copper-containing intrauterine devices are of such promise, it is imperative to extend studies of the fate of the copper before their use can be safely prescribed. Novel techniques will be required for these studies in human beings since, except possibly in rare instances, radioactive copper cannot be used, and the quantitative estimation of whether excess copper accumulation occurs in tissues requires chemical, not histochemical, analysis of serial biopsy samples (*7*).

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Hemoglobin Adaptation for Fast and Slow Water Habitats in Sympatric Catostomid Fishes

Abstract. *The oxygen equilibria of* Catostomus insignis *hemoglobins are* p*H dependent.* Catostomus clarkii *hemoglobins have some components (20 percent) whose oxygen equilibria are independent of* p*H because the alpha chains have* NH_2*-termini that are blocked and the beta chains lack the "usual" COOH-terminal histidine. Since the Bohr effect is normally a beneficial phenomenon, the maintenance of some hemoglobins without a Bohr effect must provide a physiological advantage that is habitat specific. The intrastream ecological preferences of these sympatric catostomids suggest that the hemoglobins without the Bohr effect confer an ecological advantage in a swift water habitat.*

There are 70 species of fish from the family Catostomidae. Twenty species of one genus (*Catostomus*) inhabit the western montane regions of the United States; 14 of these species are members of the subgenus *Catostomus*, while six represent the subgenus *Pantosteus* (*1*). Fishes are distributed so that only one species of a subgenus inhabits a given geographical region (that is, allopatric), although each species of one subgenus is usually found living with a member of the other subgenus (that is, sympatric) (*1*).

The subgenera *Pantosteus* and *Catostomus* can be distinguished from one another by the presence or absence, respectively, of cathodal components in the electrophoretic patterns of hemoglobins at *p*H 8.6 (*2*). I expected the characterization of the cathodal hemoglobins to be important in correlating structure and function of the hemoglobins with fish ecology. A representative pair of sympatric species, *Catostomus* (subgenus *Catostomus*) *insignis* and *Catostomus* (subgenus *Pantosteus*) *clarkii*, were collected and hemoglobins were separated by ion-exchange chromatography (see Fig. 1). Fractions were characterized by gel electrophoresis according to the method of Smithies (*3*, *4*).

The molecular weights, which ranged from 66,300 to 68,600, of single chromatographic components were determined by sedimentation equilibrium (*5*). Hence, hemoglobins from either *C. clarkii* or *C. insignis* have approximately the same molecular weight, which corresponds to the tetramer of mammalian hemoglobins. The electrophoretic banding multiplicity, therefore, cannot be attributed to polymerization through the formation of interchain disulfide bonds or to dissociation of subunits.

The ability of hemoglobin to bind oxygen is usually modified by the *p*H of its microenvironment. Within physiological range, the hemoglobin's affinity for oxygen is directly proportional to *p*H. Consequently, oxygen affinity is higher at the lungs (or gills) and lower at the tissues (lactic and carbonic acids lower the tissue *p*H). The dependence of hemoglobin oxygen affinity on *p*H is known as the Bohr effect.

Oxygen equilibria of chromatographic components were performed by the method of Riggs and Wolbach (*6*). The anodal hemoglobin components from *C.* (subgen. *P.*) *clarkii* and *C.* (subgen. *C.*) *insignis* had slightly different oxygen equilibrium curves but all anodal components demonstrated substantial sensitivity to *p*H changes (large Bohr effect). The cathodal components from *C. clarkii*, however, did not have a Bohr effect (see Fig. 1E).

The NH protons in the imidazolium ring of COOH-terminal histidine in the β chains and NH_2-termini of the α chains are known to be largely responsible (75 percent) for the Bohr effect in mammalian hemoglobins (*7–9*). Consequently, the NH_2-termini and COOH-termini of the hemoglobin components were examined for each of the species.

The α and β chains of the anodal components, fractionated by a method similar to that of Clegg *et al.* (*10*), from both species and the globins of the cathodal components from *C. clarkii* (*11*) were subjected to NH_2-terminal analysis by a modification of Edman's phenylisothiocyanate procedure (*12*). The phenylthiohydantoins of the amino acids were identified either by the methods of Jeppsson and Sjörquist (*13*) or by gas chromatography [procedure of Pisano and Bronzert (*14*)].

Carboxypeptidase A (CPA) and B

(CPB) analyses were performed on the α and β chains of the anodal components from both species and the whole globin of the cathodal components from *C. clarkii* hemoglobins (*10, 12*). The CPA procedures were those of Edmundson *et al.* (*15*), and those of CPB were based on the method of Ambler (*16*).

Quantitative sequential degradation (*11*) of the cathodal globins from *C. clarkii* hemoglobin yielded 44 percent valine in stage 1. The α chains of the anodal hemoglobins of both species were blocked with an acetyl group (*17*). The low yield at stage 1 suggested that the α chains of the cathodal hemoglobins were also blocked. Further degradation yielded an initial sequence of Val-Glu-Trp-Ser-Asp-Ser-Glu-Arg-Lys (and Gln)-Thr-Leu-Val-Ser-Val-Trp-Gly-Lys (and Arg)-Ile- (*18*). This is more like the initial sequence of anodal β chain from *C. clarkii* with Val-Glu-Trp-Thr-Asp-Ala-Glu-Arg-Ser-Ala-Ile-Leu-Ser-Leu-Trp-Gly-Lys-Ile- (*18*), than an α chain whose initial sequence is Ac-Ser-Leu-Ser-Asp-Lys-Asp-Lys-Ala-Asp-Val-Lys-Ile-Ala-Trp-Ala-Lys-Ile-. Consequently, it was tentatively concluded that the unblocked polypeptides were β chains.

The amino acids released by CPA and CPB are listed in Table 1. As in the NH_2-terminal analyses, the products liberated from the cathodal (peak 2 in Fig. 1B) and anodal (peaks 3 to 8 in Fig. 1B and peaks 1 to 7 in Fig. 1D) hemoglobins were significantly different. Equal quantities (40 percent) of Tyr and Phe (*18*) were obtained from the hemoglobins in peak 2 of Fig. 1B, while His (*18*) and Tyr were released from anodal components of both species by CPA. The common COOH-terminal sequence in most β chains is Tyr-His, but my results indicate that His is absent in the cathodal hemoglobins. Arginine, the COOH-terminus of the anodal α chain, was the major product liberated (42 percent) from the cathodal hemoglobins by CPB.

Alternate hypotheses can be made about the sequence of the COOH-termini for the cathodal hemoglobins of

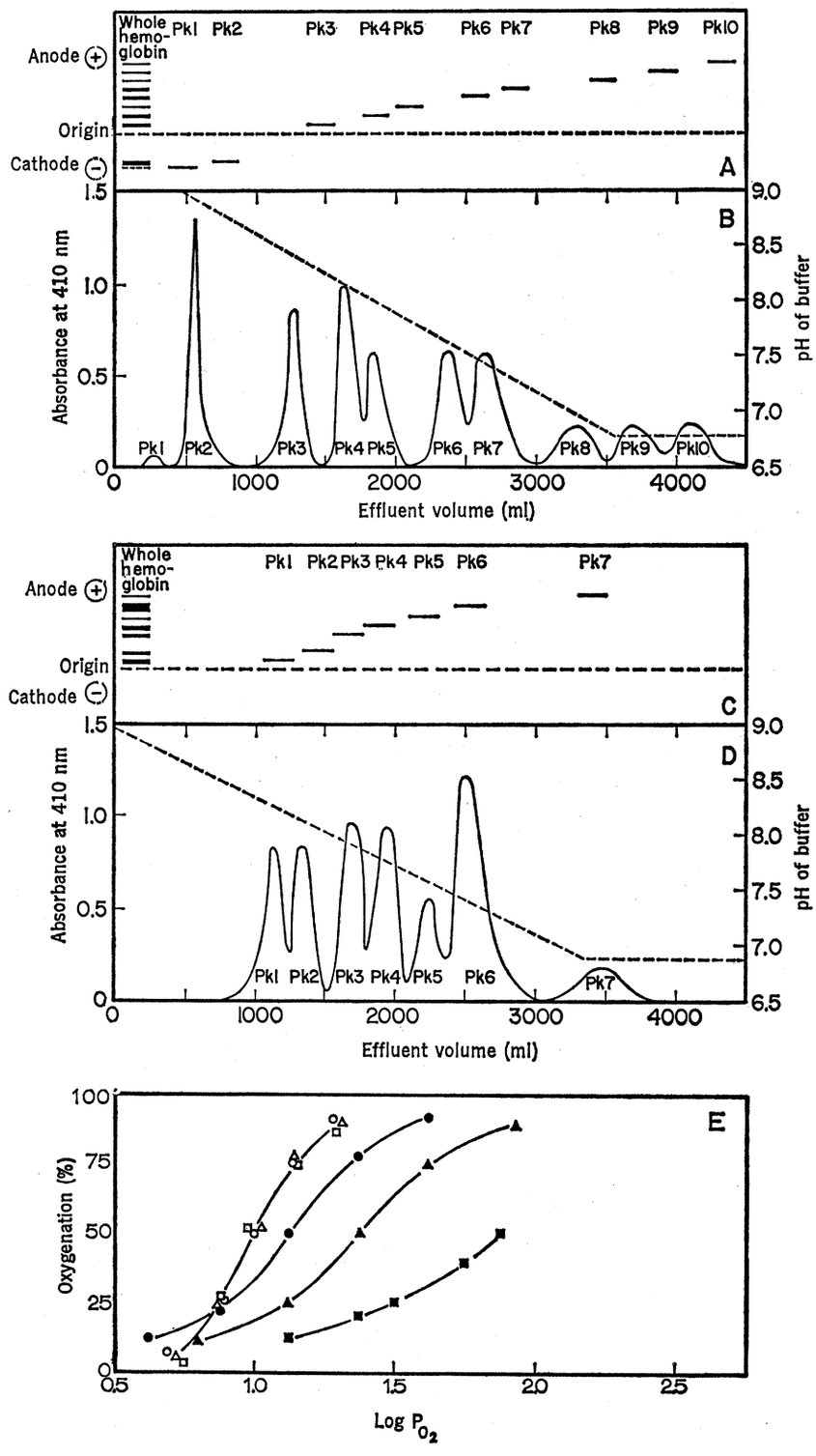

Fig. 1. (A) Starch gel electrophoresis (*p*H 8.6) of the hemoglobin components [from *Catostomus* (subgenus *Pantosteus*) *clarkii*] eluted from the column. The pattern for unfractionated hemoglobin is shown on the left side of the figure. The anode is at the top and the origin is represented by a dotted line. (B) Fractionation of *Catostomus clarkii* hemoglobins at 4°C on a column, 5 by 25 cm, of diethylaminoethyl-Sephadex A-50. Fractions of 5 ml were collected. (C) Starch gel electrophoresis (*p*H 8.6) of the hemoglobin components [from *Catostomus* (subgenus *Catostomus*) *insignis*] eluted from the column and the whole globin on the left of the figure for reference. (D) Fractionation of *Catostomus insignis* hemoglobins at 4°C on a column 5 by 25 cm, of DEAE-Sephadex A-50. Fractions of 5 ml were collected. Some components had to be rechromatographed in order to purify them adequately. (E) Oxygen dissociation curves of cathodal hemoglobins from *C. clarkii* (open symbols) and the average values of the anodal components from both *C. clarkii* and *C. insignis* (solid symbols) at *p*H 7.2 (circles), *p*H 7.0 (triangles), and *p*H 6.7 (squares). The buffer was 0.2*M* phosphate and the hemoglobin concentration was 4 mg/ml. Reactions took place at 20°C; *Pk*, peak.

Table 1. The NH_2-terminal and COOH-terminal sequences of *Catostomus clarkii* and *Catostomus insignis* hemoglobins.

	Catostomus (subgen. *Pantosteus) clarkii*		*Catostomus* (subgen. *Catostomus) insignis*
	Anodal hemoglobins	Cathodal hemoglobins	hemoglobins
	NH_2-termini		
α Chains	Ac-Ser-Leu-Ser-	Blocked*	Ac-Ser-Leu-Ser-
β Chains	Val-Glu-Trp-	Val-Glu-Trp-*	Val-Glu-Trp
	COOH-termini		
α Chains	-Lys-Tyr-Arg	Arg (CPB)	Lys-Tyr-Arg
β Chains	-Gln-Tyr-His	Tyr and Phe (CPA)	Ser-Tyr-His

*See (*11*).

C. (subgen. *P*) *clarkii* but neither hypothesis would alter the fact that His is not a COOH-terminus.

The α chains from both species had blocked NH_2-termini. However, there is a major difference between the cathodal and anodal hemoglobins. The anodal hemoglobins of both species had COOH-terminal His for the β chains, while the cathodal hemoglobins did not release His when hydrolyzed with CPA. Therefore, both the usual (*6–8*) participating Bohr groups were missing in the cathodal components, which could largely explain the absence of the Bohr effect.

These results indicate the cathodal hemoglobins of *C. clarkii* have structural modifications of the α and β chains which are manifest in their physiological function (that is, no Bohr effect). The physiological role of the cathodal hemoglobins in adaptation for a particular intrastream habitat is suggested by the primary ecological preferences of these sympatric subgenera. Fishes of the subgenus *Pantosteus* prefer fast-moving portions of the stream, while members of the subgenus *Catostomus* have a preference for the pools or sluggish water (*1*). For example, *C.* (subgen. *P.*) *clarkii* is predominantly active in the faster parts of a stream while *C.* (subgen. *C.*) *insignis* is found predominantly in the quieter pools.

Fish in fast water habitats generally have hemoglobins with low oxygen affinities and a large Bohr effect (*19*). Although a large Bohr effect is beneficial in releasing oxygen at the cellular level, it can suppress oxygen binding at the gills when the blood *p*H is sufficiently low. According to Black (*20*) the increase in lactic acid after violent exercise (for example, to escape a predator) can result in the death of hyperactive fish. Riggs (*19*) states, "The Bohr effect, deemed an advantage under normal circumstances, may thus prevent adequate oxygen from reaching the tissues." It would appear that the cathodal hemoglobins, without a Bohr effect, provide a backup system for *C.* (subgen. *P.*) *clarkii* so that swimming may be maintained after emergency exertions. If the interpretation is correct, one would predict a similar adaptation in hyperactive fishes. It is reassuring that both trout (*21*) and salmon (*22*) have been found to have some hemoglobin components devoid of a Bohr effect.

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Evoked Potential Correlates of Response Criterion in Auditory Signal Detection

Abstract. *The amplitude of a late positive component of the average evoked potential recorded from the human scalp varied systematically as a function of the observer's response criterion as defined within the context of signal detection theory. With signal intensity invariant, the P_3 component of the evoked potential increased monotonically with increasing strictness of the criterion. The results are viewed as supporting the signal detection theory approach to the analysis of discrimination behavior as well as providing further evidence of the sensitivity of P_3 to the manipulation of psychological variables.*

Various degrees of correlation between average evoked potentials and psychophysical judgments have been reported, ranging from "complete isomorphism" (*1*) to complete lack of association under an anesthetic drug (*2*). Discrepancies or lack of correspondence between physiological and behavioral data have been attributed by Donchin and Sutton (*3*) to lack of methodological precision. They particularly stressed the importance of segregating average evoked potentials according to both stimulus conditions and perceptual responses. A systematic study by Hillyard *et al.* (*4*), which satisfies these conditions, demonstrates close correspondence between a late positive component (P_3) of the auditory potential and *d'*, a measure of the sensitivity of the observer to a particular signal, based on signal detection theory (*5*). Because differences in response bias, not sensitivity, often result in altered performance, a signal detection theory approach to behavioral measurement is often su-