

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 .

SAMUEL H. BOYER, PETER HATHAWAY,
FLORA PASCASIO,

CHARLENE ORTON, JAMES BORDLEY
Division of Medical Genetics,
Johns Hopkins Hospital and
University School of Medicine,
Baltimore, Maryland

MICHAEL A. NAUGHTON
Department of Biophysics,
Johns Hopkins University
School of Medicine

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- We had been concerned with mechanisms producing different proportions of hemoglobins found in human heterozygotes (12). Earlier workers (5) failed to recognize that sheep hemoglobin C was distinct from hemoglobin B and suggested that the proportions of A and B in heterozygotes changed with anemia. Such animals seemed to provide an animal analog for an extension of our studies of human hemoglobins; thus we began the current investigation with different goals from those reported here.
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 - 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; NH₂, free amino terminus of chain; -COOH, free carboxyl terminus of chain.
- The NH₂-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 NH₂-terminal is Met, while the NH₂-terminal residue of sheep α is Val. These NH₂-terminal residues and Pro of C- β correspond to those obtained by two other groups of investigators (8, 10).
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 - One of the most attractive hypotheses accounting for the relative shortening of C- β involves deletion of several NH₂-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.
 - A- β and C- β share a common difference with respect to B- β at β -58, -75, -76, -129, and -144 (Table 1).
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 - 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.
 - Supported by NIH grant GM-10189 and by an institutional grant from the American Cancer Society. Dr. C. Wadkins performed a number of amino acid analyses.

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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-

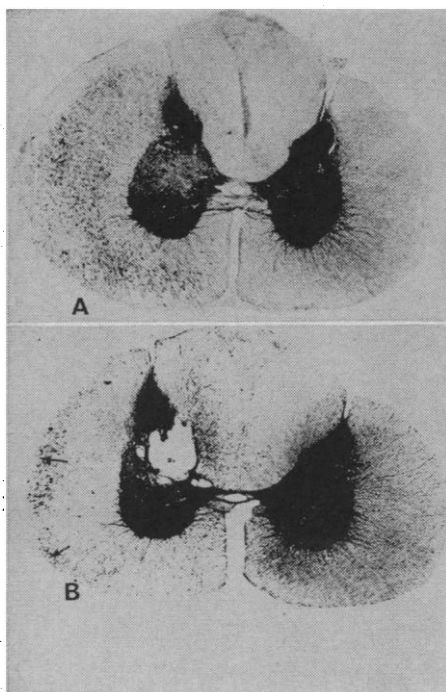


Fig. 1 (left). (A) Transverse section of spinal cord just rostral to a hemisection at C2 to C3. On the operated side many fibers stained for cholinesterase. To make these fibers clearly visible for photography, the sections were stained more heavily than usual. Thus the grey matter is over-stained. (B) Section just caudal to the lesion. The arrows point to fibers showing accumulation of cholinesterase in lateral and ventrolateral regions near the cord surface.

thetized with pentobarbitone were hemisectioned at C3, T10, or L1; 6 to 7 days after the operation each animal was again anesthetized and was perfused with saline and then with 10 percent formol-saline. The spinal cord was removed and placed in 10 percent neutral formol-saline for 4 hours at 4°C. It was then washed in distilled water and immersed in 20 percent ethanol at 4°C. Serial sections (75 μ) were cut on a freezing microtome and incubated for 45 minutes at room temperature in acetate buffer (pH 5.9) or in the same buffer containing $10^{-7}M$ ethopropazine hydrochloride or $10^{-4}M$ [(3-oxopentamethylene)di-*p*-phenylene] bis [allyldimethyl-bromide (BW 284C51). The sections were then stained for cholinesterase according to a modification of the method of Gomori (5). Acetylthiocholine iodide was used as the substrate. The stained sections were washed, dehydrated, cleared, and were then mounted in neutral balsam.

A transverse section of the spinal cord just rostral to a hemisection at C2 to C3 is illustrated in Fig. 1A. There was an accumulation of cholinesterase in many fibers on the side of the lesion but no fibers cut in transverse section were stained on the unoperated side. Similar regions showed accumulation of cholinesterase following lesions at T10 and L1. The use of inhibitors showed that the enzyme was "specific" cholinesterase. Cholinesterase appeared to be localized in regions

which carry fibers of the lateral corticospinal, rubrosegmentospinal, olivospinal, vestibulospinal, and reticulospinal tracts. It is difficult at this stage to be certain which of these tracts contain cholinesterase-staining fibers, but the results suggest that there may be many descending cholinergic fibers in the spinal cord.

A section just caudal to the lesion is shown in Fig. 1B. Fewer fibers were stained and the distribution of them was different from that found rostral to the lesion. Accumulation of cholinesterase occurred in two regions, lateral and ventro-lateral, near the surface of the cord and also in a few scattered fibers in the dorsal columns. This latter result was surprising but agrees with the observation, although in another species, that about 10 percent of the cells of the dorsal root

ganglion stain for cholinesterase (6). The lateral and ventro-lateral accumulations were present at the three levels examined, and the former appears to be in the region of the ventral spino-cerebellar tract. It is interesting to note that propriospinal regions surrounding the grey matter were unstained in both sections.

Precise identification of the ascending and descending cholinesterase-staining tracts must await further study.

D. G. GWYN

J. H. WOLSTENCROFT

Department of Anatomy and Medical Research Council, Neuropharmacology Research Unit, University of Birmingham, Birmingham, England

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Interaction of Cortex and Superior Colliculus in Mediation of Visually Guided Behavior in the Cat

Abstract. *Total contralateral hemianopia follows unilateral removal of the entire occipito-temporal neocortex in the cat. This deficit is classically ascribed to interruption of visual radiations serving cortical function ("cortical blindness") and is considered permanent. Return of vision to the hemianopic field after subsequent removal of the superior colliculus contralateral to the cortical lesion demonstrates that neither assumption is correct. The initial hemianopia is apparently due to depression of function of the colliculus ipsilateral to the cortical lesion, a depression maintained by influx of inhibition from the crossed colliculus. Thus, removal of the contralateral tectum, or splitting of the collicular commissure, abolishes this inhibition and allows the return of function in the ipsilateral colliculus, and with it the recovery from hemianopia. These findings emphasize that visually guided behavior is mediated at both cortical and midbrain levels, and that there is a marked interaction between these sites.*

Visual fields of the cat can be ascertained and measured rather accurately by means of a simple perimetric test in which animals are trained to fixate and to respond to food stimuli. When tested in this fashion with monocular masks, the horizontal visual field of each eye includes about 130°, that is, the field of the right eye extends from 100° right to 30° left of fixation. The area of binocular overlap so

measured (60°) is somewhat reduced from the actual amount by presence of the mask. Field deficits after lesions can be measured with an accuracy of about 10°. After recovery or compensation of a field deficit, bilateral stimulation can demonstrate inattention or neglect of one visual field by preferential response to stimuli in the opposite field. This and other "clinical" behavioral tests for deficits