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Genetics

Hemoglobin Lincoln Park: A βδ fusion (anti-Lepore) variant with an amino acid deletion in the δ chain-derived segment

(gene hybrid/amino acid sequence)

GEORGE R. HONIG, MIR SHAMSUDDIN, R. GEORGE MASON, AND LOYDA N. VIDA

Division of Hematology, Children's Memorial Hospital, Department of Pediatrics, Northwestern University, 2300 Children's Plaza, Chicago, Illinois 60614

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ABSTRACT **An electrophoretically slow-moving hemoglobin variant was identified in three members of a family originating from Southern Mexico. The variant, Hb Lincoln Park, made up approximately 14% of the total hemoglobin and appeared to have normal stability and functional properties. None of the individuals in whom the abnormal hemoglobin was present was anemic, but each had a mildly elevated reticulocyte count. Structural data suggest that the non-α chain of Hb Lincoln Park represents a βδ gene-fusion product, with normal β chain structure of the amino-terminal portion of the chain and δ sequences subsequently, the crossover point occurring between amino acid residues 22 and 50. An additional abnormality is the deletion of valine-137, a component of the δ gene-derived segment of the βδ chain. To account for the development of this abnormal globin chain, a series of intergenic crossovers is proposed; the first, a nonhomologous crossover between the β and δ genes, presumably gave rise to the βδ fusion gene; two additional crossovers, one of them unequal, may then have occurred between the same β and δ genes to produce the amino acid deletion.**

The Lepore and anti-Lepore hemoglobins appear to have arisen as a result of nonhomologous crossing over of the β and δ genes, with the formation of gene hybrids (1). Distinguishable variants have been identified within each of these major groups of hybrid-chain hemoglobins, depending on the crossover site within the molecule (2). The Lepore (δβ) variants are believed to have formed by a process that also produced a deletion of the normal δ and β genes (1), and these variants are accompanied by a thalassemia-like disorder with decreased hemoglobinization of the erythrocytes (3); the anti-Lepore (βδ) hemoglobins, which apparently developed without loss of the linked δ and β genes, are characteristically associated with a normal hematologic picture (2, 4).

In this report we describe the hematologic and structural features of a new anti-Lepore variant in which an amino acid deletion is present in addition to the structural characteristics suggestive of βδ hybrid gene formation. In common with other known anti-Lepore variants, the individuals in whom this variant was identified had normal hemoglobin levels and erythrocyte indices. Each affected individual, however, had an elevated reticulocyte count, possibly reflecting a mild hemolytic state related to the amino acid deletion in the abnormal hemoglobin.

METHODS

Hematologic and Hemoglobin Procedures. Hematologic measurements were made with a Coulter model S cell counter that was standardized daily with a commercial standard. Other determinations were performed by standard laboratory procedures (5). Blood samples for hemoglobin analysis were collected in EDTA. The erythrocytes were washed three times in isotonic saline and lysed with 2–3 volumes of water. The stroma were removed by centrifugation at 20,000 × *g*. Hemoglobin electrophoresis was performed in starch gel as well as cellulose acetate, with Tris/EDTA/borate, pH 8.6. For quantitation of individual hemoglobin components the hemoglobin bands were excised after cellulose acetate electrophoresis and eluted with water, and their absorbance was determined at 415 nm. Isoelectric focusing (6) was carried out in polyacrylamide columns in the pH range 6–8. Alkali-resistant hemoglobin was estimated according to Betke *et al.* (7). Oxygen equilibrium curves were determined with a blood gas analyzer, model 217, from Instrumentation Laboratories, Inc. The procedures were as described (8). For determinations of 2,3-diphosphoglycerate, protein-free filtrates were prepared from trichloroacetic acid-treated samples of whole blood. The spectrophotometric assay procedure was as described by Keitt (9).

Isolation of the Variant Hemoglobin and Globin Chain. DEAE-Sephadex column chromatography of carbon monoxide-saturated hemoglobins was performed according to Dozy *et al.* (10). Effluent fractions corresponding to the variant hemoglobin were pooled and the hemoglobin was concentrated by vacuum dialysis. Globin was prepared by precipitation in acetone/HCl at −20°, and the globin chains were separated by CM-cellulose chromatography (11). Globin representing the non-α chain was aminoethylated (11) and subsequently desalted by gel filtration through a column of Bio-Gel P-2 equilibrated with 0.5 M formic acid. The protein was recovered by lyophilization.

Peptide Isolation and Purification. The aminoethylated globin was digested with trypsin for 2 hr at 37° in NH_4HCO_3 buffer. The tryptic peptides were separated by PA-35 column chromatography with a linear pyridine/acetic acid gradient (12). Peptides requiring repurification were subjected to chromatography on a column of Dowex 50-X2 with the same buffer system.

Amino Acid Analysis. Purified samples of globin and peptides for amino acid analysis were hydrolyzed for 24 hr, or in some cases 72 hr, in 6 M HCl under reduced pressure at 110°. The analyses were performed with a Beckman–Spinco model 121M amino acid analyzer equipped with a system AA integrator. Sequence analysis of the abnormal peptide was by a subtractive Edman procedure based on the method of Gray (13) with minor modifications.

RESULTS

Case Report. The index case, a 3-year-old child, was referred for evaluation when he was found to have an electrophoretically slow-moving abnormal hemoglobin by a municipal hemoglobinopathy survey. The child had been in good health and had

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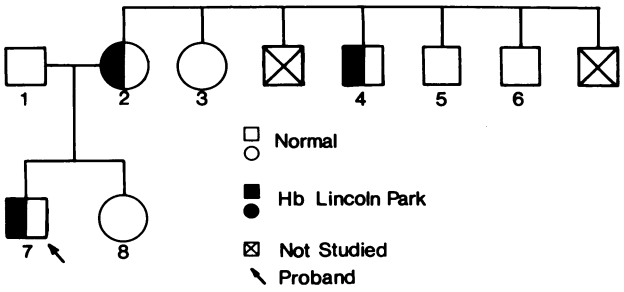

FIG. 1. Pedigree of the family.

no abnormal findings by physical examination. The mother and a maternal uncle were also found to have the hemoglobin abnormality (Fig. 1). The family are Mexican, originating from Michoacán.

Erythrocyte morphology appeared to be entirely normal in stained blood smears of the family members in whom the variant hemoglobin was identified; none of these individuals was anemic, and their erythrocyte indices were normal, but each had a mildly elevated reticulocyte count (Table 1). Family member 8, a 1-year-old child, appeared to be recovering from iron deficiency.

Hemoglobin Studies. The electrophoretic mobility of the variant hemoglobin at pH 8.6 was slightly anodal to that of hemoglobin S. A similar relationship was observed in an isoelectric focusing determination. The abnormal hemoglobin made up between 13.6 and 14.5% of the total and was accompanied by normal levels of Hb A_2 but a moderate increase in the level of alkali-resistant hemoglobin (Table 1). The oxygen affinity of a blood sample from the propositus was normal but appeared slightly increased in a sample of blood obtained from his mother (Table 1). The erythrocyte levels of 2,3-diphosphoglycerate were normal in both. Studies of freshy prepared hemolysates for evidence of hemoglobin instability (14, 15) yielded negative results.

Isolation of the Variant Hemoglobin and Globin Chain. The abnormal hemoglobin was eluted from the DEAE-Sephadex column approximately midway between hemoglobins A_2 and A (Fig. 2) and was well separated from each. Complete resolution of the α and non-α chains was achieved by CM-cellulose chromatography, with the non-α component emerging from the column slightly ahead of the normal point of elution of β^A.

Structural Studies of the Variant Globin Chain. Analyses

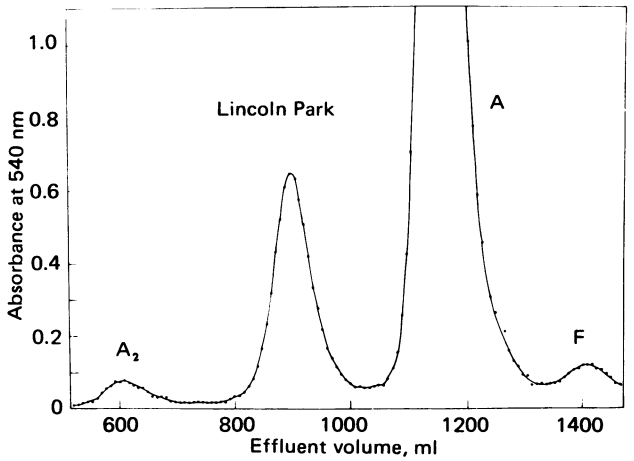

FIG. 2. Separation of the hemoglobins from a subject with Hb Lincoln Park by DEAE-Sephadex chromatography.

of hydrolyzed samples of the purified non-α globin chain (Table 2) revealed multiple differences in its amino acid composition in comparison with that of the normal β^A or δ chains. Its composition, however, corresponded closely to that expected for a $\beta\delta$ hybrid with a crossover after β-22, the single point of nonconformity being an apparent deficiency of one residue of valine per chain.

The amino acid composition of the purified tryptic peptides (Table 3) supported the impression that the variant globin chain represented a $\beta\delta$ hybrid. Peptide T-2 contained serine, and T-3 contained two residues of glutamic acid, as occur in the corresponding peptides of the β^A chain. The composition of T-5 (including three residues of serine and absence of threonine) is characteristic of this peptide in the normal δ chain, and in each of the succeeding peptides in which differences exist between the β and δ chains (T-10, T-12b, T-13, and T-14) the amino acid composition corresponded to that of the δ chain peptide.

The remaining peptides, in which the amino acid sequences are identical in the β and δ chains, had normal amino acid compositions with the exception of T-15 and T-15 + 16, in which only two of the three expected valine residues were present. The yield of valine in the T-15 peptide reached maximal values only after prolonged periods of acid hydrolysis (Table 4), suggesting that the hydrolytically resistant Val-Val bond normally present between residues 133 and 134 was re-

Table 1. Hematologic and hemoglobin composition data*

Family member†	Hb, g/dl	RBC	PCV, %	MCV, fl	MCH, pg	MCHC, %	Retic, %	$P^{50}O_2$, mm Hg	2,3-DPG, μmol/g Hb	Hb A_2, %	Alkali-resistant Hb, %	Hb Lincoln Park, %
1	16.4	5.27	45.6	84	31.3	37.3	1.2			2.7	1.6	—
2	14.1	5.03	39.7	86	28.1	32.0	2.6	25.2	16.6	1.6	4.2	14.5
3	11.7	4.05	34.4	84	28.7	33.4	1.4					—
4	15.7	5.56	44.9	81	28.3	35.3	2.9			2.8	3.6	13.6
5	14.8	4.66	41.7	88	31.8	35.6	1.6			2.9	1.3	—
6	15.2	4.97	43.3	86	30.5	35.2	1.8			3.1	1.1	—
7	14.1	5.05	39.8	76	28.2	36.8	7.0	27.0	18.9	2.3	4.9	14.1
8	13.7	5.25	39.2	72	26.3	36.4	5.0			3.2	5.0	—
Normal values								27.2 ±0.6	15.0 ±2.4			

* Variables and units of measurement: Hb, hemoglobin, g/100 ml; RBC, erythrocyte count, 10^{12}/liter; PCV, packed cell volume; MCV, mean corpuscular volume, femtoliters; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Retic., reticulocytes; $P^{50}O_2$, partial pressure of oxygen at which erythrocytes are 50% saturated; alkali-resistant Hb, alkali-resistant hemoglobin.

† Numbered as in Fig. 1.

Table 2. Amino acid composition of the non-α chain of Hb Lincoln Park*

Amino acid	Hydrolysate 24-hr	Hydrolysate 72-hr	β^A	δ	$\beta\delta$ Hybrid (β22–δ50)
Lys	11.0	11.4	11	11	11
His	6.9	7.0	9	7	7
Arg	3.9	3.7	3	4	4
Asp	14.0	13.8	13	15	14
Thr	4.8	4.4	7	5	5
Ser	6.7	5.1	5	6	7
Glu	13.0	12.7	11	12	13
Pro	4.8	5.3	7	6	5
Gly	12.8	13.1	13	13	13
Ala	15.0	15.0	15	15	15
Val	15.8	16.2	18	17	17
Met	1.8	1.0	1	2	2
Leu	18.0	18.0	18	18	18
Tyr	3.2	3.0	3	3	3
Phe	8.0	7.1	8	8	8

* The indicated values represent the number of amino acid residues per chain.

tained in this peptide. A subtractive Edman degradation analysis of a purified sample of the T-15 peptide confirmed that the amino-terminal sequence of the peptide was Val-Val; after completion of the second step of the degradation, no valine remained whereas the other amino acids normally present in this peptide were recovered in their expected ratios; the fifth step of the degradation established directly that valine-137 was deleted (Fig. 3).

Table 4. Recovery of valine from purified T-15 peptide

Hydrolysis in 6 M HCl, hr	Valine residues recovered/peptide
16	1.54
24	1.84
72	1.96
92	1.98

DISCUSSION

The results of the structural studies suggest that the non-α chain of Hb Lincoln Park represents a $\beta\delta$ hybrid with a crossover point occurring between β22 and δ50. In addition, the globin chain is shortened by one residue due to the deletion of the valine that normally occupies δ137. Although a number of hybrid-chain hemoglobins have been described (1, 2, 4, 16–19), Hb Lincoln Park represents an apparently unreported example of a globin-chain hybrid having an additional structural abnormality. Another possible example of a doubly abnormal $\beta\delta$ hybrid, however, may be represented by Hb Coventry (20). This variant, in which β141 leucine is deleted, occurred together

Table 3. Amino acid composition of tryptic peptides of the non-α globin chain of Hb Lincoln Park*

Acid	T1 β 1–8	T2 β 9–17	T3 β 18–30	T4 β or δ 31–40	T5 δ 41–59	T6 δ 60–61	T7 δ 62–65	T8 δ 66	T9 δ 67–82	T10 δ 83–95	T11 δ 96–104	T12a δ 105–112	T12b δ 113–116	T13 δ 117–120	T14 δ 121–132	T15 δ 133–144	T16 δ 145–146	T15–16 δ 133–146
Lys	1.06 (1)	0.99 (1)			1.00 (1)	0.99 (1)	1.00 (1)	1.00 (1)	1.06 (1)	0.94 (1)				1.01 (1)	1.02 (1)	0.99 (1)		1.05 (1)
His	0.98 (1)						1.01 (1)		1.05 (1)	0.87 (1)	0.92 (1)					0.95 (1)	1.05 (1)	1.82 (2)
Arg			1.00 (1)	1.00 (1)							0.96 (1)		1.00 (1)					
Asp			2.00 (2)		3.12 (3)				2.90 (3)	1.07 (1)	2.00 (2)	0.97 (1)		0.98 (1)		1.00 (1)		1.00 (1)
Thr	0.92 (1)	0.94 (1)		0.99 (1)						0.99 (1)					1.02 (1)			
Ser		0.96 (1)			2.87 (3)				0.97 (1)	1.93 (2)								
Glu	2.02 (2)		2.00 (2)	1.00 (1)	1.01 (1)					2.07 (2)	1.03 (1)				3.96 (4)			
Pro	1.07 (1)			1.00 (1)	2.01 (2)						0.98 (1)				1.02 (1)			
Gly		1.00 (1)	3.10 (3)		2.12 (2)		1.01 (1)		2.07 (2)	1.07 (1)		1.05 (1)		1.04 (1)		1.05 (1)		1.13 (1)
Ala		2.02 (2)	1.00 (1)		1.00 (1)		1.00 (1)		2.03 (2)				0.92 (1)		2.03 (2)	3.94 (4)		3.89 (4)
Val	1.00 (1)	1.08 (1)	2.98 (3)	1.90‡ (2)	1.04 (1)	1.01 (1)			1.02 (1)		1.03 (1)	2.03 (2)	1.05 (1)			1.96‡ (3)		1.96‡ (3)
Met					0.50 (1)										0.60 (1)			
Leu	1.01 (1)	1.02 (1)	1.00 (1)	2.00 (2)	1.00 (1)				3.92 (4)	2.04 (2)	1.12 (1)	2.95 (3)	1.00 (1)			1.10 (1)		1.10 (1)
Tyr				0.90 (1)											0.93 (1)		0.95 (1)	0.90 (1)
Phe					2.93 (3)				0.98 (1)	1.06 (1)	0.95 (1)			0.97 (1)	1.02 (1)			
Try†		+(1)		+(1)														

* Numbers in parentheses represent the number of residues of the amino acid present in the normal peptide.
† Tryptophan was detected by spot testing on paper.
‡ Indicated values for valine in peptides T4, T15, and T15 + T16 were obtained from samples hydrolyzed for 72 hr.

δT-15	Val	Val	Ala	Gly	Val	Ala	Asn	Ala	
βδ Lincoln Park T-15	Val	Val	Ala	Gly	[ ]	Ala	Asn	Ala	
	133	134	135	136	137	138	139	140	

FIG. 3. Sequence determination of peptide T-15, demonstrating the deletion of valine-137 residue.

with another β chain structural variant, in an individual who may also have had hemoglobin A (20). It was proposed that Hb Coventry may have arisen by nonhomologous crossover between β and δ genes, at a point near the carboxyl terminus where both chains have identical amino acid sequences (20).

Among the individuals in whom Hb Lincoln Park was identified, none had anemia, microcytosis, hypochromia, or morphological erythrocyte abnormalities, in common with other known cases of anti-Lepore variants. These findings are consistent with the βδ hybrid gene having been formed by a process that involved partial duplication of the β and δ structural genes without deletion of the normal globin genes (1).

The significance of the elevated reticulocyte counts in the individuals with Hb Lincoln Park is unclear. Although tests of hemoglobin stability failed to demonstrate an unstable fraction, a mild degree of instability, sufficient to bring about a shortening of erythrocyte survival, may nevertheless have been the principal underlying cause for the increased reticulocyte counts. It may be relevant in this regard that, of 10 known hemoglobin variants having one or more deleted amino acids (20–28), all have shown at least some degree of molecular instability.

The development of a hemoglobin mutant in which an amino acid is deleted would imply that a codon triplet was lost in the globin gene. Unequal crossing over between homologous globin genes during meiosis has been suggested as a likely mechanism to account for an event of this kind (21–23). The loss of one or more base triplets from a globin gene by unequal crossing over would also be expected to produce, at the same time, globin genes having additional codon insertions. A recently described variant, Hb Grady (29), was shown to have a reiterated sequence of three amino acids in its α chains, apparently representing an elongated gene formed by a mismatched α gene crossover of this kind.

It has been noted that a pair of adjacent identical amino acids occupies a position close to the regions of amino acid deletion in several of the known hemoglobin deletion variants (21–23, 25, 28). Assuming that these amino acid pairs represent identical adjacent codons, these regions may serve as sites for nonhomologous pairing prior to an unequal crossover. A similar relationship appears to exist in the βδ chain of Hb Lincoln Park: the deleted residue 137 is in close proximity to a pair of adjacent valine residues at 133 and 134 (see Fig. 3).

The presence in the variant globin chain of Hb Lincoln Park of both a βδ hybrid structure and an amino acid deletion suggests that the corresponding gene may have arisen as a result of two separate crossover events, one a nonhomologous crossing over involving β and δ genes and the other an unequal crossing over either between δ genes or between β and δ genes. A possible explanation for the coexistence of these structural abnormalities in the variant chain is that the two postulated crossovers may have occurred as separate, successive changes that by chance involved a single gene. This possibility would be strengthened by the identification of either the βδ hybrid or the δ chain deletion occurring in isolated form in a family member of the individuals with Hb Lincoln Park. Attempts to document variants of this kind in the available family members, however, have been unrewarding.

Although isolated mutational events with subsequent homologous crossing over may well be the most plausible explanation for the development of hemoglobin variants with separated point mutations in the same chain (30–32), the occurrence of separate successive crossovers would seem to be less probable as a mechanism for the development of the variant chain of Hb Lincoln Park. In contrast to the high frequency of certain point-mutation abnormal hemoglobins in some populations, crossover-derived variants of the βδ type are apparently extremely rare, previously having been identified in only three families (2, 4, 19). Deletion mutations are also of rare occurrence, and none thus far has been reported that involves the δ chains, although this may be related to the likelihood that an abnormality involving only Hb A_2 will escape detection.

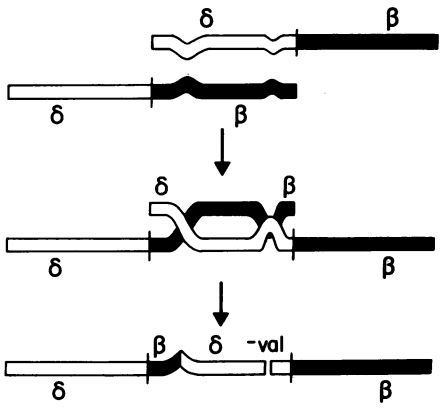

FIG. 4. Repeated crossing over between the β and δ genes during meiosis as a possible mechanism for the development of the variant globin chain of Hb Lincoln Park.

Because of the rare occurrence of βδ chain hybrids as well as deletion variants, and the exceedingly low probability therefore that both such abnormalities would develop successively in the same gene, it would seem appropriate to consider possible mechanisms that might lead to the development of both the βδ hybrid and the deletion as part of a single process. A possible mechanism that fulfills this requirement, and is consistent with the hematologic findings of the individuals in whom Hb Lincoln Park was identified, is illustrated in Fig. 4. Included in this postulated mechanism are three simultaneous crossovers; (*i*) a βδ crossover between corresponding segments of the genes for these chains; (*ii*) an unequal cross-back between the β and δ genes to produce the amino acid deletion; and (*iii*) an additional crossover that allows the normal β gene to be retained on the chromosome bearing the βδ hybrid gene (a normal β gene is assumed to be present on the chromosome from the absence of anemia or microcytosis in the individuals having the abnormal hemoglobin). From the structural data, the site of the first crossover would be localized to the region between residues 22 and 50; the second crossover presumably would be at the site of the deletion at residue 137; and the third would be expected to occur prior to the point corresponding to the carboxyl-terminal amino acid position 146, although possibly later, but in any case prior to the region representing the amino terminus of the β chain.

An alternative mechanism for the formation of a βδ gene hybrid with a deleted valine codon might be a crossover with subsequent gene conversion, followed by excision at the repair point (33, 34). As with the other proposed mechanism, an additional crossover would then be required in order to retain the normal β gene on the chromosome.

It was shown in early studies of gene recombination in *Drosophila* (35) that crossing over of a chromosome decreases the probability of another crossover occurring in an adjacent area

of the same chromosome, the phenomenon of "interference." More recent studies, which have included various different organisms (36–39), have led to the conclusion that, for very closely linked regions of a chromosome, "negative interference," the apparent facilitation of additional crossovers once a crossover has occurred, may be a phenomenon of general biological significance. Smithies (1) in 1964 noted the possible relevance of negative interference to globin gene hybrid formation and suggested that evidence of multiple crossing over events might become apparent from studies of Lepore-type hybrid chain hemoglobins. The variant chain of Hb Lincoln Park may represent an example of a process of this kind. Detailed structural studies of other crossover-derived globin chains, and of globin chains closely linked to them, may allow other examples of multiple crossover products to be identified.

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1. Smithies, O. (1964) *Cold Spring Harbor Symp. Quant. Biol.* **29,** 309–319.
2. Badr, F. M., Lorkin, P. A. & Lehmann, H. (1972) *Nature New Biol.* **242,** 107–110.
3. White, J. M., Lang, A., Lorkin, P. A., Lehmann, H. & Reeve, J. (1972) *Nature New Biol.* **235,** 208–210.
4. Ohta, Y., Yamaoka, K., Sumida, I. & Yanase, T. (1971) *Nature New Biol.* **234,** 218–220.
5. Cartwright, G. E. (1963) Diagnostic Laboratory Hematology (Grune & Stratton, New York).
6. Drysdale, J. W., Righetti, P. & Bunn, H. F. (1971) *Biochim. Biophys. Acta* **229,** 42–50.
7. Betke, K., Marti, H. R. & Schlicht, I. (1959) *Nature* **184,** 1877–1878.
8. Honig, G. R., Gunay, U., Mason, R. G., Vida, L. N. & Ferenc, C. (1976) *Pediatr. Res.* **10,** 613–620.
9. Keitt, S. (1971) *J. Lab. Clin. Med.* **77,** 470–475.
10. Dozy, A. M., Kleihauer, E. F. & Huisman, T. H. J. (1968) *J. Chromatogr.* **32,** 723–727.
11. Clegg, J. B., Naughton, M. A. & Weatherall, D. J. (1966) *J. Mol. Biol.* **19,** 91–108.
12. Jones, R. T. (1964) *Cold Spring Harbor Symp. Quant. Biol.* **29,** 297–308.
13. Gray, W. R. (1967) in *Methods in Enzymology,* ed. Hirs, C. H. W. (Academic, New York), Vol. 11, pp. 469–475.
14. Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beaven, G. H. & White, J. C. (1964) *Br. J. Haematol.* **10,** 388–402.
15. Carrell, R. & Kay, R. (1972) *Br. J. Haematol.* **23,** 615–619.
16. Ostertag, W. & Smith, E. W. (1969) *Eur. J. Biochem.* **10,** 371–376.
17. Huisman, T. H. J., Wrightstone, R. N., Wilson, J. B., Schroeder, W. A. & Kendall, A. G. (1972) *Arch. Biochem. Biophys.* **153,** 850–853.
18. Schroeder, W. A., Huisman, T. H. J., Hyman, C., Shelton, J. R. & Apell, G. (1973) *Biochem. Genet.* **10,** 135–147.
19. Lehmann, H. & Charlesworth, D. (1970) *Biochem. J.* **119,** 42P.
20. Casey, R., Lang, A., Lehmann, H. & Shinton, N. K. (1976) *Br. J. Haematol.* **33,** 143–144.
21. Jones, R. T., Brimhall, B., Huisman, T. H. J., Kleihauer, E. & Betke, K. (1966) *Science* **154,** 1024–1027.
22. Bradley, T. B., Wohl, R. C. & Rieder, R. F. (1967) *Science* **157,** 1581–1583.
23. deJong, W. W. W., Went, L. N. & Bernini, L. F. (1968) *Nature* **220,** 788–790.
24. Shibata, S., Miyaji, T., Ueda, S., Matsuoka, M., Juchi, I., Yamada, K. & Shinkai, N. (1970) *Proc. Jpn. Acad.* **46,** 440.
25. Praxedes, H. & Lehmann, H. (1972) *Proceedings of the Fourteenth International Congress of Hematology, Sao Paulo.*
26. Wajcman, H., Labie, D. & Schapira, G. (1973) *Biochim. Biophys. Acta* **295,** 495–504.
27. Cohen-Solal, M., Blouquit, Y., Garel, M. C., Thillet, J., Gaillard, L., Creyssel, R., Gibaud, A. & Rosa, J. (1974) *Biochim. Biophys. Acta* **351,** 306–316.
28. Lutcher, C. L., Wilson, J. B., Gravely, M. E., Stevens, P. D., Chen, C. J., Lindeman, J. G., Wong, S. C., Miller, A., Gottleib, M. & Huisman, T. H. J. (1976) *Blood* **47,** 99–112.
29. Huisman, T. H. J., Wilson, J. B., Gravely, M. & Hubbard, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3270–3273.
30. Bookchin, R. M., Nagel, R. L., Ranney, H. M. & Jacobs, A. S. (1966) *Biochem. Biophys. Res. Commun.* **23,** 122–127.
31. Adams, J. G. & Heller, P. (1973) *Blood* **42,** 990.
32. Goossens, M., Garel, M. C., Auvinent, J., Basset, P., Ferreira Gomes, P. & Rosa, J. (1975) *FEBS Lett.* **58,** 149–154.
33. Stadler, D. R. (1973) *Annu. Rev. Genet.* **7,** 113–127.
34. Meselson, M. S., & Radding, C. M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 358–361.
35. Morgan, T. H., Sturtevant, A. H. & Bridges, C. B. (1925) *Bibliogr. Genet.* **2,** 1–262.
36. Chase, M. & Doermann, A. H. (1958) *Genetics* **43,** 332–353.
37. De Serres, F. J. (1958) *Cold Spring Harbor Symp. Quant. Biol.* **23,** 11–118.
38. Pritchard, R. H. (1960) *Genet. Res.* **1,** 1–24.
39. Sinclair, D. A. (1975) *Genet. Res.* **26,** 127–185.