Hemoglobin variants

CLASSIFICATION OF HEMOGLOBIN VARIANTS

- Hb variants are classified according to the type of mutation.
- Single point mutations in α-globin chains give rise to substitution of one amino acid residue.
- As an example, Hb SanDiego [β109(G11) Val→Met] has a methionine residue instead of the normal valine at position 109 of the β -chain.
- Hemoglobin C Harlem [β 6(A3) Glu→Val; β 73(E17) Asp-Asn] is an example of an Hb variant in which two amino acid residues are substituted, namely, valine replacing glutamic acid at position 6 and asparagine replacing aspartic acid at position 73 of the β - chain

- **Deletion:** Hb variants arise from the deletion of one to five amino acid residues in the globin chain. *Hb Vicksburg* [β 75(E19) Leu0] is an example in this category of Hb having a deletion of leucine in position 75 of the - β chain.
- **Insertion:** Hbs arise from insertion of one to three amino acid residues into the globin chain. *Hemoglobin Grady* is an example in this category with an insertion of a three amino acid residue sequence (glutamine-phenylalanine-threonine) between positions 118 and 119 of the α chain.
- **Deletion-insertion Hbs :** arise from the deletion of a portion of the normal amino acid residue sequence and the insertion of another sequence, with resultant lengthening or shortening of the globin chain. An example of this type of Hb variant is *Hb Montreal*, in which the three normal amino acid residues between positions 72 and 76 of the - β globin chain are replaced with a four amino acid residue sequence.

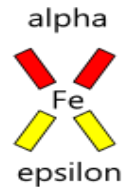
- **Elongation Hbs** result from a single base pair mutation or frameshift at the 3' end of exon 3 or the 5' end of exon 1 of the α2- or the β globin chain. The elongation hemoglobin, **Hb Constant Spring** has an additional 31 amino acid residues joined at position 142 (the carboxy terminal) of the - α chain.
- **Fusion Hbs** result from the fusion of an α- or β globin chain with a portion of another globin chain.
- **Hemoglobin Lepore-Hollandia** results from the fusion of the first 22 amino acid residues of the δ-chain with the amino acid sequence from position 50 onward of normal β globin.

NORMAL HEMOGLOBIN (Hg) VARIANTS

Hemoglobin A
(Adult hemoglobin)
2 alpha | 2 beta
(> 95% of Hg in adults)

Hemoglobin A_2
(Adult variant hemoglobin)
2 alpha | 2 delta
(1.5 - 3% of Hg in adults)

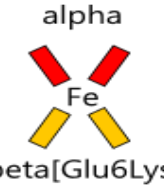
Hemoglobin F
(Fetal hemoglobin)
2 alpha | 2 gamma
(0.5% of Hg in adults)



Hemoglobin E
(Embryonic hemoglobin)
2 alpha | 2 epsilon
(present in early gestation)

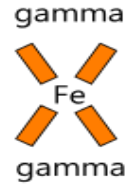
ABNORMAL HEMOGLOBIN (Hg) VARIANTS

Hemoglobin S
(Sickle cell disease)
2 alpha | 2 beta[Glu6Val]



Hemoglobin C
(Hemoglobin C disease)
2 alpha | 2 beta[Glu6Lys]

Hemoglobin H
(Heterozygous alpha thalassemia)
4 beta chains



Hemoglobin Bart's
(Homozygous alpha thalassemia)
4 gamma chains

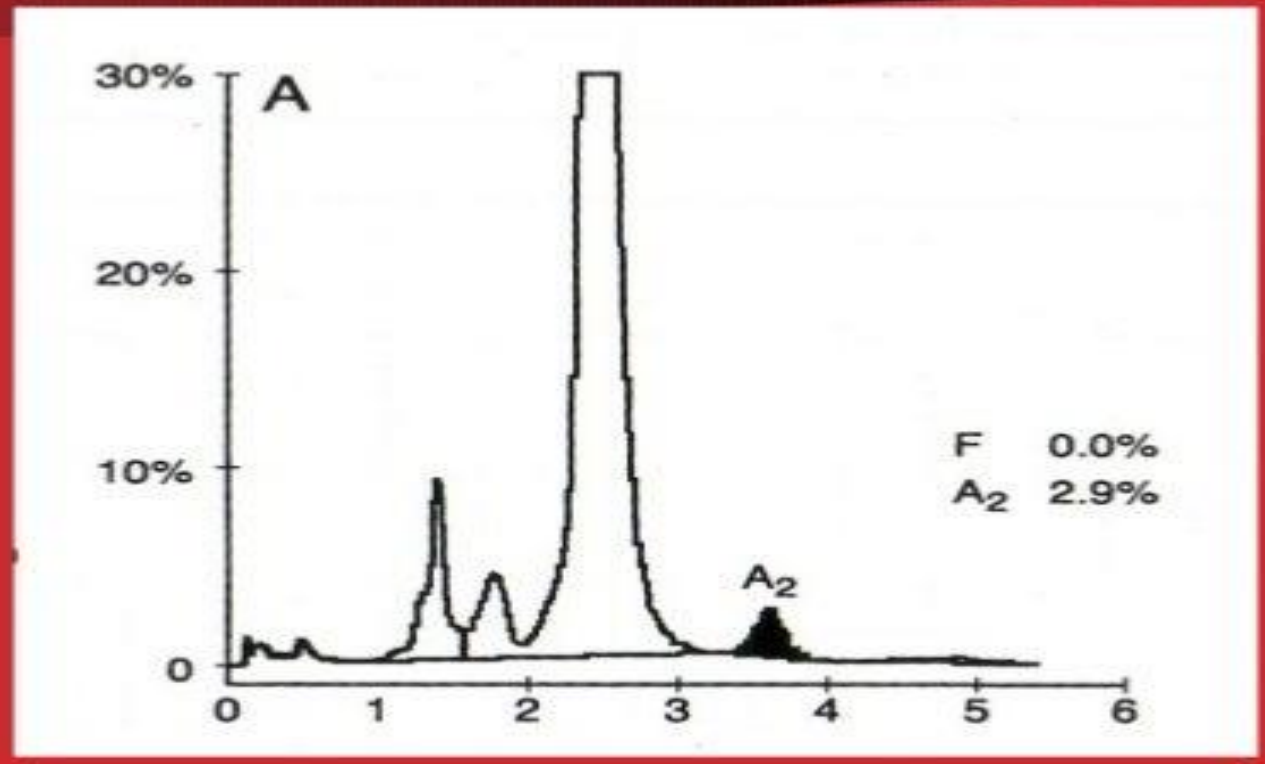
Hemoglobin chains

- α - alpha
- β - beta
- γ - gamma
- δ - delta
- ε - epsilon
- Beta[Glu6Lys] - beta globulin with glutamic acid replaced by lysine at the 6 position
- Beta[Glu6Val] - beta globulin with glutamic acid replaced by valine at the 6 position

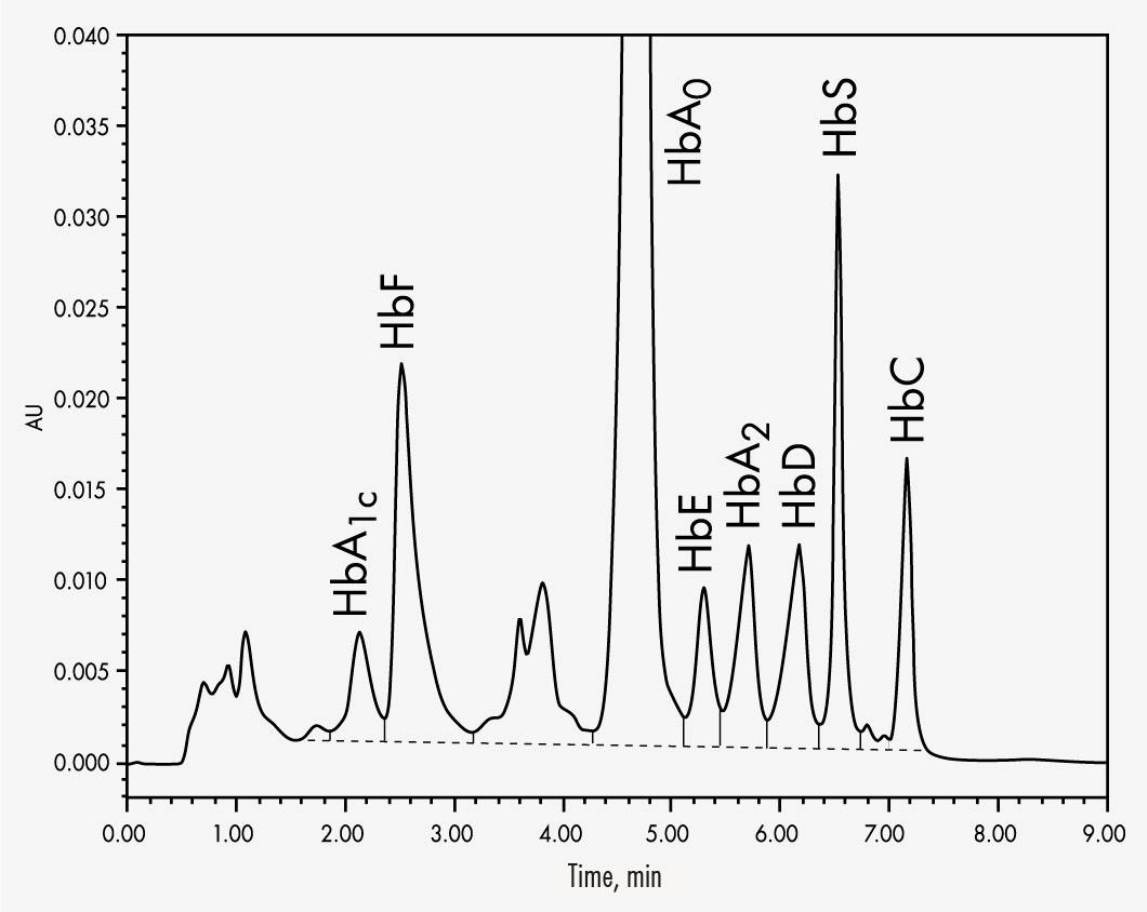
Normal hemoglobin variant

Designation	Tetrameric structure	Hemolysate (%)	
		Adult	Newborn
Adult			
HbA	$\alpha_2\beta_2$	95-98	20-30
HbA_2	$\alpha_2\delta_2$	2-3	0.2
Fetal			
HbF	$\alpha_2\gamma_2$	<1	80
Embryonic			
Gower 1	$\zeta_2\epsilon_2$	0	0
Gower 2	$\alpha_2\epsilon_2$	0	0
Hb Portland	$\zeta_2\gamma_2$	0	0

Normal HPLC pattern



High performance liquid chromatography



Hemoglobin variants

- In α-chain variants, the variant usually forms less than 25% of the total Hb because the mutation typically occurs in only one of the four genes that code for the α-globin chain.
- For β-chain variants in the heterozygous state, the variant forms 25% - 50% of the total Hb. Based on the mutation of only one of the β-globin chain genes, the Beta-chain variant should form 50% of total Hb.

Hemoglobin S [β6(A3)Glu→Val].

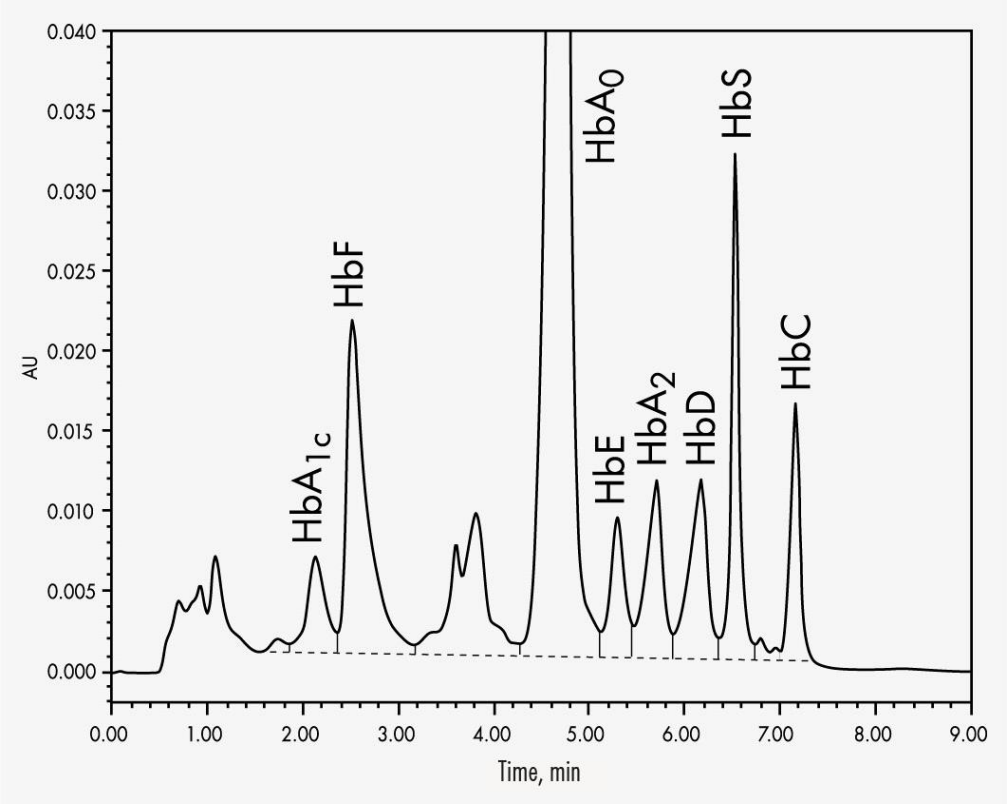
- homozygous state
- heterozygous state

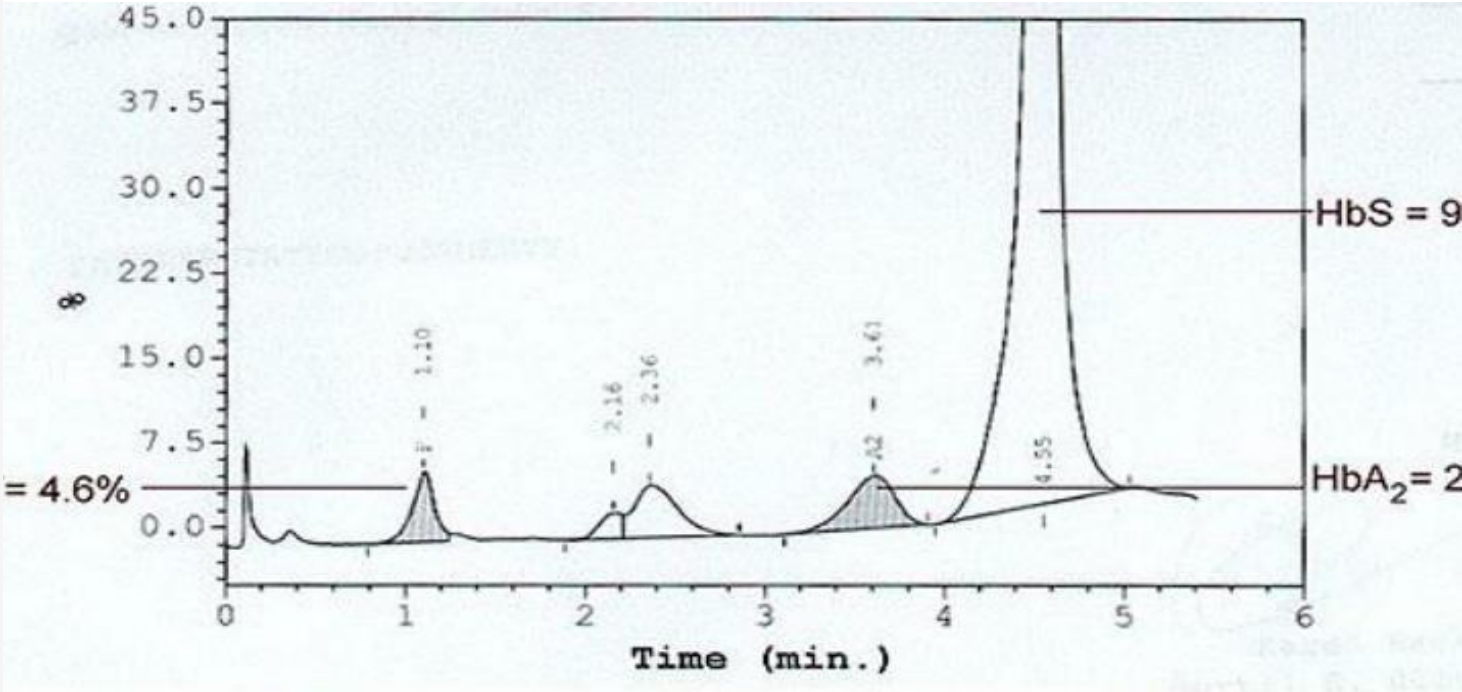
- It is the most widespread of the Hb variants and arises from a substitution of valine for glutamic acid at position 6 in the A helix of the β -globin chain.
- Hb S is found in high frequency in West and North Africa, the Middle East , and the Indian subcontinent.
- Approximately 30% of African and 8% in Americans are heterozygous for Hb S, and homozygous Hb S is found in 1 in 500 newborns in this group. .
- The widespread distribution of the single point gene mutation responsible for the synthesis of Hb S in areas where P falciparum malaria is endemic is due to protection of Hb S heterozygotes from the worst manifestations of the malaria.

Homozygous Hemoglobin S (Hb SS)

- In homozygous Hb S, a valine-for-glutamic acid substitution occurs on both β-globin chains because of the inheritance of mutated β -globin chain genes from both parents.
- This condition is described as "**sickle cell anemia**" or "**sickle cell disease"** because of the sickle-shaped RBCs that occur when a “sickle cell crisis" occurs.
- It sometimes is written as β S β S.

- HPLC analysis of a hemolysate of a individual homozygous for Hb S shows no Hb A peak and a small Hb A2 peak.
- The apparent Hb A2 concentration may be falsely increased because of the presence of glycated Hb S.
- Hemoglobin S forms 85 to 90% of the total Hb.





- The Hb F concentration is variable, with females having higher concentrations than men
- 10 to 25%: The highest Hb F concentrations are found in individuals from the Middle East and the Indian subcontinent with the Arab-Indian haplotype.
- 5 to 6%: Low Hb F concentrations are found in the West African Cameroon (sometimes called Senegal) haplotype.
- 6 to 7% : The remaining haplotypes, the Benin and Bantu, have Hb F concentrations
- Increased concentrations of Hb F mitigate to some extent the clinical manifestations of sickle cell anemia.

Electrophoresis:

- at both alkaline and acid pH shows a single large band in the Hb S position with small bands at the Hb A, and Hb F positions.
- The sickle cell screen test is positive

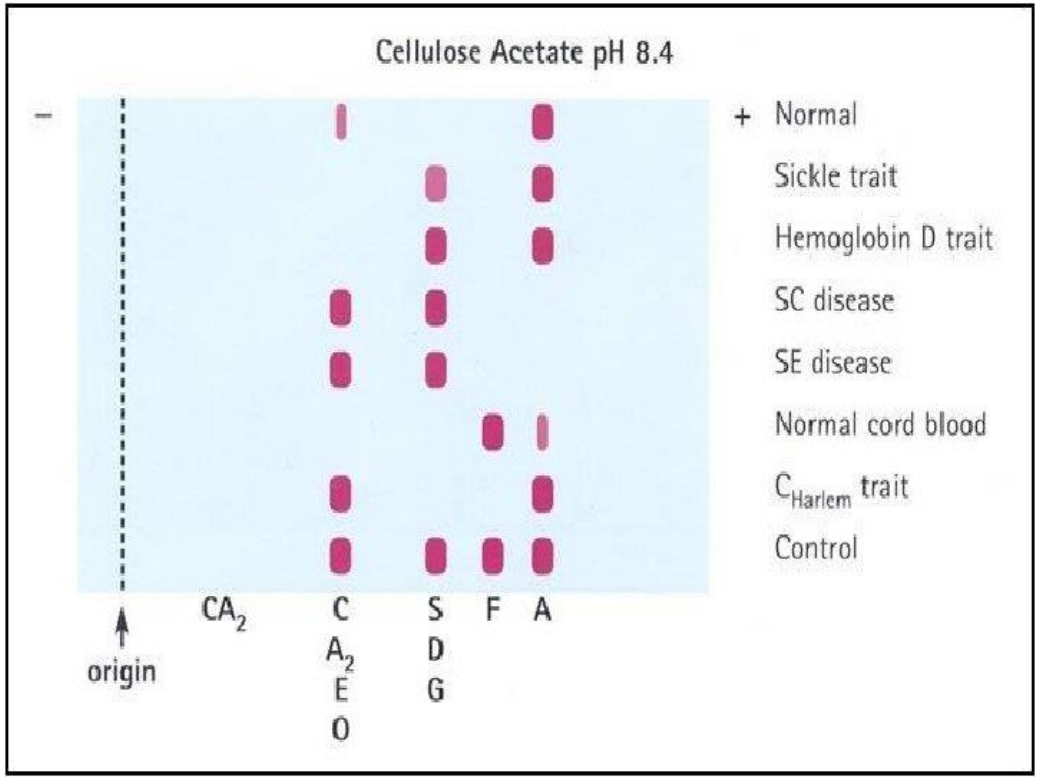
CBC analysis :

- moderate to a major decrease in Hb concentration (60 to 100 g/L) with normal to increased MCV and MCH.
- In individuals with a concurrent thalassemia, the Hb is further decreased and both MCV and MCH are lowered.
- In the neonate,

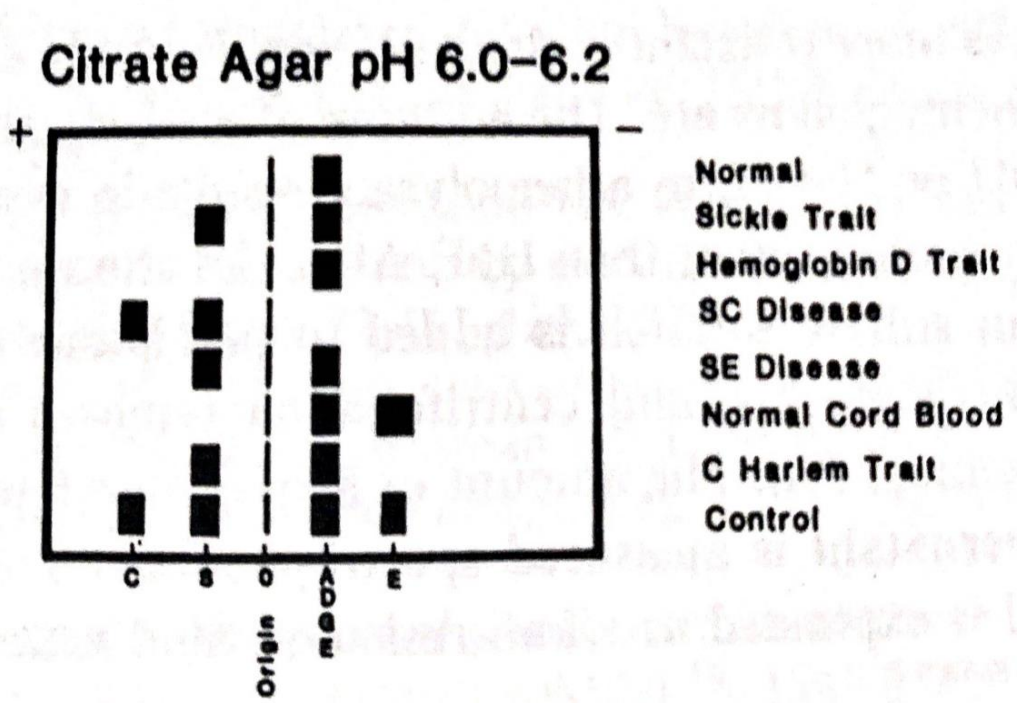
peripheral blood smear : sickle and target cells and Howell-Jolly bodies.

Electrophoresis - At both alkaline and acid pH shows Large band in S position and small band in A and F position .

Alkaline pH



acid pH



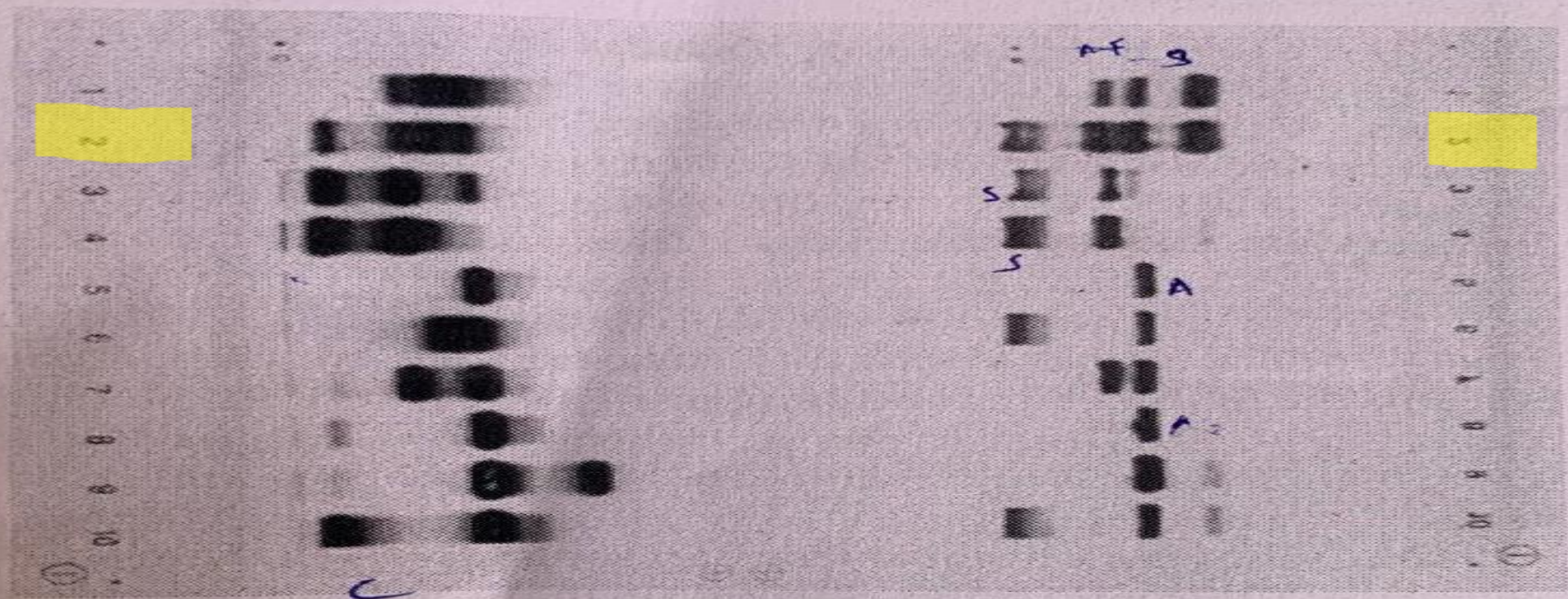


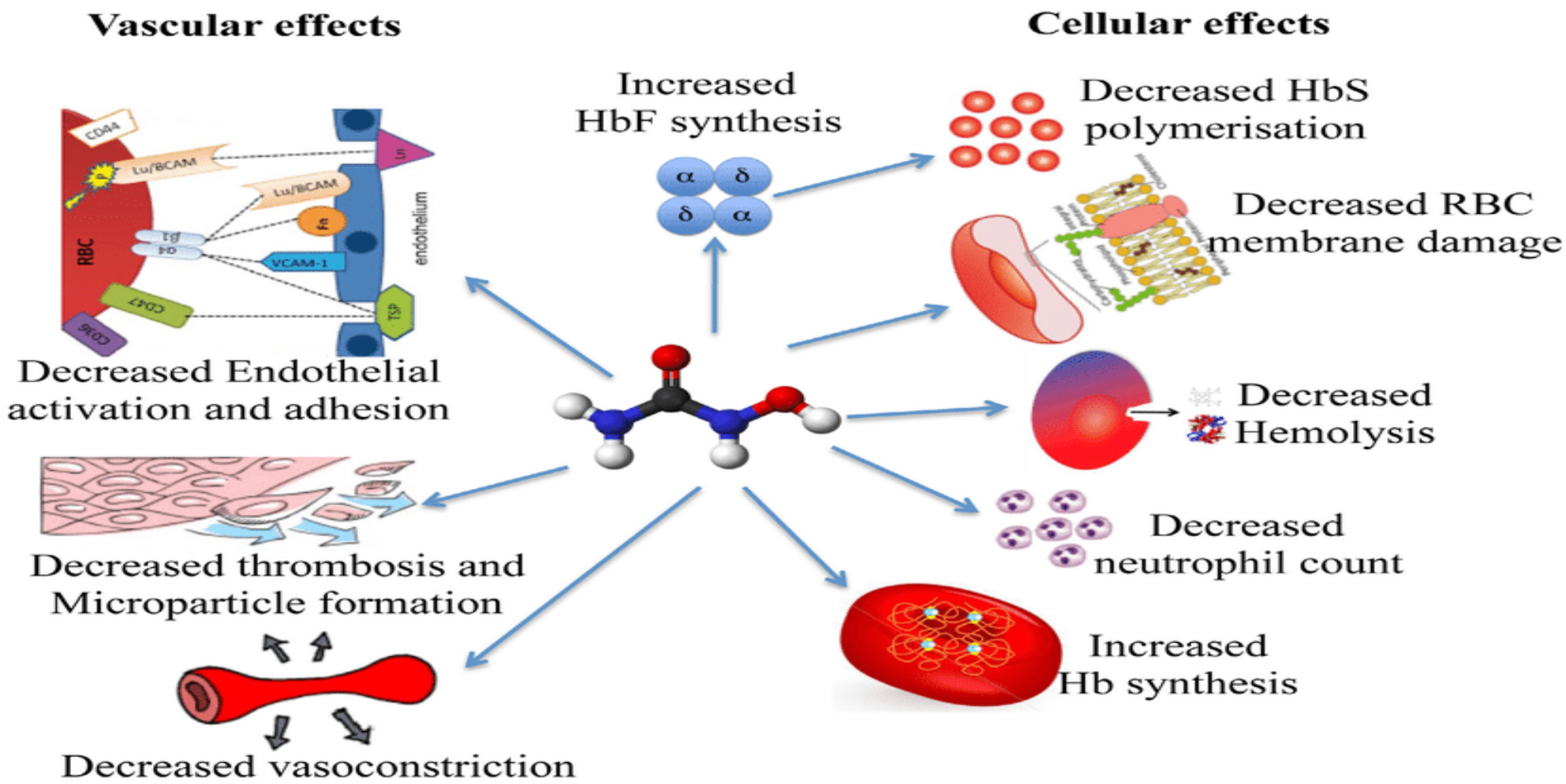
Figure 32-11 Alkaline *(left)* and acid *(right)* electrophoresis of various hemoglobinopathies. *Lane 1,* Hb S, Hb FA control. *Lane 2,* HB S, Hb F, HbCA control. *Lane 3,* Transfused SC disease. *Lane 4,* SC disease. *Lane 5,* Hb A (normal). *Lane 6,* Hb Presbyterian. *Lane 7,* Hb S. *Lane 8,* Raised Hb A_2 (β-thalassemia trait). *Lane 9,* Hb J Baltimore. *Lane 10,* Hb C.

- As a patient's age increases, these features of hyposplenism become increasingly evident.
- In the adult sickle cells :30 to 40%.
- Howell-Jolly bodies, target cells, Pappenheimer bodies, boat-shaped cells, and nucleated RBCs are noted.
- Platelet count and neutrophil counts are elevated.

Treatment of children with homozygous HbS :

- Hydroxyurea with an increase in the quantity of HbF sometimes to 25%.
- In adults, transfusion is the usual treatment, and the patient is re-transfused when the Hb A concentration falls to 20% of the total hemoglobin.

Hydroxyurea effect



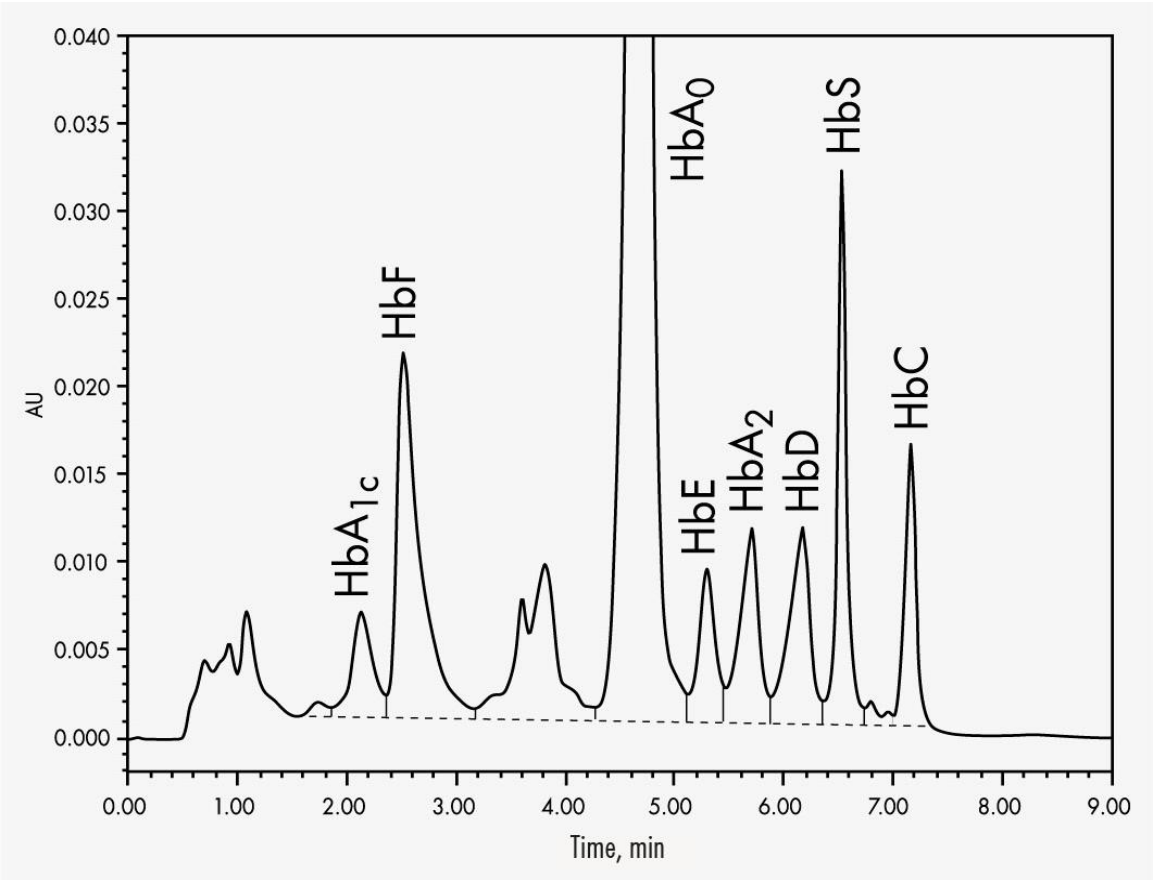
How does sickle cell protect against malaria?

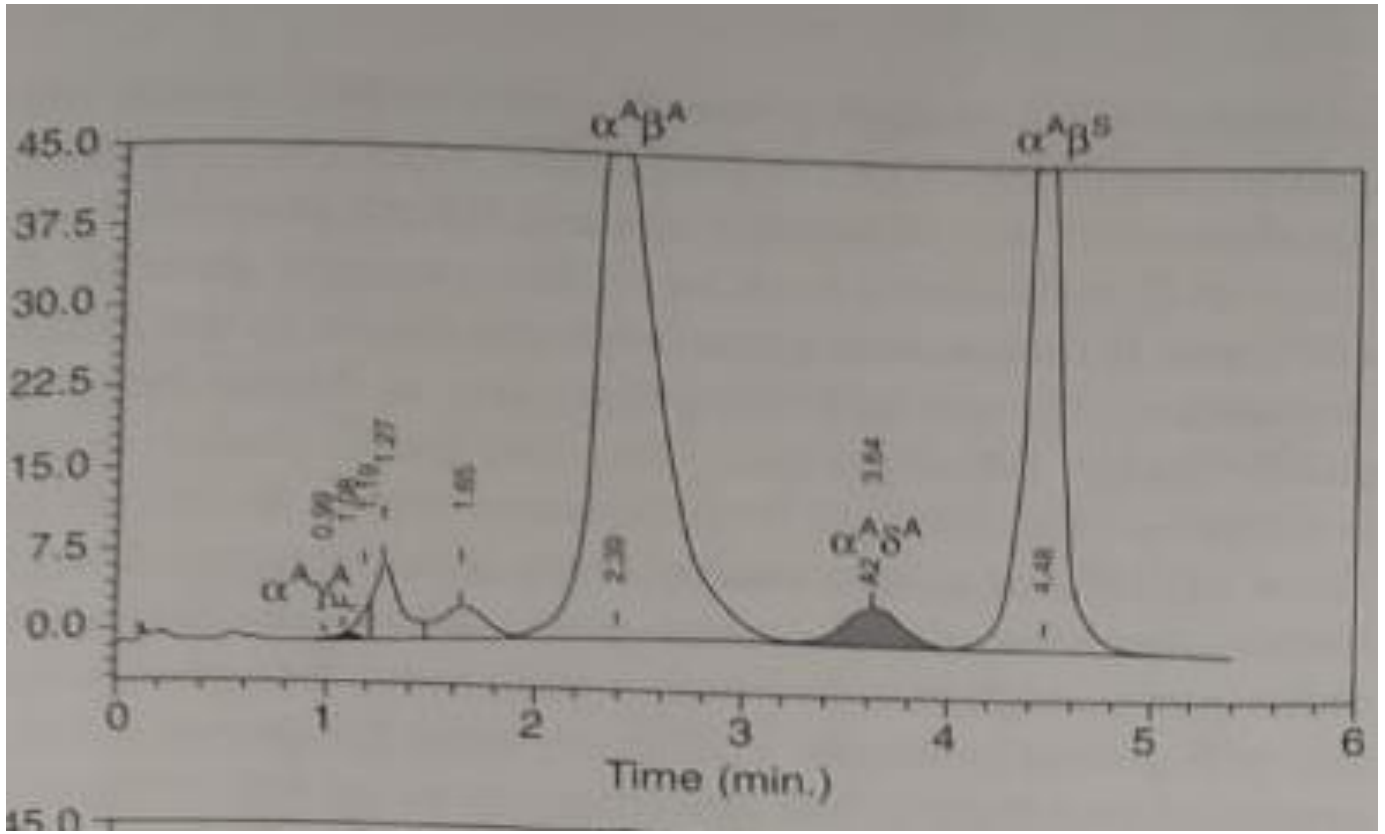
- The infected RBCs will sickle and then be destroyed by the spleen Lower oxygen states due to hemoglobin S in infected cells interfere with parasite growth.
- In those with sickle cell trait, the infected RBC isn't able to stick as easily to the walls of the blood vessel, which is one of the ways malaria causes illness.
- Decreased parasite growth may allow more time for the immune system to react to and destroy the infected RBCs

Heterozygous Hemoglobin S (Hb S Trait)

HPLC analysis : peaks in the Hb A and S positions, with 40% of the total Hb found in the Hb S peak.

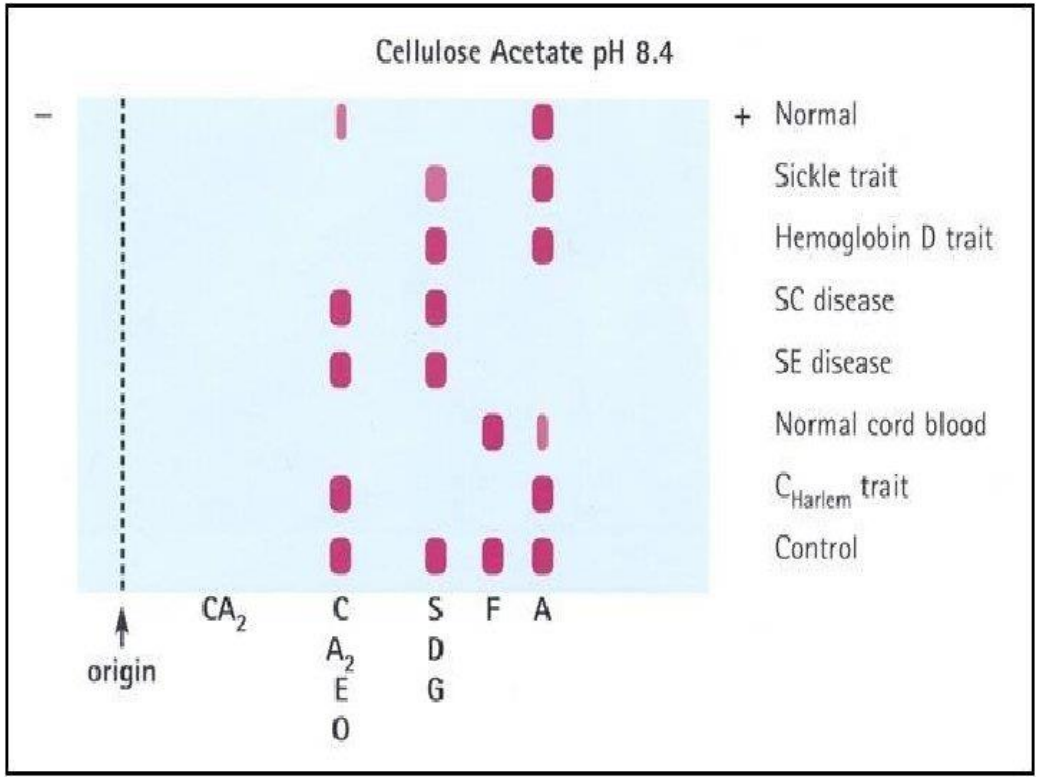
- Hb S concentrations less than 30% are suggestive of coinheritance of α-thalassemia. Hb F concentration is variable.



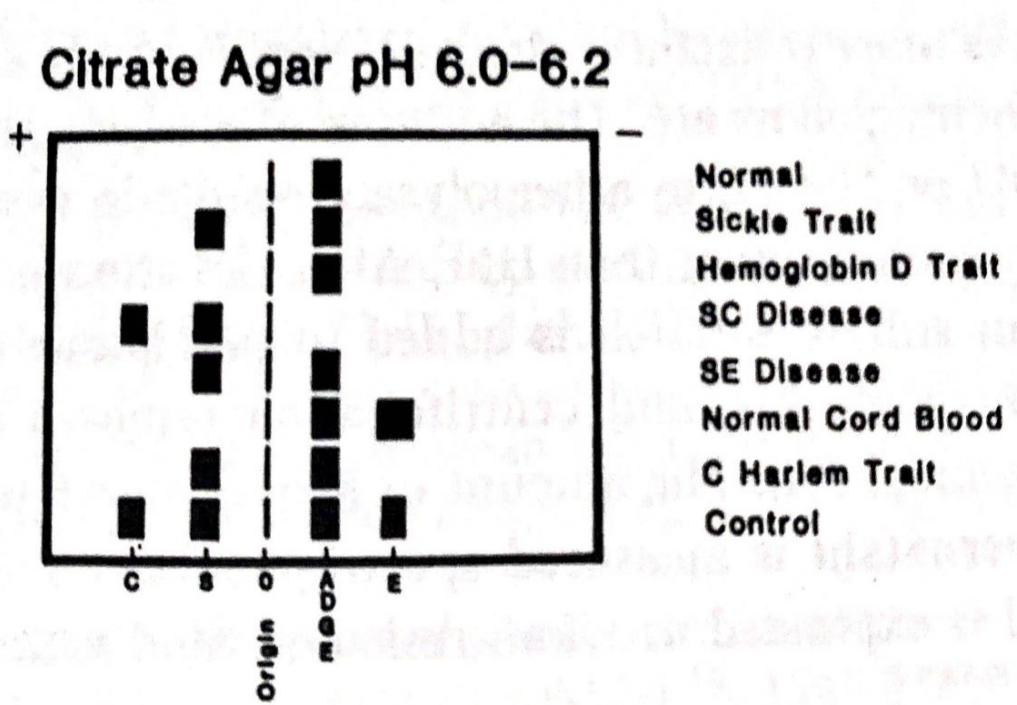


Electrophoresis - At both alkaline and acid pH shows bands in the A and S positions

Alkaline pH



acid pH



- Patients are often asymptomatic,
- CBC analysis : Slightly decreased concentration of Hb
- Genetic counseling should be considered because coinheritance of two β-globin gene abnormalities may contribute to a sickle cell disorder.

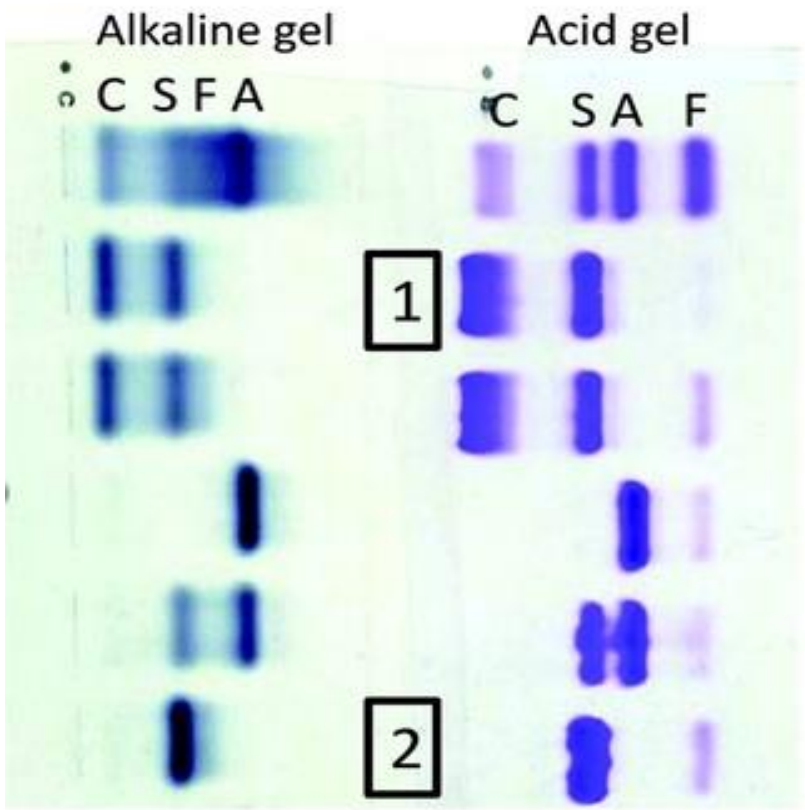
Hemoglobin SC (Hb SC Disease)

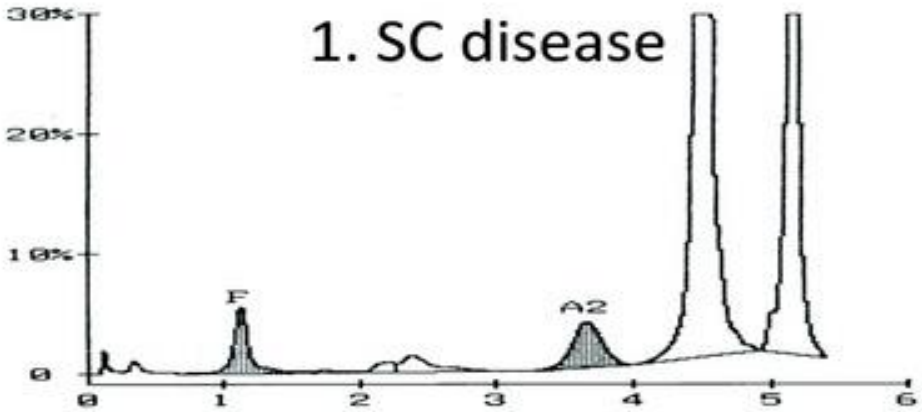
- SC disease arises when both β-globin chains are substituted at position 6 with valine (Hb S) or lysine (Hb C).
- On HPLC and capillary electrophoresis,

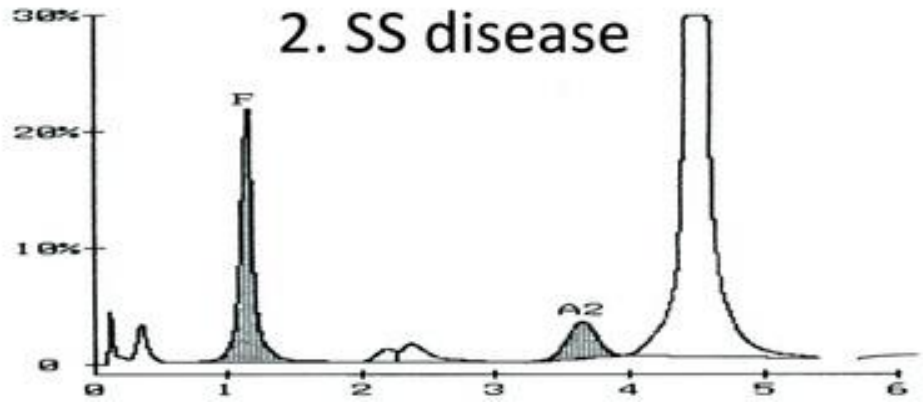
  peaks : S and C positions,
- Electrophoresis:

  at both alkaline and acid pH shows bands in the S and C positions
- sickling test is positive.

Hemoglobin SC (Hb SC Disease) Electrophoresis and HPLC







Hemoglobin SD.

- Hb S may be coinherited with Hb D (SD disease).
- Individuals with this disease have similar but milder clinical presentation when compared with that of sickle cell disease (Hb SS).

HPLC analysis shows two peaks

- Hb S position : 38 to 42% of the total Hb,
- Hb D position : 43 to 45% of the total Hb.
- Hb F concentration is usually within the reference interval, although concentrations as high as 14% have been observed in some individuals with SD disease.

Electrophoresis:

- Alkaline electrophoresis shows a band in the S position.
- Acid electrophoresis shows bands in the S and A positions.
- The sickling test is positive.

CBC analysis:

- greatly decreased concentration of Hb with normal to slightly elevated MCV.
- peripheral blood smear - Target, boat-shaped, nucleated, red, and sickle cells-together with anisocytosis and poikilocytosis

Fetal hemoglobin

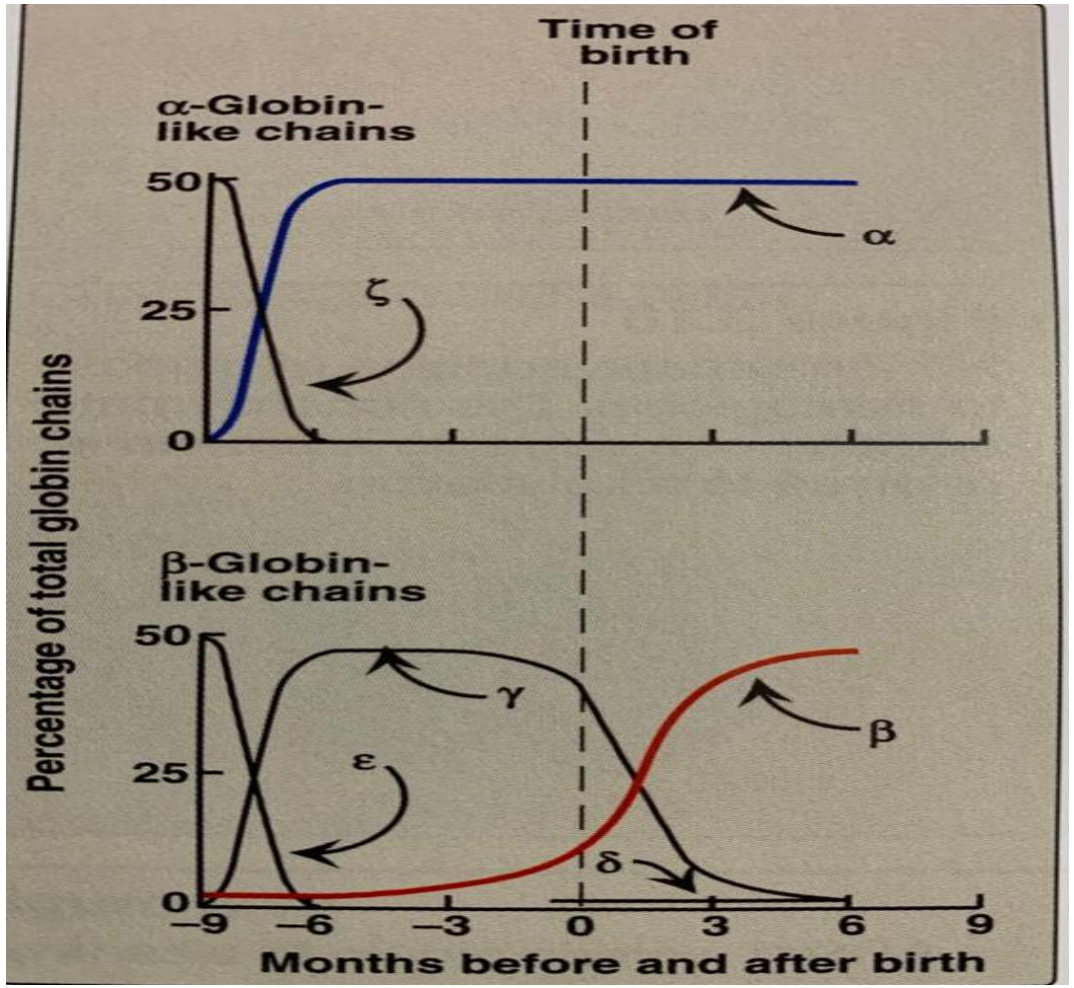
- HbF is a tetramer consisting of two α chains identical to those found in HbA, plus two y chains (α 2y2). The y chains are members of the β -globin gene family

A) HbF synthesis during development: In the first month after conception, embryonic hemoglobins :

- Hb Gower 1 : (ζ 2 ε 2),
- Hb Grower 2 : (α 2 ε 2) are synthesized by the embryonic yolk sac.
- In the 5th week of gestation, the site of globin synthesis shifts, first to the liver and then to the marrow, and the primary product is HbF.

Designation	Tetrameric structure	Hemolysate (%)	
		Adult	Newborn
Adult			
HbA	$\alpha_2\beta_2$	95-98	20-30
HbA_2	$\alpha_2\delta_2$	2-3	0.2
Fetal			
HbF	$\alpha_2\gamma_2$	<1	80
Embryonic			
Gower 1	$\zeta_2\epsilon_2$	0	0
Gower 2	$\alpha_2\epsilon_2$	0	0
Hb Portland	$\zeta_2\gamma_2$	0	0

- HbF is the major hemoglobin found in the fetus and newborn, accounting for ~60% of the total hemoglobin in the RBC during the last months of fetal life .
- HbA synthesis starts in the bone marrow at about the 8th month of pregnancy and gradually replaces HbF.



B**) 2,3-BPG binding to HbF**:

- Under physiologic conditions, HbF has a higher oxygen affinity than does HbA as a result of HbF only weakly binding 2,3-BPG

[Note: The y-globin chains of HbF lack some of the positively charged amino acids that are responsible for binding 2,3-BPG in the β-globin chains.]

- Because 2,3-BPG serves to reduce the oxygen affinity of hemoglobin, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA.
- The higher oxygen affinity of HbF facilitates the transfer of O2 from the maternal circulation across the placenta to the RBC of the fetus.

Hemoglobin S/O Arab. Coinheritance of Hb S and Hb O Arab presents a similar or somewhat milder clinical presentation to sickle cell disease and is found in the Middle East and North Africa

Hemoglobin S/G Philadelphia

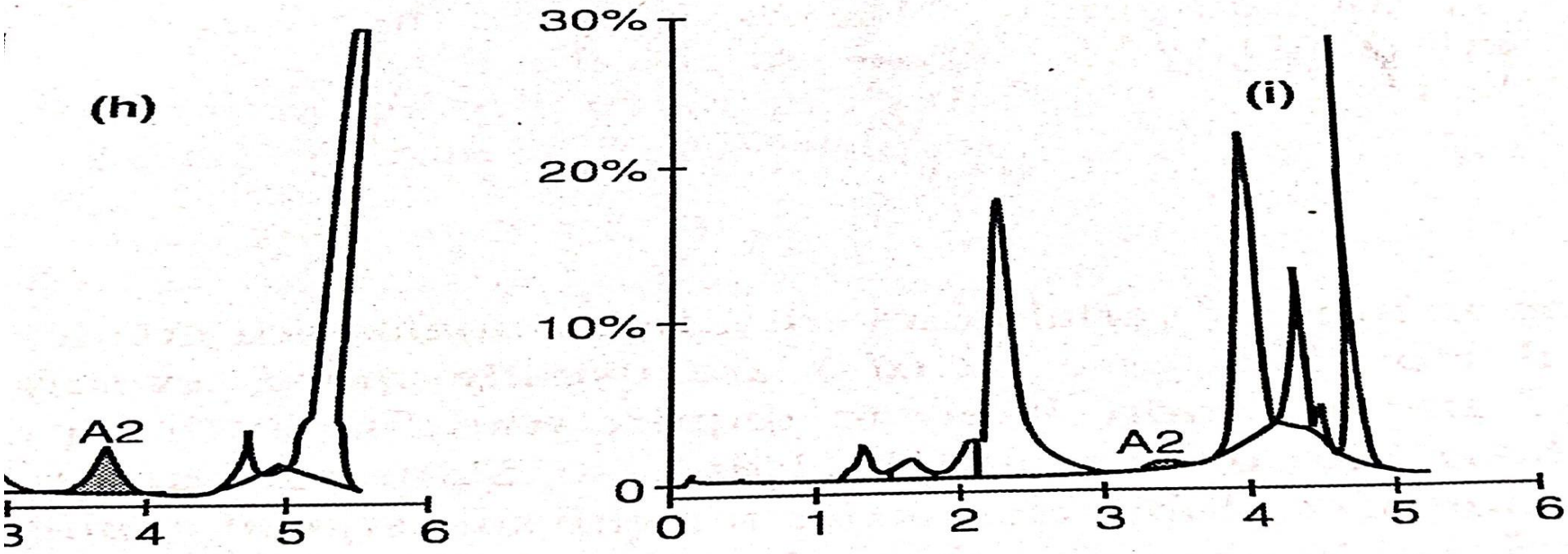
- One or more abnormal α globin chains can combine with Hb S.
- In African Americans and west Africans , the combination of Hb G Philadelphia [α68(E17) Asn→ Lys] with Hb S is prevalent.
- HPLC analysis on blood samples from these individuals shows at least two major peaks and two smaller peaks:

1. The two major peaks: Normal α- chain with Normal β-chain

   Abnormal α-chain with the normal β-chain.

2. The two smaller peaks : Normal α- chain with the abnormal β-chain Abnormal α-chain with the abnormal β-chain.

HPLC : Hemoglobin S/G Philadelphia



atography (HPLC) chromatograms
t program for (a) Hb Bart's; (b) β^0-
zygous E; (d) Hb H; (e) homozygous S;
l (i) Hb S-Hb G Philadelphia. *(From Clarke*

- Amount of Hb G-Philadelphia (generally 16% to 20%) is significantly less than the expected 25%, again because of the difference in the rate of assembly of the variant αG-globin chain with the normal βA-globin chain.

A. The minor peak following the Hb G-Philadelphia peak (αGβA) represents αGδA,

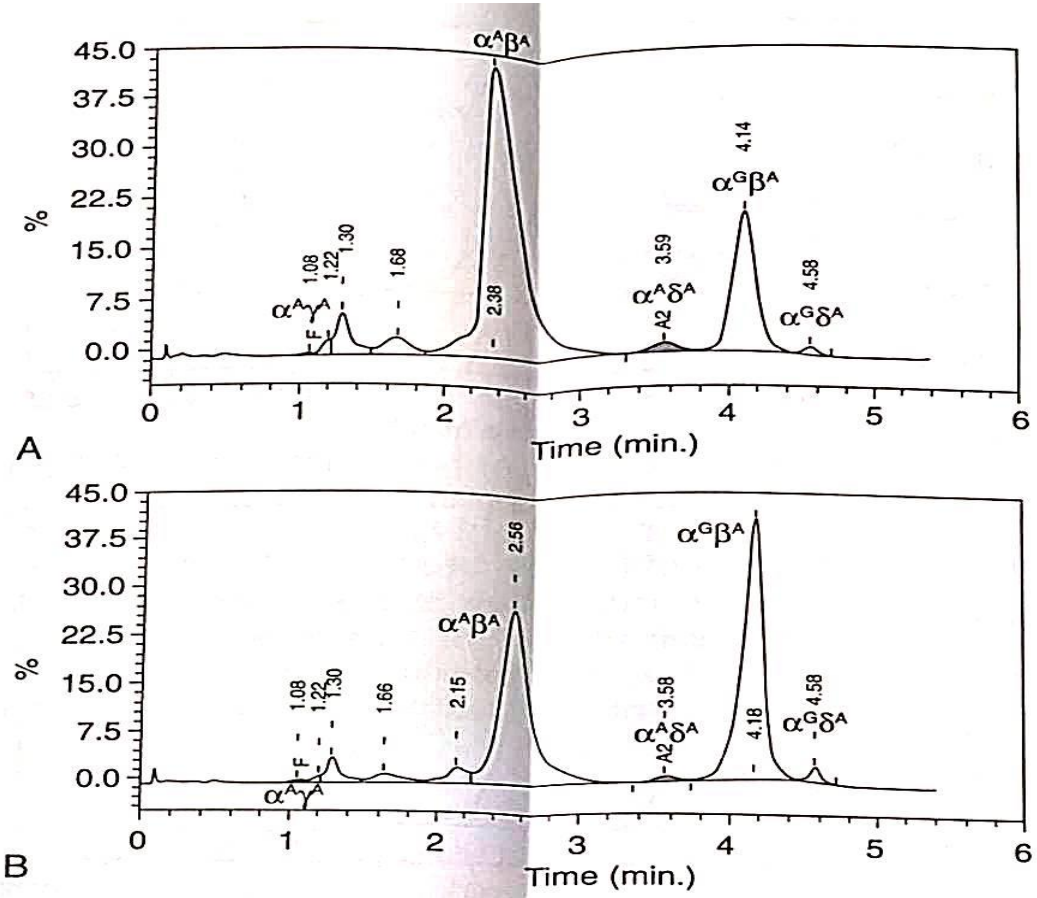


Fig. 5-6 Sample chromatograms on the BioRad Variant II of patients for **(A)** Hb G-Philadelphia trait (αAγA = 0.2%, αAβA = 79.3%, αAδA = 1.2%, αGβA = 18.8%, αGδA = 0.5%) and **(B)** heterozygous for Hb G-Philadelphia and α-thalassemia (αAγA = 0.3%, αAβA = 67.9%, αAδA = 0.8%, αGβA = 29.2%, αGδA = 1.1%). Time (minutes) represents the retention time (RT) for each fraction to elute. The RT for each fraction is shown at the peak.

Electrophoresis:

- At alkaline Ph, major bands in the A and S positions with a minor band in the C position.
- At acid pH, bands are seen in the A and S positions.

CBC analysis :

slightly decreased Hb concentration with normal MCV and MCH.

α- or β-Thalassemia can be coinherited with Hb G Philadelphia and Hb S.

- In these cases, CBC analysis results in markedly decreased MCV and MCH with reduced Hb concentration.

Hemoglobin C [β 6(A3)Glu Lys]

- Hb C arises from a substitution of lysine for glutamic acid at position 6 of the β-globin chain.
- Hb C may be found in the homozygous (Hb C disease, β C β C) or heterozygous (Hb C trait) state.
- Hb C is commonly found in West Africa and the Caribbean.
- It is the second most commonly studied, after Hb S, of all Hb variants.

Homozygous Hb C

- HPLC analysis : large peak in the C position, with Hb C forming 90 to 95% of the total Hb.
- Hb F concentrations are variable.
- Glycated Hb C is found as a small peak eluting before the Hb C peak.
- Electrophoresis at alkaline and acid pH shows a single band in the C position

Homozygous Hb C

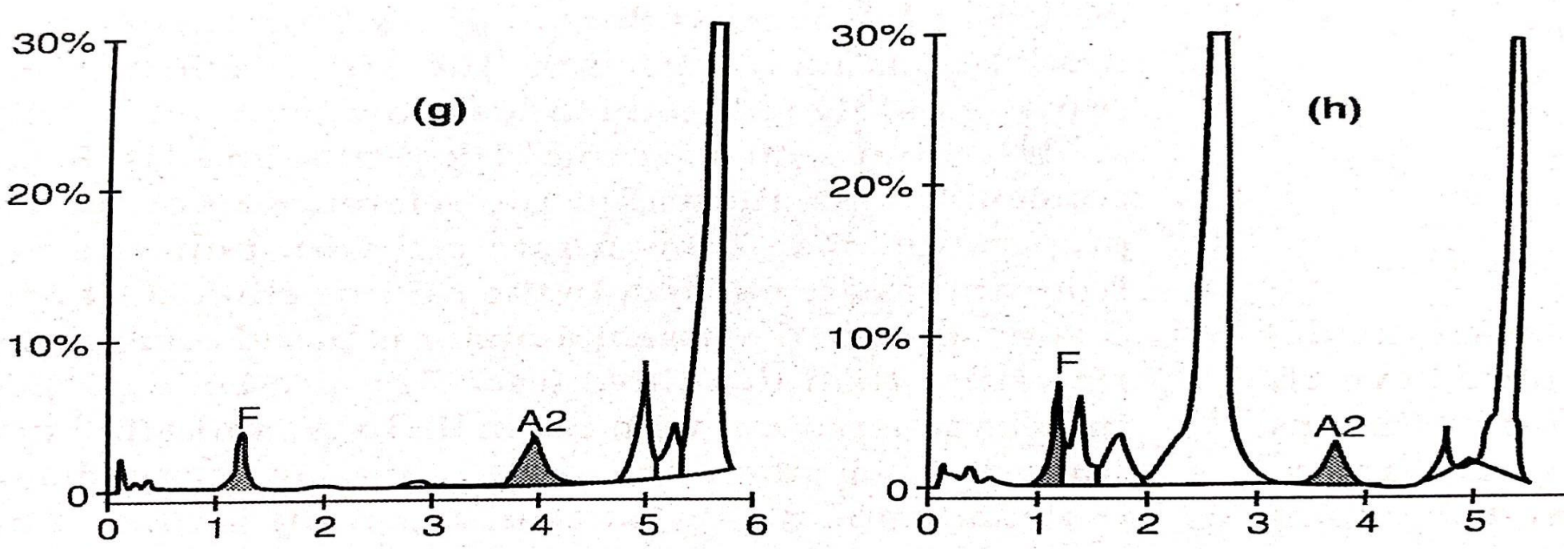
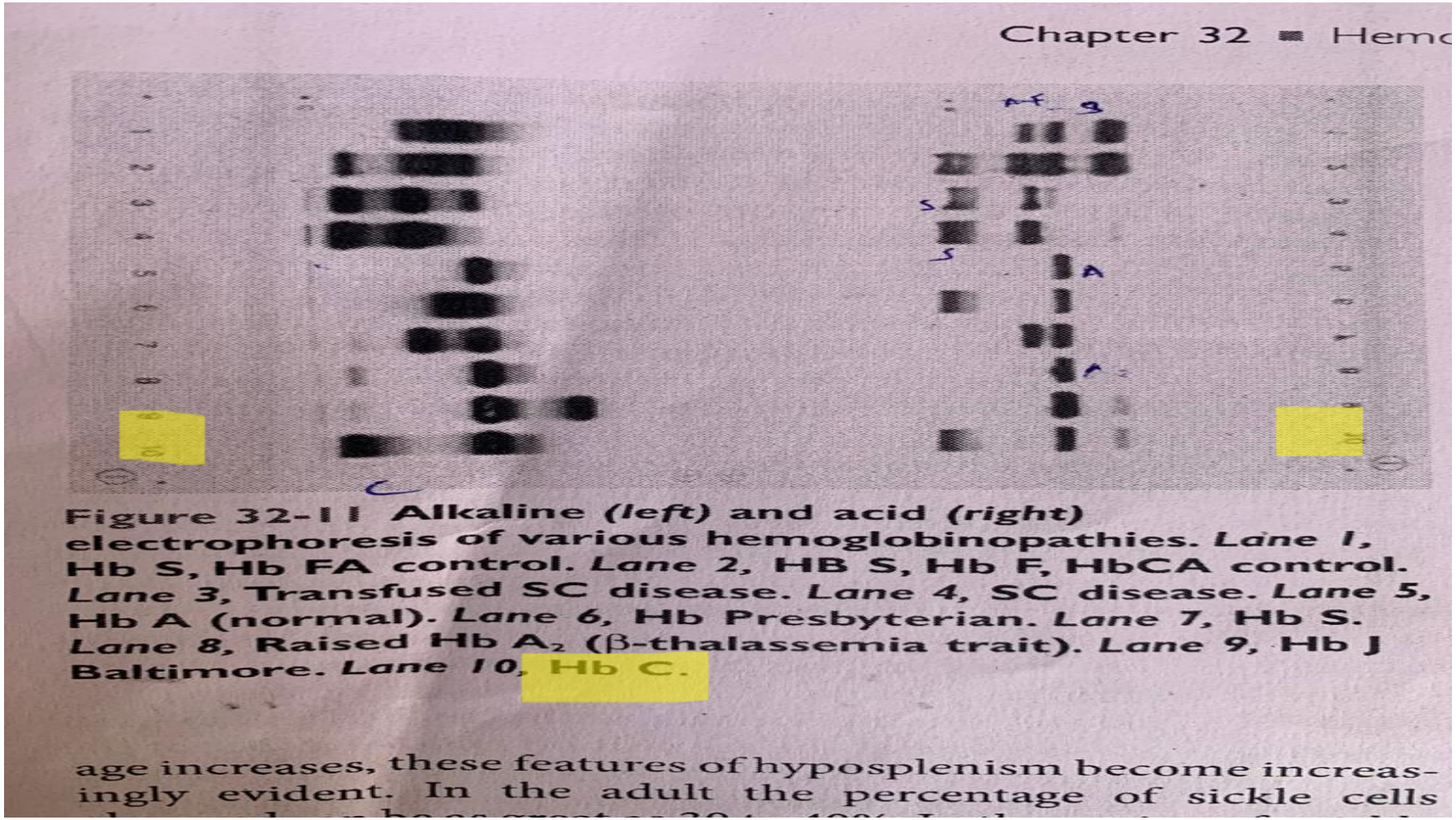


Figure 32-7 High-performance liquid chromatography (HP
obtained on the Bio-Rad Variant β-Thal short program for
thalassemia major; (c) B^+-thalassemia homozygous E; (d) H
(f) S trait; (g) homozygous C; (h) C trait; and (i) Hb S-Hb

Homozygous Hb C



Chapter 32 ■ Hemo

Figure 32-11 Alkaline (*left*) and acid (*right*) electrophoresis of various hemoglobinopathies. *Lane 1*, Hb S, Hb FA control. *Lane 2*, HB S, Hb F, HbCA control. *Lane 3*, Transfused SC disease. *Lane 4*, SC disease. *Lane 5*, Hb A (normal). *Lane 6*, Hb Presbyterian. *Lane 7*, Hb S. *Lane 8*, Raised Hb A_2 (β-thalassemia trait). *Lane 9*, Hb J Baltimore. *Lane 10*, Hb C.

age increases, these features of hyposplenism become increasingly evident. In the adult the percentage of sickle cells

- Mild to moderate anemia is the most common clinical presentation.

CBC analysis :normal or slightly decreased Hb concentrations with a normochromic and normocytic red cell morphology.

- An increase in polychromasia may be present, and the reticulocytes may contribute to an increase in the MCV.
- Peripheral blood smear : Numerous target cells with occasional nucleated RBCs and characteristic irregular contracted red cells (sometimes called pyknocytes).
- Hb C crystals may be seen, and bilirubin concentrations may be slightly elevated.
- Red cell survival and osmotic fragility are decreased.

Heterozygous Hemoglobin C (Hb C Trait).

- HPLC analysis , capillary electrophoresis, and electrophoresis at both alkaline and acid pH : bands in A and C positions, with Hb C forming 38 to 45% of the total Hb.
- CBC analysis: Target cells and generally is normochromic with the MCV near the lower limit of the reference interval .
- Asymptomatic.
- Genetic counseling may be useful when prospective parents have abnormalities in the β-globin gene.
- Hb C may be coinherited with both α- and β-thalassemia, and the concentration of Hb C is related to the number of functioning α-genes. With only two functioning α-genes, the Hb C concentration can fall to 32% of the total Hb.
- The Hb F concentration is often increased.

Heterozygous Hemoglobin C

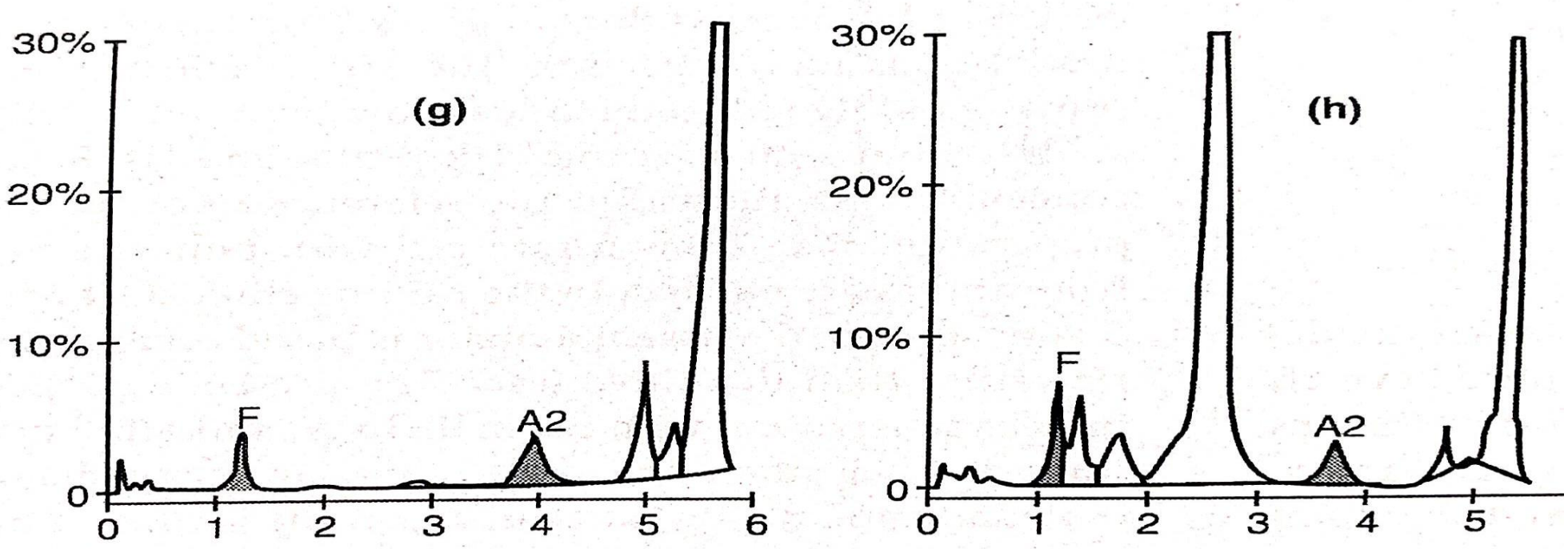


Figure 32-7 High-performance liquid chromatography (HP obtained on the Bio-Rad Variant β-Thal short program for thalassemia major; (c) B^+-thalassemia homozygous E; (d) H (f) S trait; (g) homozygous C; (h) C trait; and (i) Hb S-Hb

Heterozygous Hemoglobin C

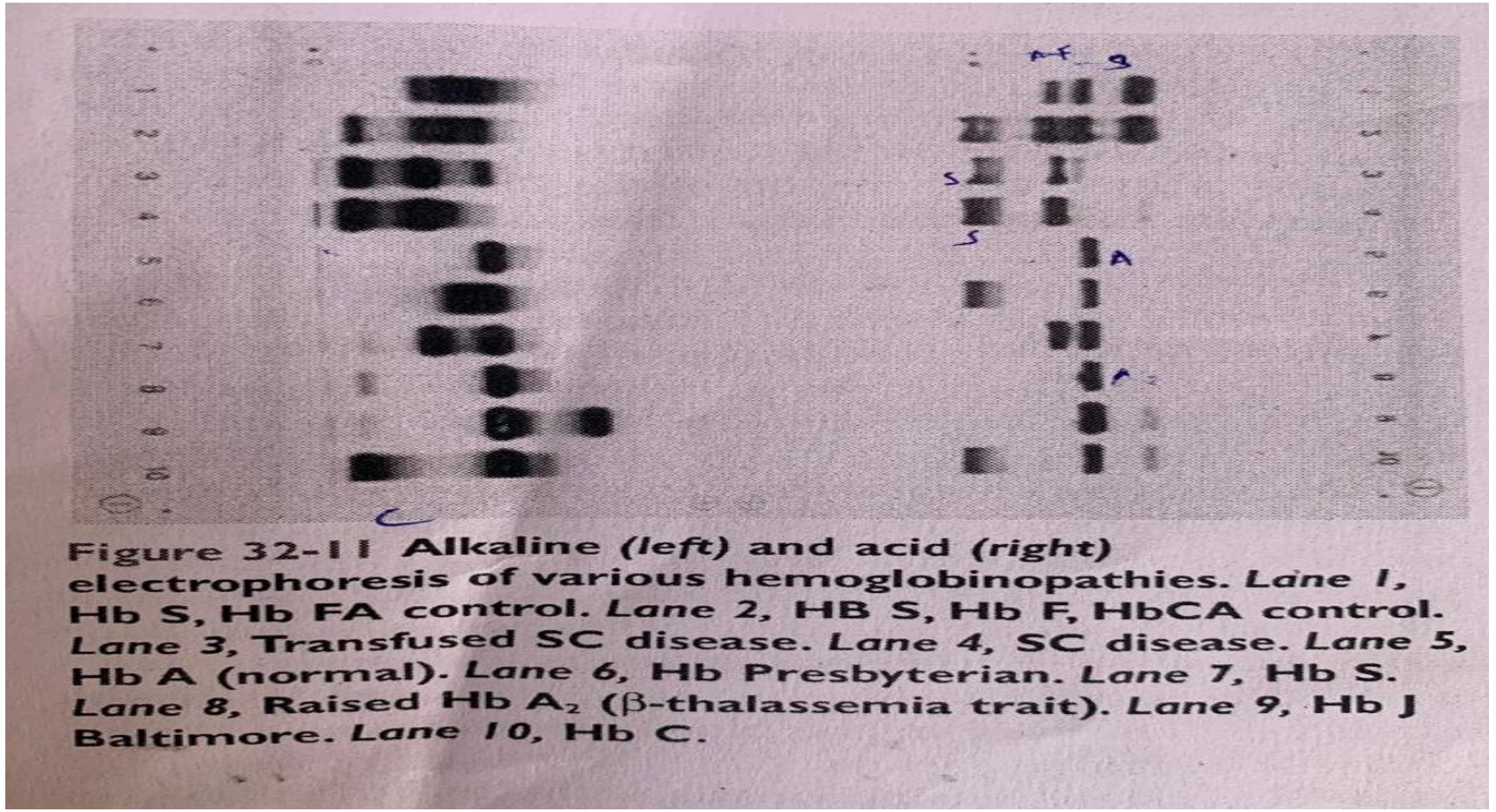


Figure 32-11 Alkaline *(left)* and acid *(right)* electrophoresis of various hemoglobinopathies. *Lane 1,* Hb S, Hb FA control. *Lane 2,* HB S, Hb F, HbCA control. *Lane 3,* Transfused SC disease. *Lane 4,* SC disease. *Lane 5,* Hb A (normal). *Lane 6,* Hb Presbyterian. *Lane 7,* Hb S. *Lane 8,* Raised Hb A_2 (β-thalassemia trait). *Lane 9,* Hb J Baltimore. *Lane 10,* Hb C.

Hemoglobin D Punjab [β121 (GH4) Glu→Gln].

- Hb D Punjab is an Hb variant in which glutamic acid at position 121 of the β globin chain is replaced with glutamine.
- The names *Hb D Los Angeles* and *Hb D Punjab* are used to describe this variant, with the former name used more often in North America and the latter in the United Kingdom.
- Hb D Punjab is found in the Punjab region of the Indian subcontinent, especially in Sikhs of the Lycus Valley.
- Large-scale immigration from this area to the United Kingdom, the United States, and Canada has widened the distribution of Hb D Punjab.
- Hb D Punjab is also found in Caucasians whose foreparents lived in the Indian subcontinent at the time of the British Raj.
- Hemoglobin D Punjab is found in both heterozygous (Hb D Punjab trait) and homozygous (Hb D Punjab disease, β D β D) states.

Hemoglobin D Punjab homozygous:

Electrophoresis:

at alkaline pH shows a band in the S position,

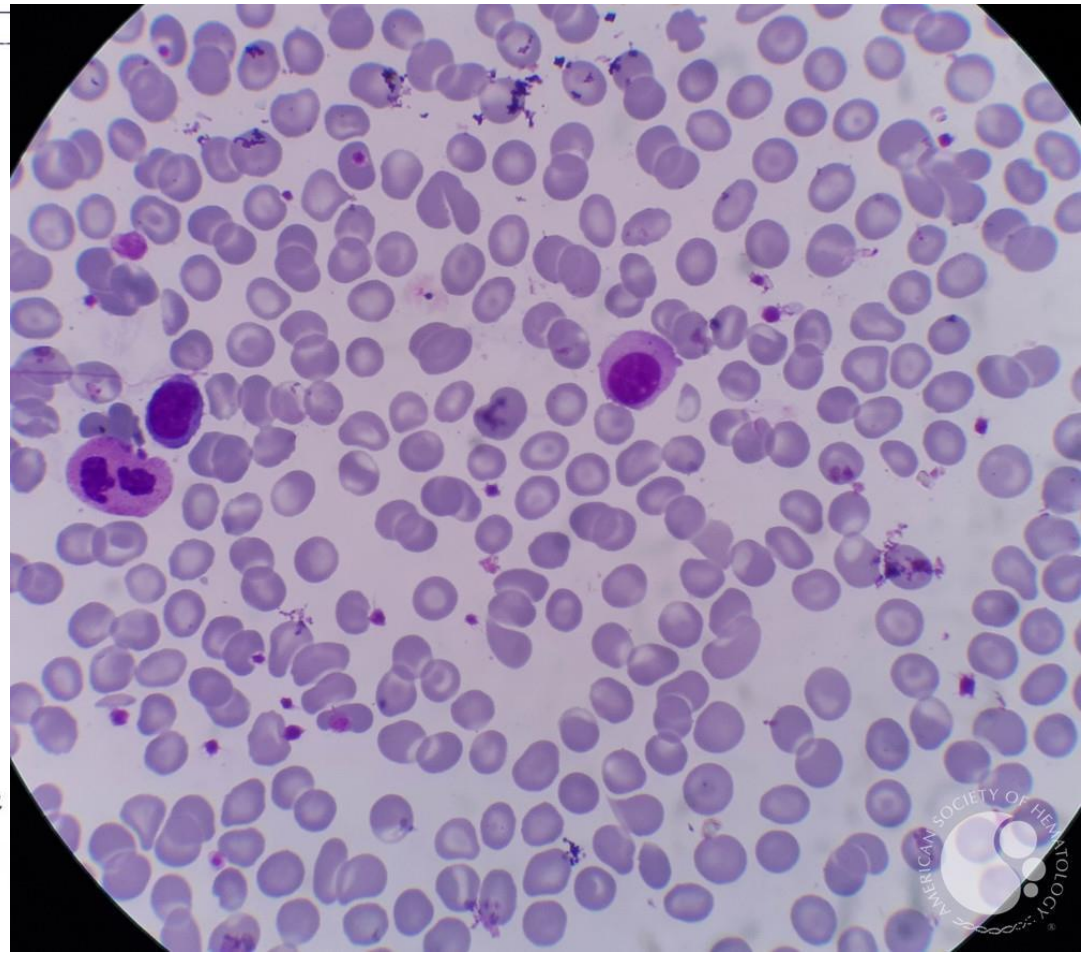
- Acid pH band migrates S to the A position.

CBC analysis :

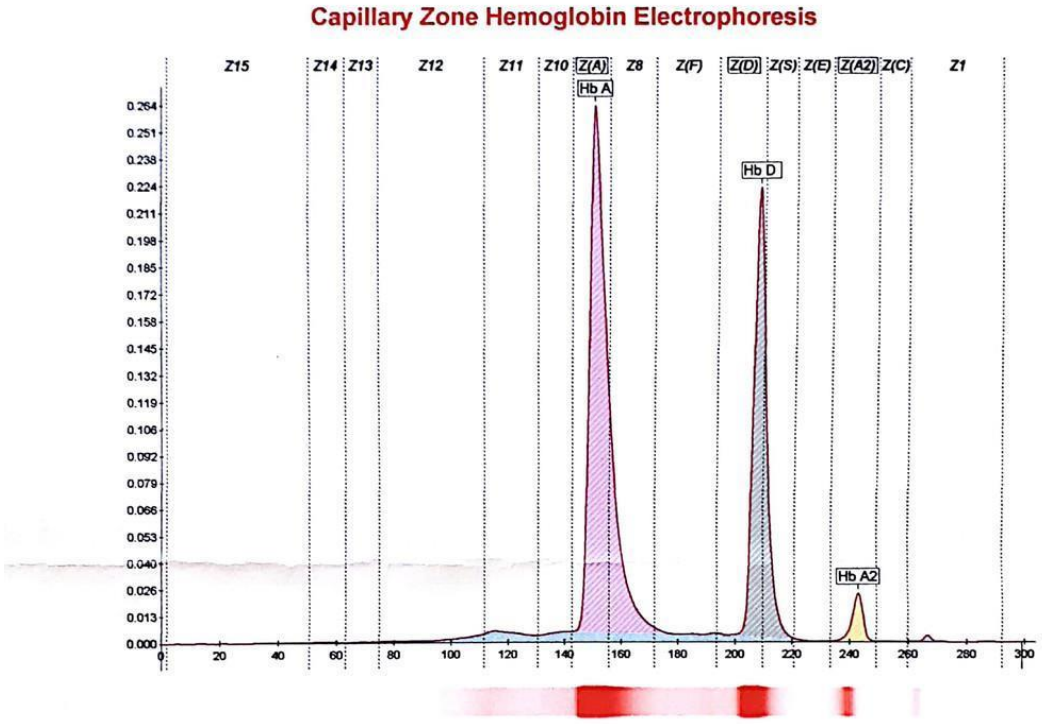
- a mild decrease in Hb concentrations, MCV, and MCH
- peripheral blood smear: target cells
- Mild anemia.

Hemoglobin D Punjab homozygous:

Parameter		Result	Unit	Ref.range
WBC		7.31	10^3/uL	4.00-10.00
Neu#		3.57	10^3/uL	2.00-7.00
Lym#		3.08	10^3/uL	0.80-4.00
Mon#		0.52	10^3/uL	0.12-0.80
Eos#		0.11	10^3/uL	0.02-0.50
Bas#		0.03	10^3/uL	0.00-0.10
Neu%	L	48.8	%	50.0-70.0
Lym%	H	42.2	%	20.0-40.0
Mon%		7.1	%	3.0-8.0
Eos%		1.5	%	0.5-5.0
Bas%		0.4	%	0.0-1.0
RBC		5.07	10^6/uL	3.50-5.50
HGB		12.9	g/dL	12.0-16.0
HCT		39.1	%	35.0-50.0
MCV	L	77.2	fL	80.0-100.0
MCH	L	25.4	pg	27.0-31.0
MCHC		33.0	g/dL	32.0-36.0
RDW-CV		13.3	%	11.5-14.5
RDW-SD		39.5	fL	35.0-56.0
PLT		266	10^3/uL	150-450
MPV		8.8	fL	7.0-11.0
PDW		16.1		15.0-17.0
PCT		0.234	%	0.108-0.282
P-LCC		77	10^9/L	30-90
P-LCR		29.0	%	11.0-45.0



Hemoglobin D Punjab homozygous:



Fractions	%	Ref. %
Hb A	61.6	
Hb D	35.3	
Hb A2	3.1	

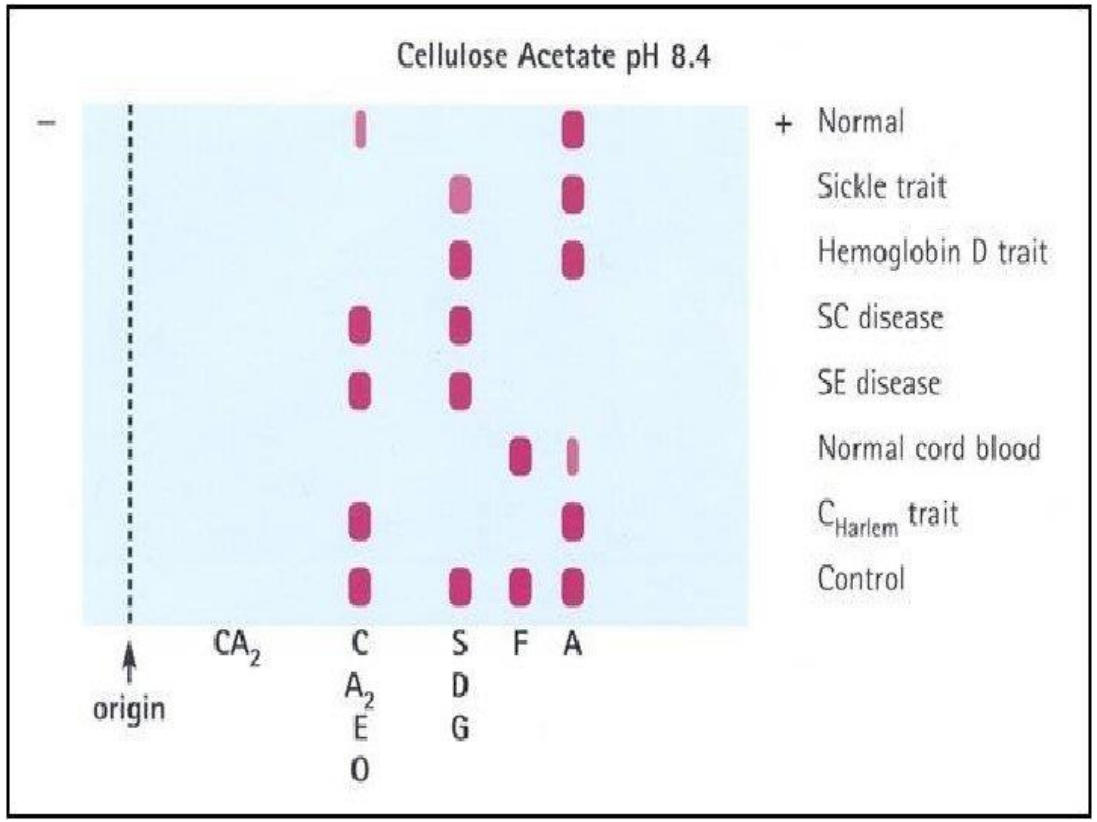
HPLC analysis :

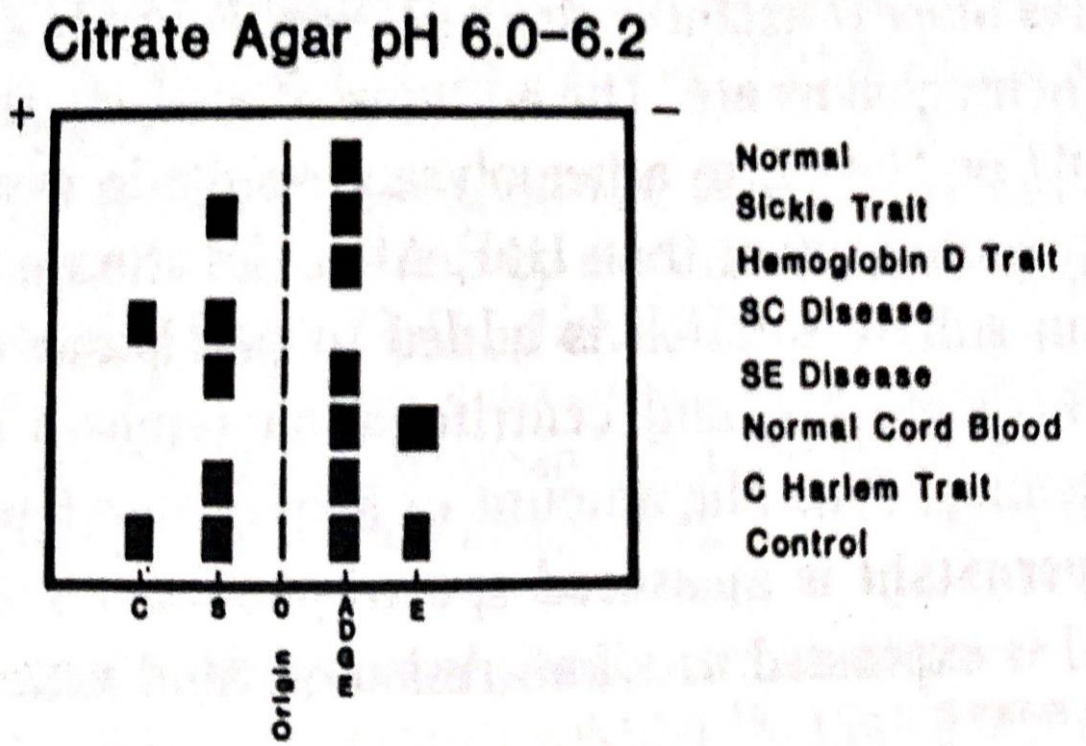
- normal or marginally raised Hb F and Hb A2 peaks
- Large peak in the Hb D position forming more than 90% of the total Hb.

Hb D Punjab trait

- Electrophoresis at alkaline pH shows two bands-one in the A position and the other in the S position.
- At acid pH, a single band in the A position is noted.
- CBC analysis is unremarkable except for the presence of target cells on the blood smear.
- Individuals with Hb D Punjab trait are clinically asymptomatic.

Electrophoresis : at alkaline pH shows two bands-one in the A position and the other in the S position.
At acid pH, a single band in the A position is noted.





- HPLC analysis on individuals with Hb D Punjab trait:

- two peaks-one at the A position and the other at the D position with Hb D forming 30 to 40% of the total Hb.
- The Hb F and Hb A, concentrations are within or slightly above the reference intervals.

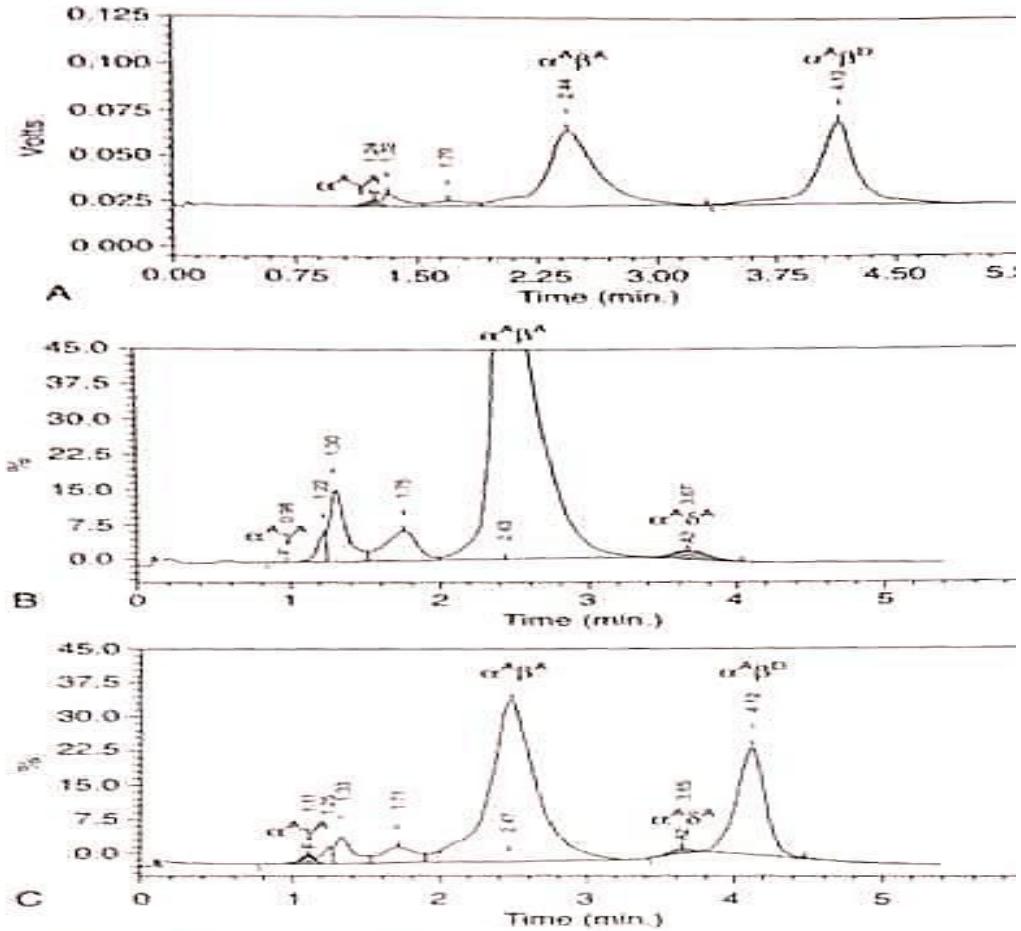


Fig. 5-7 Sample chromatograms on the BioRad Variant II
patients for **(A)** Hb D-Punjab trait (αAγA = 1.1%, αAβA
56.3%, αAβD = 42.6%), **(B)** α-thalassemia trait (αAγA = 0.1
αAβA = 98.0%, αAδA = 1.9%), and **(C)** heterozygous f
Hb D-Punjab trait and α-thalassemia (αAγA = 1.2%, αAβA
55.4%, αAδA = 0.9%, αAβD = 25.1%). Note the absence
Hb A2 in 7A caused by co-elution of the variant hemoglob
fraction. In the absence of Hb A2 the variant system labe
the vertical axis as Volts. Time (minutes) represents the rete
tion time (RT) for each fraction to elute. The RT for each fra
tion is shown at the peak. The peak seen at RT = 1.3
represents Hb A1c.

- Coinheritance of Hb D Punjab (both heterozygous and homozygous states) with β-thalassemia is common.
- CBC analysis : Decreased concentrations of Hb

  Decreased MCV and MCH.
- Peripheral blood smear : Target and irregular contracted cells together with hypochromia and anisocytosis .
- Individuals with coinheritance of Hb D Punjab and β-thalassemia present with a notable compensated anemia.

Hemoglobin D Iran [b22(B4)Glu-Gln]

- Hb D Iran is a β-globin chain variant in which glutamine replaces glutamic acid at position 22 of the β-globin chain.
- On HPLC analysis, peaks are seen in the A and A2 positions with quantification for

On Electrophoresis :

- Alkaline electrophoresis shows two bands-one in the A position and the other in the S position
- Acid electrophoresis, a single band in the A position is noted.
- Individuals with Hb D Iran are asymptomatic.

Hemoglobin E [b26(B8) Glu- Lys]

Hb E is a β-chain variant with lysine replacing glutamic acid at position 26 of the β -globin chain.

- Hb E is found in both homozygous and heterozygous states and may be combined with β -thalassemia.
- It is widespread in the Far East, including Southern China, Cambodia, Thailand, and Laos.
- Hb E is increasingly found in the United States and Canada and is caused by emigration from this area.
- It may be thought of as the "thalassemic variant , as some of the features of the CBC resemble thalassemia especially in the homozygous state

- HPLC analysis: homozygous Hb E (Hb E disease, β E β E) shows a single peak (>90% of the total Hb) coeluting with Hb A2.
- Hemoglobin F is within or marginally above the reference interval.
- On Alkaline electrophoresis : a single band in C position,

  Acid electrophoresis : it migrates to the A position
- CBC : normal to marginally decreased Hb concentrations

  low MCV and MCH.
- Peripheral blood smear: Target cells.
- Iron studies are normal.

Homozygous Hb E : Asymptomatic, mild anemia may be present.

HPLC : homozygous Hb E

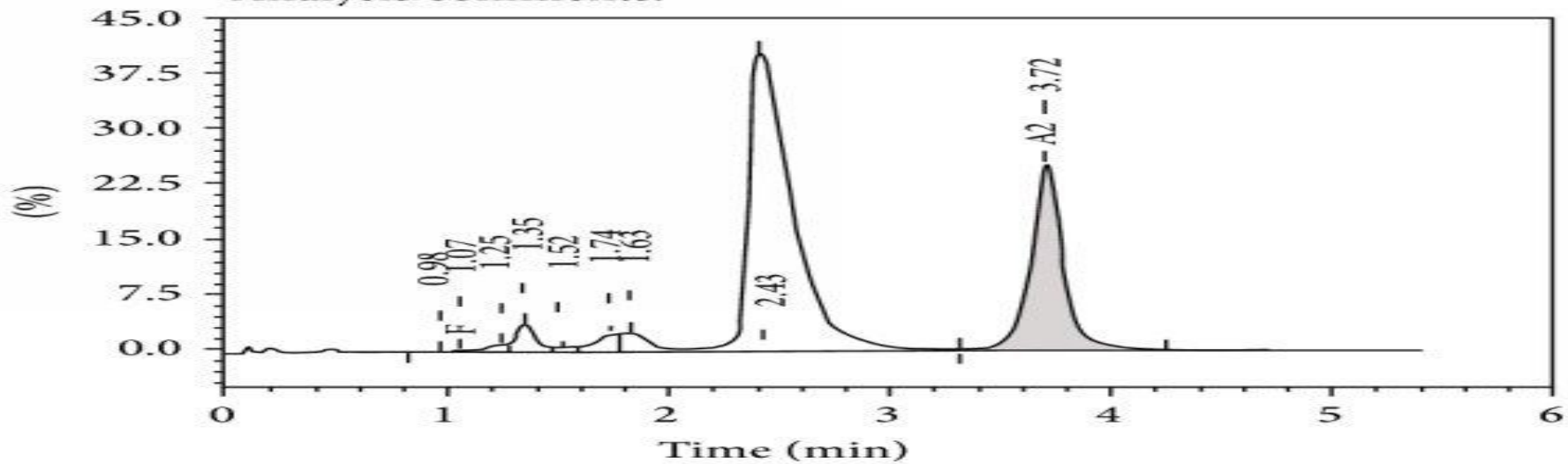
Peak name	Calibrated area (%)	Area (%)	Retention time (min)	Peak area
Unknown	—	0.0	0.98	1013
F	0.2	—	1.07	6584
Unknown	—	0.7	1.25	19627
P2	—	2.7	1.35	76646
Unknown	—	0.7	1.52	18463
Unknown	—	2.0	1.74	55348
P3	—	2.2	1.83	62326
A0	—	63.3	2.42	1778805
A2	24.8*	—	3.72	791409

Total area: 2,810,221

F concentration = 0.2%
A2 concentration = 24.8*%

*Values outside of expected ranges

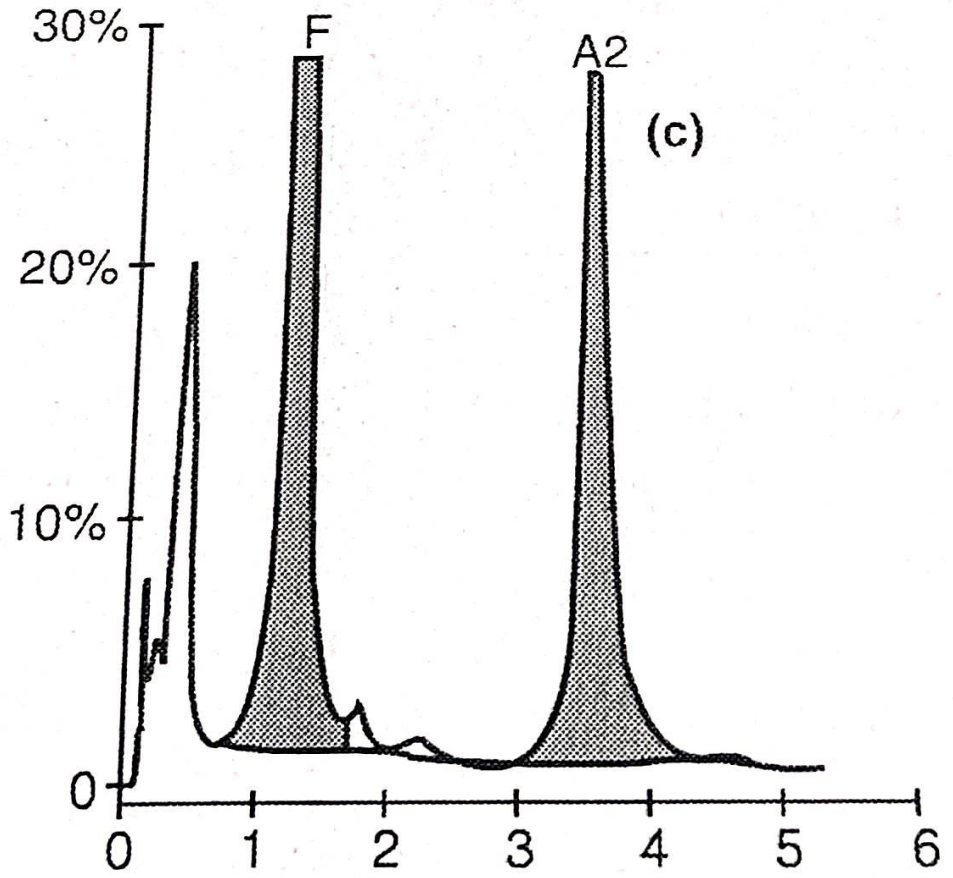
Analysis comments:



- HPLC analysis: heterozygous Hb E reveals two peaks-one in the A position and the other in the A2 position
- Hb E forms approximately 30% of the total Hb.
- Capillary electrophoresis resolves Hb E from Hb A2.
- Hb A2 is higher in patients with Hb E than in patients without a hemoglobin variant or thalassemia as the result of decreased synthesis of the abnormal ß-globin chain, allowing for increased binding between the excess α-globin and 8-globin chains producing Hb A.
- CBC analysis : normal Hb concentrations and low MCV.
- peripheral blood smear: Target cells. Iron studies are normal.
- Heterozygous Hb E : Asymptomatic, although slight anemia may be present.

- Coinheritance of Hb E and thalassemia produces an anemia of variable severity.
- HPLC on a patient with coinheritance of β-thalassemia and homozygous E
- increased Hb F concentration.
- **Coinheritance of homozygous Hb E and β-thalassemia** : severe anemia , reduced - Hb concentrations, MCV, and MCH
- Numerous target cells are noted on the peripheral blood smear, together with microcytosis, anisocytosis, hypochromia, and a few nucleated red cells, Iron studies are normal.
- In the most severe cases, the clinical presentation is similar to that of βº-thalassemia, and transfusion may be the only therapy.

HPLC on a patient with coinheritance of β-thalassemia and homozygous E



- **Coinheritance of heterozygous Hb E with α- thalassemia** produces a less severe anemia with low Hb and MCV and MCH.
- peripheral blood smear: Target cells, microcytosis and hypochromia. Patients who are pregnant may need to be monitored closely, although transfusion is not usually required.

Hemoglobin O Arab [b121(GH4) Glu-Lys]

- Hb O Arab is a β-chain variant with lysine replacing glutamic acid at position 121 of the β-globin chain.
- Hb O Arab is found in a wide variety of ethnic groups in North Africa and Eastern Europe and is not confined, nor is it even common, among Arab populations.
- Hb O Arab has been found in both heterozygous and homozygous states.

HPLC analysis :

homozygous Hb O Arab shows a single band between the S and C positions, with Hb O Arab forming more than 90% of the total Hb.

Electrophoresis

- At alkaline pH shows a band close to the C position.
- At acid pH, a band is seen between the A and S positions (but closer to A).

CBC analysis : normal or marginally low Hb concentration, MCV, and MCH.

- The peripheral blood smear shows slight microcytosis.

- No unusual hematologic features are noted in individuals with heterozygous Hb O Arab.
- HPLC analysis: two peaks-one in the A position and the other eluting close to the C position and forming 30 to 40% of the total Hb.
- Electrophoresis:

  At alkaline electrophoresis : bands in the A and close to the C position.

  At acid electrophoresis : two bands are noted-one in the A position and the other in a position between the A and S positions.

Hybrid Hemoglobins

- Hybrid Hbs, or crossover Hbs, describe a group of Hb variants in which one of the globin chains is a hybrid of amino acid sequences of two other globin chains.
- The term crossover Hb is sometimes used because there is a point in the amino acid sequence at which there is crossover from the amino acid sequence of one globin chain to another globin chain.
- Similar findings to thalassemia
- Production of the hybrid globin chain is reduced. Hb Lepore is the prototypical hybrid Hb.

Hemoglobin Lepore.

- Hb Lepore is classified as a δβ- hybrid Hb variant on the basis that the non-α-chain is a hybrid of δ- and β-globin chains.
- It is unique in that it is the only hemoglobinopathy named after the family name of the index case.
- δβ -Hybrid Hbs arise because there are deletions of part of the 3’ portion of the δ-globin gene and in the 5' portion of the β-globin chain with resultant formation of a δβ -fusion gene. Three distinct variations of Hb Lepore have been described.

- In **Hb Lepore-Hollandia** [δβ -hybrid (δ through 22; β from 50)], a variant found in Canada and Papua New Guinea, fusion occurs of the first 22 amino acid residues of the δ-globin chain with the amino acids from position 50 onward of the β -globin chain.
- In **Hb Lepore-Baltimore** [δβ - hybrid (δ through 50: β from 86)], found mainly in individuals of Spanish ancestry, the first 50 amino acid residues of the δ -globin chain are fused with amino acid residues from position 86 of the β -globin chain.
- In **Hb Lepore-Boston- Washington** [δβ -hybrid (δ through 87; β from 116)], the most common Hb Lepore, the first 87 amino acid residues of the δ-globin chain are fused with amino acid residues from position 116 onward of the β -globin chain.
- Hb Lepore-Boston- Washington, sometimes called Hb Lepore-Boston, is found mainly in individuals of Italian descent, although it has been found in individuals from Eastern Europe.

- HPLC analysis of blood from individuals with Hb Lepore shows greatly elevated Hb A_2 concentration with marginally reduced Hb A.
- The Hb A_2 concentration is usually greater than 10% of the total Hb and is falsely increased because of the coelution of Hb A, and Hb Lepore.

Electrophoresis

- At alkaline pH : band in the S position for Hb Lepore- Boston-Washington and in a position between the A and S positions for the other Hb Lepore variants.
- At acid Ph: a single band is present in the A position for all Hb Lepore variants.
- Hb A_2 and Hb Lepore are resolved on capillary electrophoresis.

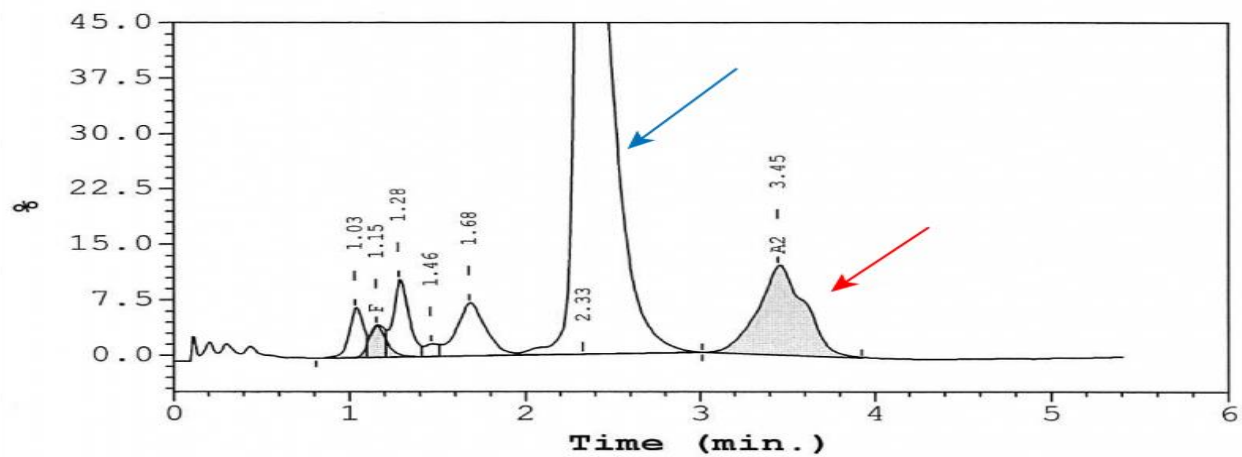
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	2.3	1.03	47451
F	1.8*	---	1.15	37248
P2	---	3.8	1.28	80162
Unknown	---	0.6	1.46	12096
P3	---	4.8	1.68	100429
Ao	---	73.7	2.33	1545734
A2	12.2*	---	3.45	275117

Total Area: 2,098,237

F Concentration = 1.8* %
A2 Concentration = 12.2* %

*Values outside of expected ranges

Analysis comments:



CBC analysis :

Homozygotes

- reduced - Hb, MCV, and MCH.
- Hematologic findings are very similar to those of β-thalassemia major or intermedia.

Heterozygotes

- reduced MCV and MCH.
- Hematologic findings are very similar to those of β-thalassemia trait.
- Iron studies are normal in both heterozygotes and homozygotes.

Elongation Hemoglobins

- Elongation Hbs, of which there are 13, including seven α-chain and six β-chain variants, result from lengthening of the C or N terminus of either globin chain.
- The most important, from a clinical perspective, are the five C terminal α-chain variants in which the terminal codon TAA is changed and an amino acid sequence is added.
- The prototypical elongation Hb is Hb Constant Spring. In this variant, the C terminal TAA codon is changed to CAA in the α-gene, and a 31 amino acid sequence is added at the C terminal end to give an α-globin chain length of 173 amino acid residues, rather than the normal 142 residue length.
- This increase in length results in instability of the Hb variant, and synthesis of this elongated globin chain is reduced.

- Hemoglobin Constant Spring is found in South East Asia, especially in Vietnam, Cambodia, and Laos, and is found in both heterozygous and homozygous states.
- Patients with Hb Constant Spring present with slightly reduced Hb, MCV, and MCH
- peripheral blood smear : hypochromia and microcytosis .
- Iron studies are often normal
- The blood used for analytical procedures should be as fresh as possible.
- Samples older than 24 hours should not be used.

HPLC analysis :

Hb Constant Spring demonstrates a small peak in the C position,

- homozygote 4 to 6% of the total Hb
- Heterozygote 1 to 3% .

Electrophoresis:

- at alkaline pH, a small band migrating cathodally to the application point may be seen.
- This electrophoretic mobility is unique in that it is the only Hb variant that moves toward the cathode rather than the anode.

Thank you