may favor animals with β^A and β^C. It is also possible that a portion of the present day A-B-C polymorphism is the product of genetic isolation followed by admixture. For example, ancestors of domestic sheep may have become isolated into two or more groups. Thereafter the successive mutations whereby β^B differs in the same way from both β^A and β^C could be confined to a portion of the species. Homozygosity for β^B could be produced either by selective advantages peculiar to new environments or by a combination of advantage and genetic drift. Present day heterogeneity might result from the admixture of such β^B/β^B animals from one isolate with $\beta^A - \beta^C/\beta^A - \beta^C$ animals persistent in another isolate. Such processes as these provide a means whereby both $\beta^A - \beta^C$ and β^B can persist in the species. Thus these mechanisms complement the third evolutionary scheme which seems to explain the observed differences and similarities between the three β-chains, but does so only at the apparent cost of complete displacement of $\beta^A - \beta^C$ by β^B.

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21. Abbreviations used in this report are: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Asx, aspartic acid or asparagine, identity not established; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Glx, glutamic acid or glutamine, identity not established; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, Lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; NH_2-, free amino terminus of chain; -COOH, free carboxyl terminus of chain.
22. The NH_2-terminal segments of A-β and B-β chains are Met-Leu-Thr; in the chain in lamb hemoglobin that is not the α chain, the NH_2-terminal is Met, while the NH_2-terminal residue of sheep α is Val. These NH_2-terminal residues and Pro of C-β correspond to those obtained by two other groups of investigators (*8, 10*).
23. V. M. Ingram, *Nature* **189**, 704 (1961).
24. R. Brimacombe, J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, T. Jaouni, *Proc. Nat. Acad. Sci. U.S.* **54**, 954 (1965).
25. D. Beale and H. Lehmann, *Nature* **207**, 259 (1965).
26. One of the most attractive hypotheses accounting for the relative shortening of C-β involves deletion of several NH_2-terminal region codons coincident with the duplication which produced the gene for this chain. If this occurred, it is also possible that portions of the gene concerned with initiation of transcription or translation were affected to the extent that C-β synthesis is limited to the anemic state.
27. A-β and C-β share a common difference with respect to B-β at β-58, -75, -76, -129, and -144 (Table 1).
28. J. V. Evans and H. M. Turner, *Nature* **207**, 1396 (1965).
29. Although attractive, the third scheme is not without flaws. For example, recent mutations affecting solely B-β might be expected to render that chain not only different from A-β and C-β, but also different from the homologous residue of other species. This is not always the case, as illustrated by the Pro at B-β-58; Pro at this position is ubiquitous in the hemoglobin chains of other species (*18*) while it is Ala of A-β- and C-β-58 that is unique. Similarly A-β- and C-β-76 rather than B-β-76 bear the unique residues. Such findings, although potentially explicable by reversion of B-β to antecedent types of residues, demand considerable specificity of the selective process.
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Ascending and Descending Cholinergic Fibers in Cat Spinal Cord: Histochemical Evidence

Abstract. *The distribution of fibers staining for cholinesterase in the spinal cord of the cat was examined after hemisection at the level of the third cervical segment (C3), of the tenth thoracic segment (T10), or of the first lumbar segment (L1). An accumulation of cholinesterase was found in many fibers of the cord both rostral and caudal to the lesion, the distribution being different in the two regions. These experiments indicate that there are ascending and descending cholinergic fibers in cat spinal cord.*

Studies of the distribution of cholinesterase following section or damage to cholinergic nerve fibers have shown that the enzyme accumulates on the proximal side of the lesion (*1*). With use of this result, the course of cholinesterase containing fibers in the brain has been determined from the sites of accumulation of the enzyme after lesions had been made in the central nervous system (*2*). Confirmation that the tracts observed by this technique are most probably cholinergic has been obtained for fibers which travel in the fimbria to innervate the hippocampus (*3*) and for fibers which ascend from subcordial structures to the cerebral cortex (*4*). Thus the technique of studying the distribution of cholinesterase after lesions have been made can be used to provide evidence of the presence and course of cholinergic fibers.

The spinal cords of four cats anes-