Hemoglobin McKees Rocks ($\alpha_2\beta_2{}^{145\,\text{Tyr}\rightarrow\text{Term}}$)

A HUMAN "NONSENSE" MUTATION LEADING TO A SHORTENED β-CHAIN

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ABSTRACT The first example of the premature termination of a polypeptide chain in man appears to be Hb McKees Rocks, β145 Tyr→Term, discovered in polycythemic members of a Caucasian family. Point mutation has apparently occurred at the codon for Tyr β145 from UAU to a "nonsense" codon, UAA or UAG, resulting in a shortened polypeptide chain with Lys 144 as its carboxyl-terminal amino acid. Evidence for this structural conclusion is the absence of tryptic peptide βT-15 from "fingerprints" of the abnormal β-chain, the finding of C-terminal Lys, and the similarity between the functional properties of this variant hemoglobin and those of *des* Tyr(145)-His(146)β hemoglobin resulting from carboxypeptidase-A digestion of normal human hemoglobin. Hb McKees Rocks has markedly abnormal properties: its oxygen affinity is the highest of the human variants described to date; its Bohr effect is reduced; it is devoid of subunit cooperativity; and it is unaffected by 2,3-diphosphoglyceric acid. These properties are probably the consequences of decreased stability of the T quaternary conformation and are partially restored in the presence of the strong allosteric effector inositol hexaphosphate.

INTRODUCTION

More than 200 mutant human hemoglobins have been described (1). These have arisen as the result of amino acid substitutions, deletions, duplications, unequal crossing over, frame shifts, and in several instances, mutation of the normal terminator codon leading to an extended polypeptide chain. To date, however, no examples in man of premature termination of a polypeptide chain have been described that correspond to the "nonsense" mutations which have been observed in bacteria. In such instances, a codon that specifies an amino acid becomes one that signals termination of the polypeptide chain. The present work describes the first example in man in which such an event has probably occurred.

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METHODS

Hematologic studies. Blood was collected from carriers in EDTA or heparin. Hematologic values were obtained according to standard clinical procedures. Electrophoresis of hemolysates employed Mylar-backed cellulose acetate sheets and a pH 8.6 Tris-borate buffer (Helena Laboratories, Beaumont, Tex.) and agar gel, using pH 6 citrate buffer (2). Blood methemoglobin and carbon monoxide (CO)[1]-hemoglobin were estimated spectrophotometrically (3). 2,3-Diphosphoglyceric acid (DPG) measurements were made by the method of Nygaard and Rörth (4).

Hemoglobin purification. Cells were washed three times in NaCl (0.9 g/100 ml), mixed with an equal volume of deionized, distilled water, and lysed by repeated freeze-thaw. After removal of stroma by centrifugation, the hemolysate was diluted with an equal volume of Tris-maleate (5) buffer, gassed with CO, and loaded onto a column of carboxymethyl-Sephadex C-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). Buffers were 0.05 M Tris, the

[1] *Abbreviations used in this paper:* CPA, carboxypeptidase-A; HbCPA, hemoglobin digested with CPA; DPG, 2,3-diphosphoglyceric acid; CO, carbon monoxide; P_{50}, oxygen pressure at half-saturation of hemoglobin; IHP, inositol hexaphosphate; bis-Tris, bis-(2-hydroxyethyl)iminotris-(hydroxymethyl)methane.

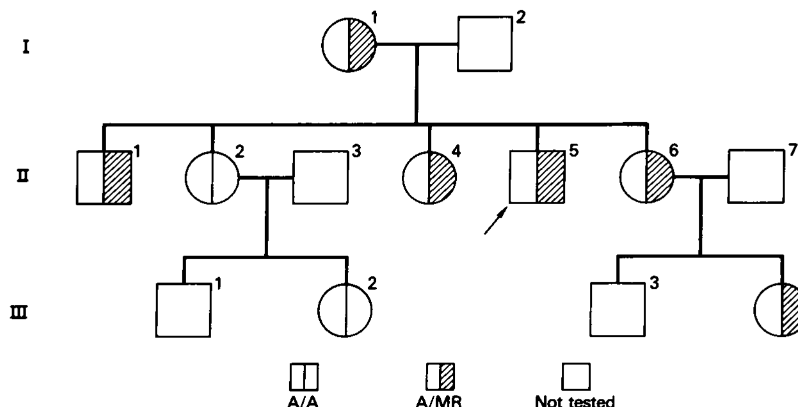

FIGURE 1 Pedigree of the family which carries Hb McKees Rocks.

pH of which had been adjusted to the desired value with 1 M maleic acid. To prepare the column, resin was suspended in starting buffer (pH 6.7) and packed into a 2.5 × 40-cm column (Pharmacia Fine Chemicals, Inc.) that was maintained at 4°C. It was washed overnight before applying approximately 200 mg of hemoglobin in 3 ml of buffer. A linear gradient between pH 6.7–7.0 (500 ml of each buffer in two chambers) was then used to separate the two hemoglobins. After elution of Hb McKees Rocks with pH 7 buffer, the pH of the buffer was increased to 7.2 to elute the Hb A. The pooled hemoglobin fractions were again gassed with CO, concentrated by pressure filtration (Amicon PM 10 membrane, Amicon Corp., Scientific Sys. Div., Lexington, Mass.) and stored at 4°C until use. Identification of the peaks and an estimation of purity could be achieved by agar-citrate electrophoresis.

Structural studies. Globin was prepared from concentrated hemoglobin solutions by acid-acetone precipitation (6), and component polypeptide chains were isolated by the method of Clegg et al. (7). After *S*-aminoethylation (8) and gel filtration (G25 fine, 0.09% formic acid), the purified globin chains were lyophilized.

Tryptic digests of globin or aminoethyl-globin samples were prepared (9), and 50–100-μg samples were applied to 0.1-mm cellulose plates (Polygram Gel-300, Brinkman Instruments, Inc., Westbury, N. Y.) that had been soaked and blotted in pH 6.4 buffer (pyridine-glacial acetic acid-water, 10:0.4:90 by volume). Wicks (wick A, Brink-

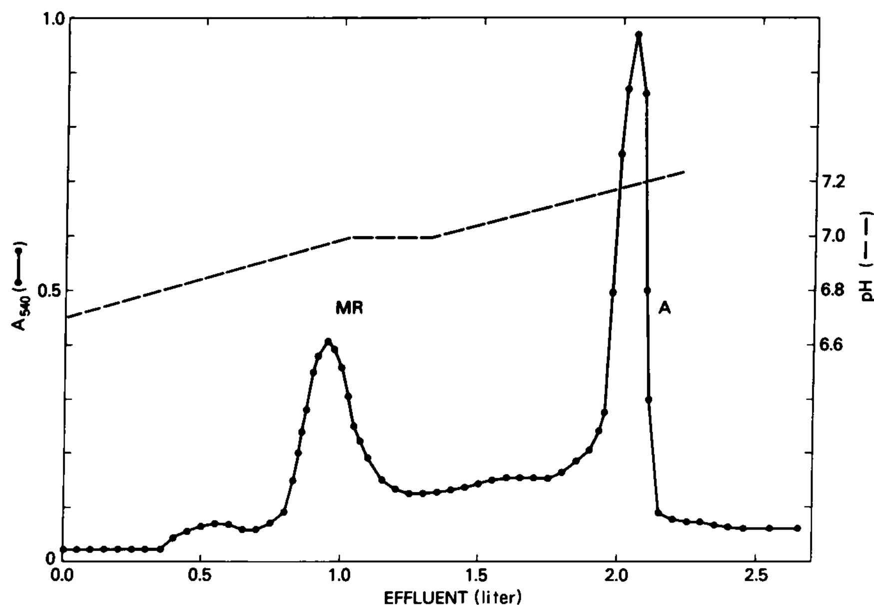

FIGURE 2 Isolation of Hb McKees Rocks. 200 mg of hemoglobin was applied to a 2.5 × 30-cm carboxymethyl Sephadex (C50) column which was operated at 96 ml/h at 4°C with Tris-maleic acid buffers (Methods). A linear gradient between pH 6.7 and 7 (- - - - -) was used to separate the two hemoglobins. Hb McKees Rocks was eluted with pH 7 buffer and Hb A with pH 7.2 buffer.

TABLE I
Hematologic Values from Members of the Family Carrying Hb McKees Rocks

Subject	Age	Sex	Genotype	Hb	Hct	2,3-DPG*
				g/100 ml	*%*	*μM/μM Hb*
I 1	57 yr	F	A/MR	17.9	59	1.15
II 1	36 yr	M	A/MR	17.8	56	1.03
2	30 yr	F	A/A	15.4	47	0.75
4	36 yr	F	A/MR	21.2	64	0.92
5	23 yr	M	A/MR	17.8	55	0.80
6	27 yr	F	A/MR	18.4	60	0.94
III 2	9 yr	F	A/A	13.7	44	0.75
4	16 mo	M	A/MR		50	

* Normal range, 0.8-1.0 μM/μM hemoglobin.

man Instruments, Inc.) were soaked in the same buffer, blotted, and applied to the plates in a double thin-layer electrophoresis apparatus (Desaga, Brinkman Instruments, Inc.) which was cooled to 11°C by circulating water. The sample, in approximately 5 μl, was applied near the corner of the plate, and electrophoresis was carried out at 25 V/cm for 75 min The double chamber allowed the simultaneous electrophoresis of two samples which could be compared. After electrophoresis, the plates were dried in an oven or 20 min at 60°C, cooled, and ascending chromatography at right angles to the electrophoresis was carried out for 6 h with pyridine-isoamyl alcohol-water (35: 35:28, by volume) (10). The peptide spots were located by dipping the dried plates in 0.2% ninhydrin in acetone (by volume) that contained 0.03 volume of the pH 6.4 buffer. Specific stains for His or Tyr could be done directly on the unstained plates or after decolorization of the ninhydrin spots (10).

Peptides were isolated from preparative fingerprints (11) which used 5 mg of tryptic digest per run. The ninhydrin positive spots were cut out, eluted in 1% (by volume) formic acid, lyophilized, hydrolyzed (6 M HCl:2-mercaptoethanol, 2,000:1 by volume, 110°C for 22 h *in vacuo*), and their amino acid compositions were determined with a Beckman 121 amino acid analyzer, (Beckman Instruments, Inc., Fullerton, Calif.).

Approximately 5-mg samples of the purified β-chain were analyzed for their C-terminal amino acid by hydrazinolysis (12). Purified β-chains were digested with carboxypeptidase-A (CPA) (13), and the liberated amino acids were quantitated using the Beckman model 121 amino acid analyzer.

Blood oxygen equilibria. Blood was collected in EDTA anticoagulant and kept at 4°C until use approximately 1 h later. The method was a modification of that of Rossi-Bernardi et al. (14). 2 ml of blood was placed in a tonometer (IL-237, Instrumentation Laboratories, Lexington, Mass.) and equilibrated with 5% CO_2 in nitrogen or 30 min at 37°C. Deoxygenated blood (0.5 ml) was placed into a cuvette (Advanced Products, Milan, Italy) which was fitted with O_2 and CO_2 electrodes and was maintained at 37°C. 2 μl catalase (250 IU, Calbiochem, San Diego, Calif.) was added, and stirring was begun with a magnetic mixer. When P_{O_2} and P_{CO_2} readings were stable, H_2O_2 (0.26 M in 0.154 M NaCl, 0.0001 M EDTA) was delivered into the mixture by a constant-speed pump (Razell Scientific Instruments, Inc., Stamford, Conn.). The pump rate was chosen so that full saturation of the hemoglobin was achieved in about 10 min. During the run, P_{CO_2} was held constant by the addition of 0.4 M NaOH from a micrometer syringe. Approximately 10–15 μl was required for a typical run. Full saturation was taken as the point at which the rise in P_{O_2} was linear with time. The resulting data, a plot of P_{O_2} vs. time, was manually converted to O_2 content vs. P_{O_2} using experimentally determined correction values for the difference between P_{O_2} readings in air and blood, hemoglobin, carboxyhemoglobin, and methemoglobin concentrations. Control experiments indicated that during a run there were no changes in these values or in 2,3-DPG concentration.

Hemoglobin oxygen equilibrium. Concentrated, stripped Hbs were exchanged into 0.05 M bis(2-hydroxy-ethyl)iminotris(hydroxymethyl)methane, (bis-Tris), 0.1 M NaCl, (the pH of which had been adjusted to 7.2 with HCl) in a Sephadex G-25 (fine) column at 4°C, which had been equilibrated with CO. The CO was removed by flash photolysis as previously described (15), and the oxyhemoglobin was mixed with a ferredoxin (16) system and kept at 4°C. Under these conditions, no methemoglobin could be detected for 3 days after photolysis. The pentacyclohexylammonium salt of 2,3-DPG (Calbiochem) was converted to the free acid by the method of Benesch et al. (17), and titrated to pH 7 with 0.1 M NaOH. Inositol hexaphosphate (IHP) (Sigma Chemical Co., St. Louis, Mo.) was dissolved directly in water and adjusted to pH 7 with 0.1 M HCl. Continuous hemoglobin oxygen equilibrium curves were obtained by the automatic method of Imai et al. (18), using a Cary 118C spectrophotometer (Cary Instruments, Monrovia, Calif.). The oxygen electrode amplifier, thermister, and variable light path cell assembly were obtained from the Johnson Foundation, University of Pennsylvania, Philadelphia. The Beckman 39065 Polarographic electrode was fitted with Teflon membrane 5937 (Yellow Springs Instrument Co., Yellow Springs, Ohio). Spectrophotometric studies were done in this cell at controlled temperature and oxygen tension. The volume of the hemoglobin solution was 5.5 ml, and when DPG or IHP was present not more than 50 μl was added so that dilution was not significant. Visible spectra of the reaction mixtures were scanned before and after deoxygenation, and no methemoglobin could be detected (19).

For the estimation of the fractional content of Hb McKees Rocks in a hemolysate, equilibrium curves were obtained for the hemolysate and the purified hemoglobins under identical conditions. From the data for the two purified hemoglobins, a curve was fit to the hemolysate data by a least-squares iterative procedure using the MLAB facility at the National Institutes of Health (20).

TABLE II
Release of Amino Acids from 1 mg each of β^A and β^{MR} Chains during Digestion with CPA, 30°C

		Time		
		30	60	120
			min	
His, *μM*	β^A	60	120	122
	β^{MR}	0	0	0
Tyr, *μM*	β^A	67	122	154
	β^{MR}	0	0	0

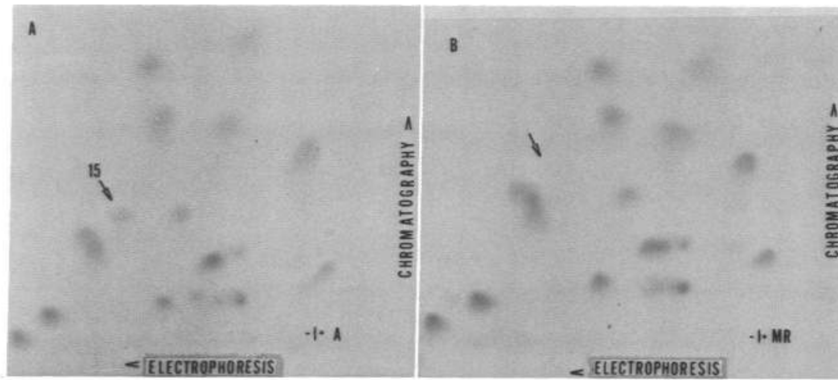

FIGURE 3 Microfingerprints of β^{A}- and β^{MR}-globin chains. 100 μg of tryptic digest was applied to 0.1-mm cellulose thin layers and a two-dimensional separation was carried out as described in the text. The arrow in the β^{A} fingerprint (A) designates βT-15 which is absent in the β^{MR} fingerprint (B).

RESULTS

Family study. The proband, a 23-yr-old Caucasian male (Fig. 1: II-5) was hospitalized because of diabetes mellitus. In the course of his evaluation the Hb was found to be 17.8 g/100 ml, and the hematocrit was 55%. No cause for the polycythemia could be determined. Although cellulose acetate electrophoresis at pH 8.6 was normal, electrophoresis on citrate agar at pH 6 revealed a fast component with the mobility of Hb F. The ensuing family study revealed that his pregnant sister (II-6) had a markedly elevated hematocrit (the actual value is unknown), and venesection was performed before her delivery. The pregnancy and delivery were normal, and the child (III-4) was subsequently found to be a carrier of the abnormal Hb.

The hematologic values obtained from several family members (Table I) showed a difference between the Hbs and hematocrits of adult heterozygotes and normals. No significant differences were noted between males and females. DPG levels of all family members tested were normal. No history of heart disease, sudden death, stroke, exercise intolerance, or any difficulties

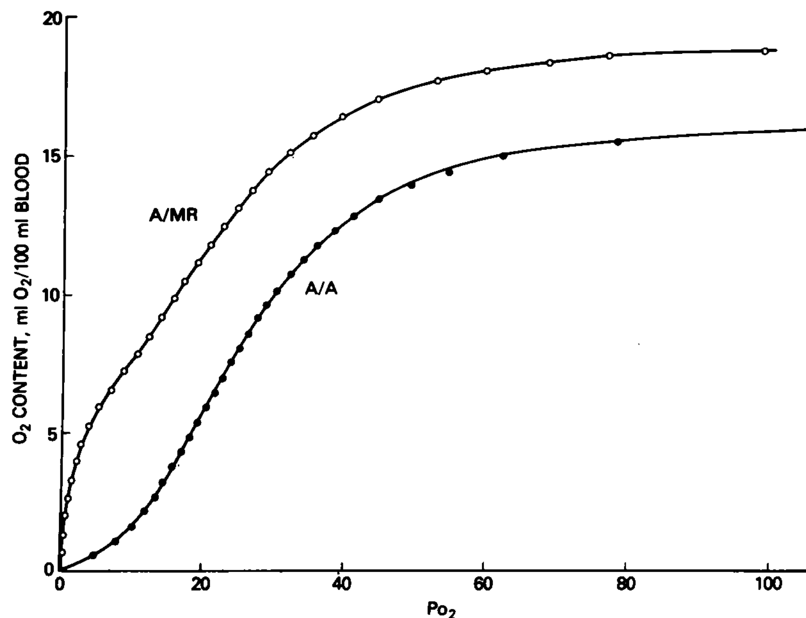

FIGURE 4 Oxygen equilibria of whole blood. pH and Pco_2 were held constant during oxygenation. pH = 7.45, Pco_2 = 35 mm Hg, 37°C. Points were taken from the continuous tracing of Po_2 vs. time; oxygen content and Po_2 were calculated.

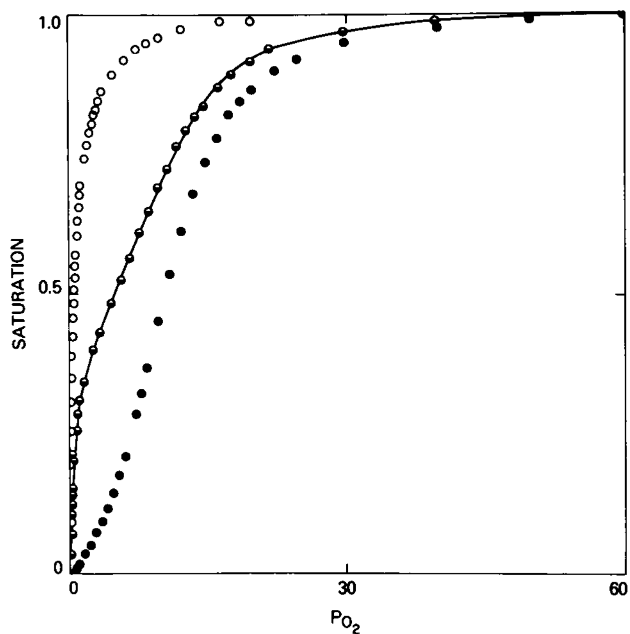

FIGURE 5 Oxygen equilibria of purified Hb A, MR, and a hemolysate from subject II-1 (Fig. 1). pH 7, 30°C, 0.05 M Bis-Tris, 0.1 M NaCl. All hemoglobin concentrations 8 μM (tetramer). ○, Hb McKees Rocks; ●, Hb A; ◒, the mixture. The line through the hemolysate points was computed assuming 46% Hb MR (see Methods).

TABLE III
The Effect of DPG and IHP on Hb A and Hb McKees Rocks

*Hb	Condition	*n*	P_{50}	P_{50}/P_{50} stripped
			mm Hg	
A	Stripped	2.78	10.0	—
	60 μM DPG	3.00	20.3	2.0
	60 μM IHP	2.85	100.0	10.0
MR	Stripped	1.04	0.66	1.0
	60 μM DPG	1.00	0.66	1.0
	60 μM IHP	1.81	2.29	3.5

* Hemoglobin concentrations 15 μM (tetramer). 0.05 M bis-Tris, 0.1 N NaCl, 30°C, pH 7.

during pregnancy could be discovered, and all of the affected members were asymptomatic except for mild ruddiness of complexion.

Structure of Hb McKees Rocks. Hb McKees Rocks, named for the place of residence of the proband, could be isolated with greatest purity using carboxymethyl-Sephadex chromatography (Fig. 2). This method allowed the purification of large quantities of the variant hemoglobin in approximately 36 h, and when the CO derivative was employed methemoglobin formation was

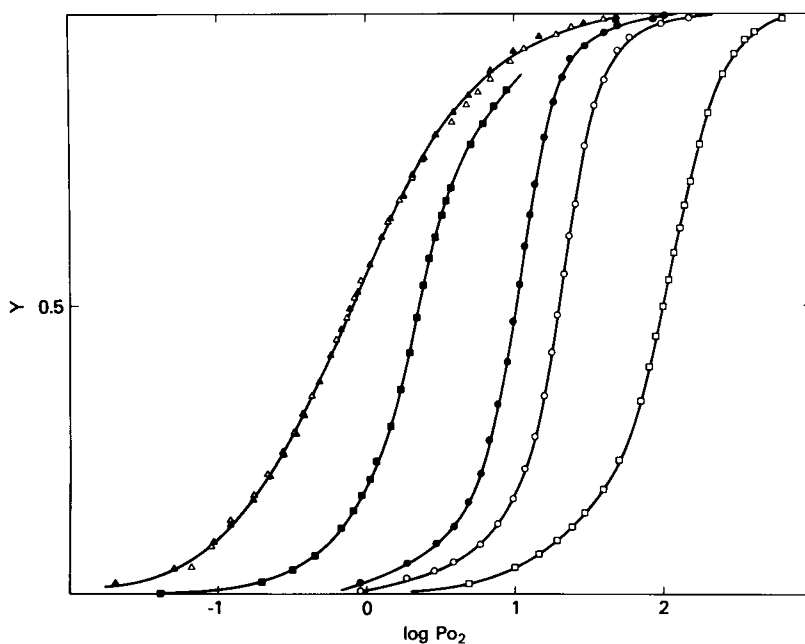

FIGURE 6 The effect of DPG and IHP on the oxygenation of Hb A and Hb McKees Rocks. All hemoglobin concentrations 8 μM, 0.05 M Bis-Tris, 0.1 M NaCl, 30°C. ●, Hb A; ○, Hb A + 60 μM DPG; □, Hb A + 100 μM IHP. ▲, Hb McKees Rocks; △, Hb McKees Rocks + 60 μM DPG; ■, Hb McKees Rocks + 100 μM IHP.

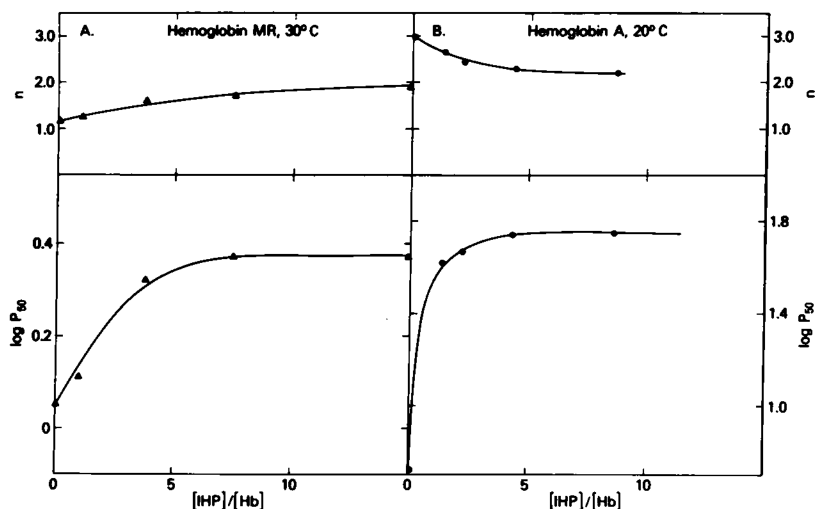

FIGURE 7 The effect of IHP on Hb A and Hb McKees Rocks. Log P_{50} and Hill's parameter "n" are plotted as a function of [IHP]/[hemoglobin]. The Hb McKees Rocks data (A) were obtained at 30°C and the Hb A data (B) at 20°C. All experiments were at pH 7.0, 0.05 M bis-Tris, 0.1 M NaCl. (Hb McKees Rocks concentration was 8 μM; Hb A concentration was 14 μM (tetramer).

not encountered. Poor separation of β^{MR} and β^{A} was noted by urea-carboxymethyl cellulose chromatography; β^{MR} eluted between γ- and β-chains in this system. Therefore, in the final structural analyses only Hb McKees Rocks which had undergone repurification on carboxymethyl-Sephadex was used.

Peptide βT-15 could not be located on the microfingerprints of the β^{MR} chain (Fig. 3), and specific stains for His and Tyr failed to show any new spots. All other peptides of the β^{A} and β^{MR} chains from the same subject were analyzed for amino acid composition and were found to have the expected values.

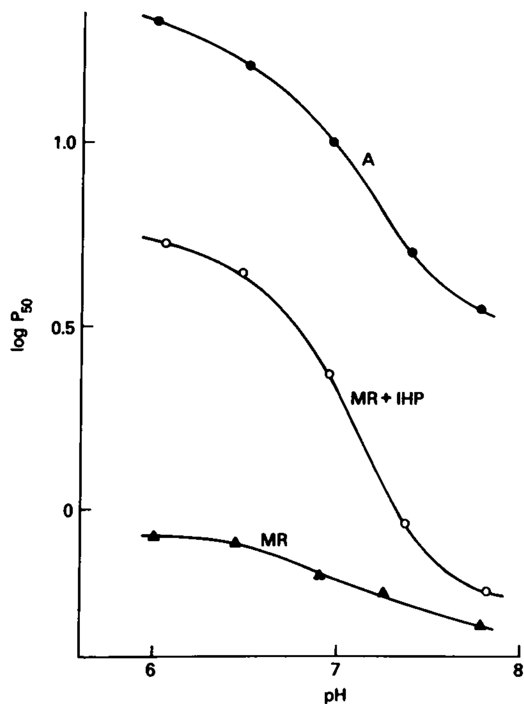

FIGURE 8 The Bohr effect of Hb A and Hb McKees Rocks. Each point represents a single oxygenation curve, done at 30°C, 0.05 M bis-Tris, 0.1 M NaCl.

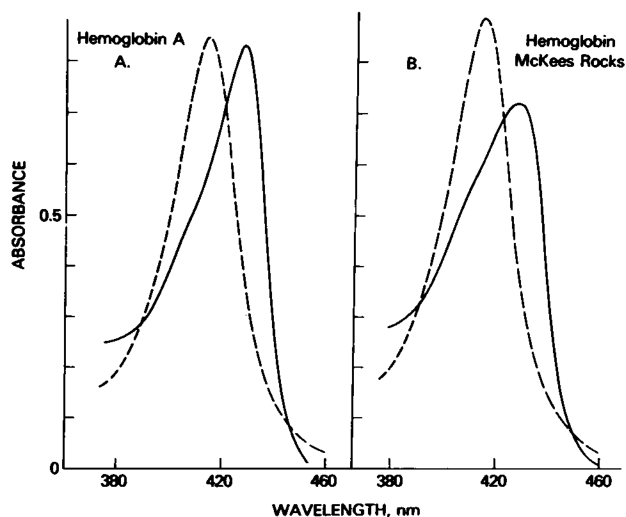

FIGURE 9 Soret absorption spectra of Hb A and Hb McKees Rocks. Scans were obtained at 30°C, pH 7. Deoxygenation was by equilibration of the hemoglobin with nitrogen with monitoring of the P_{O_2}. Sodium dithionite was added to ensure complete deoxygenation. ———, deoxyhemoglobins; - - - - -, oxyhemoglobins.

Digestion of the β^A chain with CPA resulted in the release of equivalent amounts of His and Tyr, while no amino acids were released after up to 2 h digestion of the β^{MR} chain (Table II). Hydrazinolysis of the two β-chains yielded only histidine from β^A and only Lys

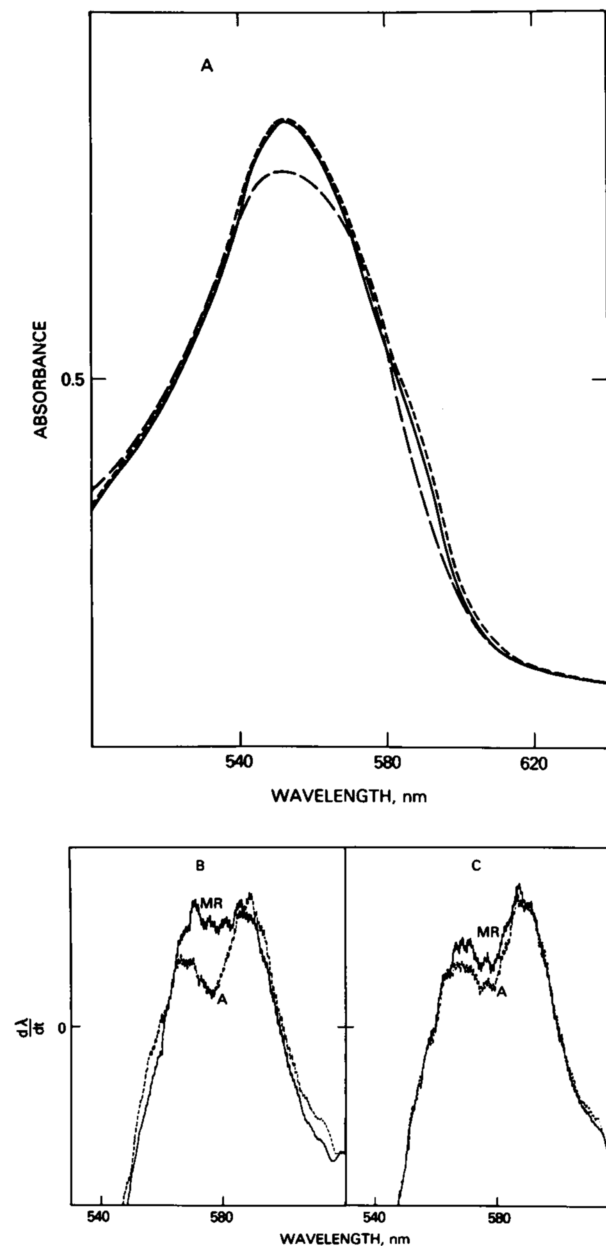

FIGURE 10 Visible absorption spectra of Hb A and Hb McKees Rocks. (A) ——; deoxyHb A; — — —, deoxyHb McKees Rocks; - - - -, Hb McKees Rocks + 100 μM IHP. (B) differential absorption spectra, deoxyhemoglobins. (C) differential absorption spectra in the presence of 100 μM IHP. pH 7, 0.05 M Bis-Tris, 0.1 M NaCl, hemoglobin concentration 8 μM (tetramer).

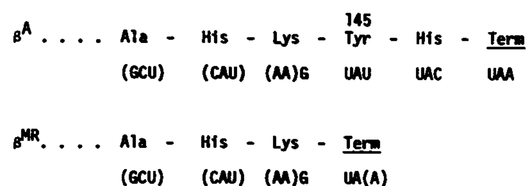

FIGURE 11 The proposed structure of Hb McKees Rocks. Experimentally determined nucleotide sequences (28) are not in parentheses; those in parentheses are consistent with the genetic code, but not proven. The proposed point mutation, U→A could equally be U→G.

from β^{MR}. On the basis of these data, it was concluded that the carboxyl-terminal amino acid of Hb McKees Rocks β-chain was Lys 144.

Whole blood oxygen equilibria. Whole blood oxygenation curves for subject II-1 and a normal subject are shown in Fig. 4. Oxygenation of Hb McKees Rocks occurred separately from that of Hb A in the blood of the heterozygote as suggested by the biphasic nature of the curve. Because of this biphasic feature, the oxygen pressure at half-saturation of Hb. (P_{50}) (10 mm Hg for MR/A blood) has little physiologic meaning.

Oxygen equilibria of purified Hb McKees Rocks and hemolysate. The oxygen equilibrium studies done with purified Hb A, McKees Rocks, and a hemolysate are shown in Fig. 5. The hemolysate curve is clearly biphasic, and the computer fit of the data for pure Hb A and MR to the hemolysate data suggested that Hb MR accounted for 46% of the total. The biphasic feature of the hemolysate curve indicates that there was no interaction between the two hemoglobins (21) and suggests that mixed deoxytetramers of the $\alpha_2{}^A\beta^A\beta^{MR}$ type do not occur to an appreciable extent under the conditions studied. A similar argument can be offered for whole blood from the data of Fig. 4, where two separate components are also apparent.

The influence of H^+, DPG, and IHP on the oxygenation of Hb A and Hb McKees Rocks. The addition of an eightfold excess of DPG to a solution of Hb McKees Rocks had no effect on its oxygenation curve (Fig. 6). However, the same concentration of DPG decreased the oxygen affinity of Hb A considerably (Table III). IHP decreased oxygen affinity of both Hbs A and Hb McKees Rocks and increased Hill's parameter, "n", for the latter. The IHP effect was concentration-dependent (Fig. 7), but the maximum P_{50} and n values were 2.29 and 1.81, respectively, still considerably less than the values for Hb A. The Bohr effect of Hb McKees Rocks was much less than that of Hb A but could be increased by the addition of IHP (Fig. 8). These results are essentially the same as those obtained with the chemically modified Hb CPA (22), a further confirmation of the assignment of structure. The decrease

TABLE IV

Some Physical Properties of Hemoglobins with Alterations near the β-Carboxyl-Terminus

Hb	Substitution	P_{50}(Variant)/P_{50}(HbA)	n	DPG effect	Bohr effect	Conditions*	Reference
						C pH	
Little Rock	H21 (143) His → Gln	2/5	2.5	↓	Normal	20°, 7	31
Abruzzo	H21 (143) His → Arg	1.3/1.8	2.0	↑	Normal	20°, 7	40
Syracuse	H21 (143) His → Pro	0.34/3	1.1	↓	↓	20°, 7.2	32
Andrew-Minneapolis	HC1 (144) Lys → Asn	3.3/18.5	2.4	Normal	↓	37°, 6.95	33
Bethesda	HC2 (145) Tyr → His	1.1/3.9	1.2	↓	↓	20°, 7.2	34
Rainier	HC2 (145) Tyr → Cys	2/12.5	1.4	Normal	↓	33°, 6.26	35
Osler	HC2 (145) Tyr → Asp	0.32/6.6	1.1	↓	↓	25°, 7.2	36,41
McKees Rocks	HC2 (145) Tyr → Term	0.56(30°C)/10(20°C)	1.0	↓	↓	30°, 7.0	—
Hiroshima	HC3 (146) His → Asp	0.5/8	1.4	Normal	↓	20°, 7	37
α^{SH}	—	0.45/8.7	1.0	—	—	20°, 7.0	36
β^{SH}	—	0.24/16.3	1.0	—	—	20°, 7.2	34
‡Hb CPA	—	0.44(30°C)/10(20°C)	1.0	↓	↓	7.0	22

* Buffers for the data for Hb Syracuse, Bethesda, Osler, McKees Rocks, α^{SH}, β^{SH} were 0.5 M bis-Tris, 0.5 M NaCl; for Hb Abruzzo and CPA, 0.1 M bis-Tris. Buffers for the remainder of the Hbs are not given in the references.
‡ Hb A which has been digested with CPA. Its structure is identical to that of Hb McKees Rocks.

in cooperativity of Hb A after the addition of IHP has been noted by others (23).

Absorption spectra. The absorption spectra for isolated Hb A and Hb McKees Rocks were identical for oxygenated samples but different for deoxygenated samples (Figs. 9, 10). The decrease in the maximal absorption in the Soret band (Fig. 9) observed for Hb McKees Rocks was identical to that observed with Hb CPA (3, 24). This difference was unaffected by the addition of DPG or IHP. A further difference was noted in the deoxy spectrum at 590 nm: in Hb A, but not Hb McKees Rocks, a small shoulder was noted (Fig. 10*a*). This difference could be demonstrated easily by examination of the derivative of the absorption spectrum (Fig. 10*b*) and was almost abolished by the addition of 100 μM IHP (Fig. 10*c*). These data are consistent with the notion that a conformational alteration occurs in the deoxy structure of the Hb McKees Rocks and that this alteration can be partially restored by the addition of IHP.

DISCUSSION

The evidence for the structural alteration of Hb McKees Rocks (β145 Tyr→Term, Fig. 11) is based on several types of data. The clear identification of carriers (Fig. 1, Table I) by electrophoresis, the clinical finding of erythrocytosis, and the finding of 46% Hb McKees Rocks in the lysate of a carrier support the view that this is not the result of enzymatic or other degradative reactions during isolation of the hemoglobin. The electrophoretic behavior of the variant hemoglobin is identical to that of Hb CPA (22). Tryptic peptide βT-15 is missing from fingerprints of the abnormal B-chain, but βT-14 and all other peptides of the β-chain are present and have the expected amino acid compositions. The C-terminal amino acid is Lys as suspected by CPA digestion and confirmed by hydrazinolysis. Finally, the functional properties of Hb McKees Rocks are identical with those reported for Hb CPA (*22, 25–27*).

We cannot be certain of the exact genetic alteration which gave rise to Hb McKees Rocks, but the mechanisms proposed for other abnormal Hb (1) can be invoked. First, deletion of UAUUAC, the mRNA sequence coding for Tyr 145 and His 146 could have occurred. At least eight such deletions have been observed in the human β-chain. Second, unequal crossing over between adjacent chromosomes could have occurred with resulting removal of the Tyr-His sequence. Third, deletion of an AUUACU sequence starting with the second nucleotide of the triplet for β145 Tyr could result in the termination codeword UAA after Lys 144β. Fourth, insertion of an A into the Tyr 145 triplet could have occurred. Other specific deletions, insertions, or duplications can be imagined. We favor the point mutation hypothesis, as indicated in Fig. 11, because point mutations appear to be common; they account for the bulk of the human variants described to date (1). The transition U → A proposed in the figure could equally be the transversion U → G.

The functional studies reported here serve to confirm that the structure of Hb McKees Rocks is identical with that of Hb CPA. The very high oxygen affinity, absent DPG reactivity, diminished Bohr effect, and the restoration of these properties by the addition of IHP are in close agreement with previous studies of Hb CPA and need not be described in detail here, since

they have been dealt with extensively in the literature on Hb CPA (*22, 25–27, 29,* 30).

Table IV summarizes functional data for all of the human mutants known to affect the C-terminal portion of the β-chain and whose properties have been studied. The properties of these mutants confirm the importance of this region. The oxygen affinity of Hb McKees Rocks is nearly identical to that of Hb CPA but is higher than that of isolated chains, an observation that is difficult to explain on structural grounds. Only Hb Osler has oxygen affinity that is elevated to approximately the same degree (36).

The "defect" in Hb McKees Rocks function can be viewed as a shift in the T-R equilibrium toward the R structure because of instability of the T structure: DPG binding, cooperativity, and the alkaline Bohr effect are all dependent upon an intact, stable T structure. IHP binds preferentially to the deoxy structure but with a much greater affinity than does DPG (38) and the greatest shift of the McKees Rocks equilibrium curve in the presence of IHP is at the bottom end (Fig. 6). Spectral changes are also associated with the addition of IHP to deoxyHb McKees Rocks (Fig. 10). These findings suggest that nearly all of the molecules take up the R conformation in deoxyHb McKees Rocks.

Because of the high oxygen affinity of Hb McKees Rocks and its lack of reactivity with DPG, red cells from carriers can be viewed as a limiting case for high affinity variants: blood P_{50} is about 10 mm Hg under arterial conditions. One can calculate that if 46% of the hemoglobin in red cells were a component which remained oxygenated under all conditions, blood P_{50} would still be about 9 mm Hg. Carriers of Hb Syracuse (32), Bethesda (34), Osler (36), and McKees Rocks have blood P_{50} values which are reduced to a similar degree. The value P_{50} has little significance in terms of estimating the oxygen-delivering capacity of the blood, since the Hb McKees Rocks curve is biphasic.

The transport of oxygen from lung to tissue sites is a complex process, depending on pulmonary function, blood flow, blood oxygen affinity, and tissue P_{O_2}. It is known that in all mammals, blood oxygen affinity and resting tissue P_{O_2} are maintained within rather close limits (39). Thus, the optimal oxygen requirement for metabolizing tissues is probably rather stringently controlled, and it might be expected that alteration in blood oxygen affinity to the extent seen with Hb McKees Rocks might have serious if not lethal consequences. To the contrary, we have observed that there are essentially no clinical consequences apart from compensatory erythrocytosis in any of the carriers of Hb McKees Rocks.

All mammals have evolved fetal hemoglobins that ensure a favorable oxygen gradient at the placenta for the maintenance of adequate fetal oxygenation. The high oxygen affinity β-chain variants, (Table IV) are the only known instances in which during pregnancy, maternal blood oxygen affinity is higher than that of the fetus. Yet, there appears to be no fetal wastage in these families, and in the present study, pregnancy and birth were observed to be without complication except for erythrocytosis in the mother. Thus, further physiological studies of these fascinating families could provide new insight into normal and pathological mechanisms of the regulation of oxygen delivery.

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