

HETEROGENITY OF HEMOGLOBIN

- I. DEVELOPMENTAL
- II MINOR-COMPONENT
- III GENETIC

DEVELOPMENTAL

- Embryonic $\epsilon_2 \epsilon_2$
 $\alpha_2 \epsilon_2$

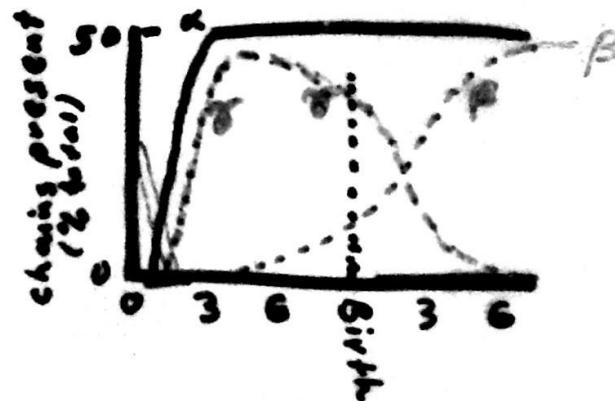
- Fetal Hb ... HbF

$\alpha_2 \gamma_2$

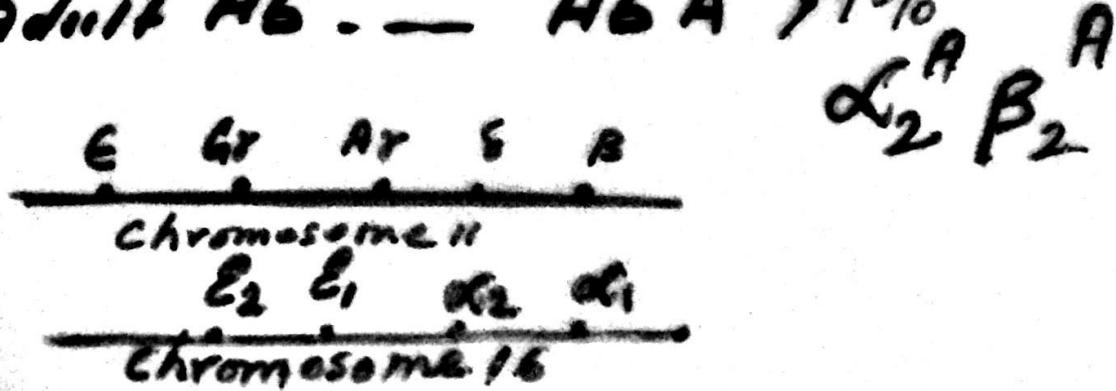
- HbF differ from HbA a.a. sequence
 in 37 a.a.

- 2,3-BPG binding

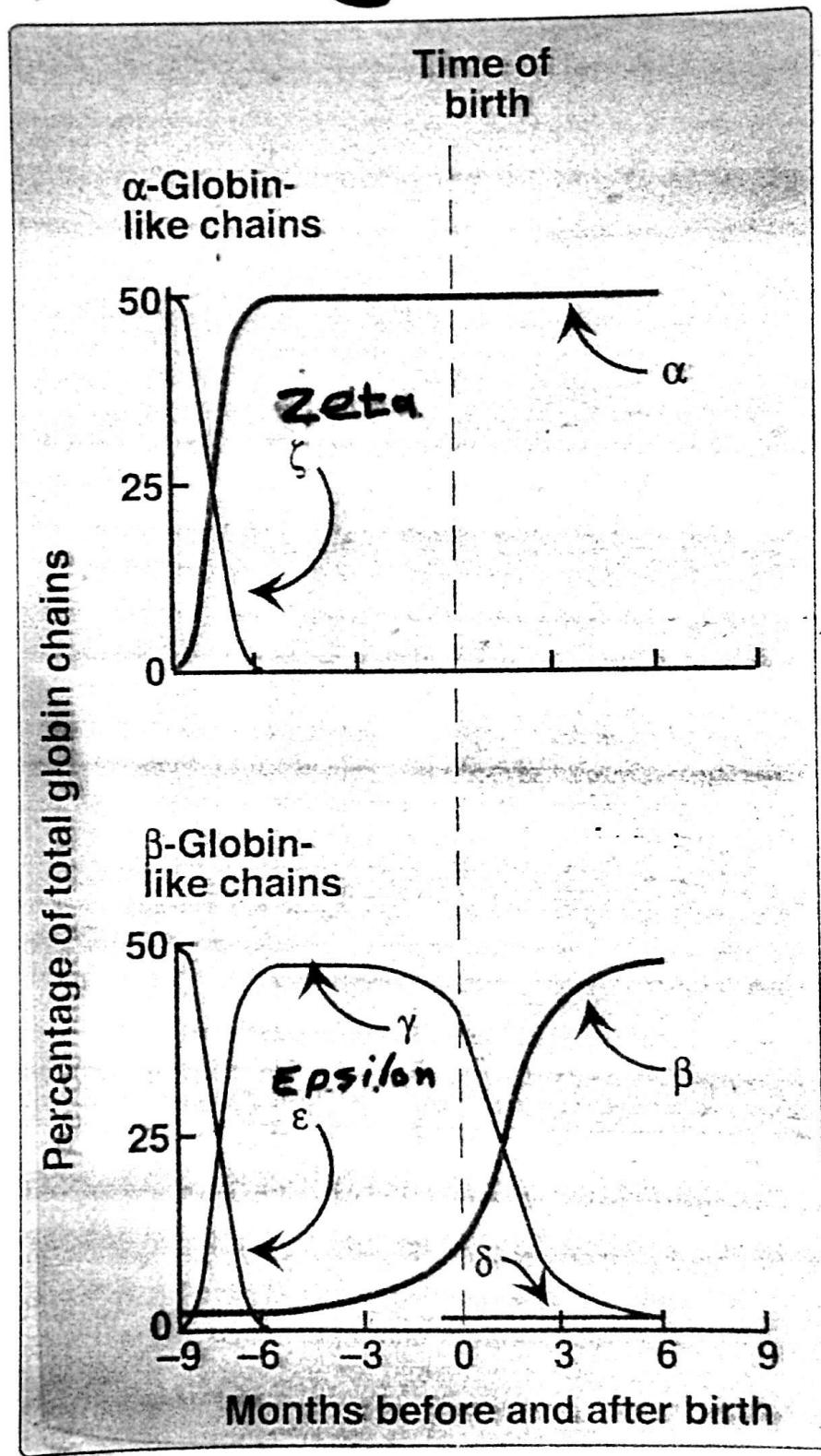
- Affinity for O₂



- Adult Hb -- HbA > 90%



Developmental changes in Hemoglobin



II Minor Component Hemoglobins

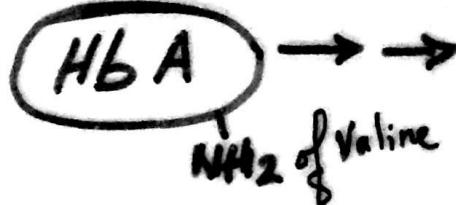
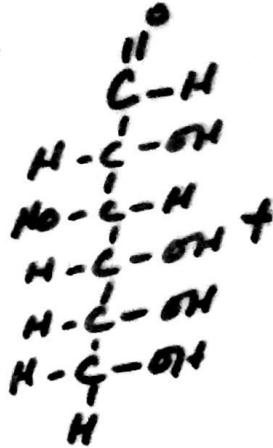
- Hb A₂ $\alpha_2 \delta_2$ 2.5%
appears about 12 weeks after birth

- Hb A_{1c} - - - 5%

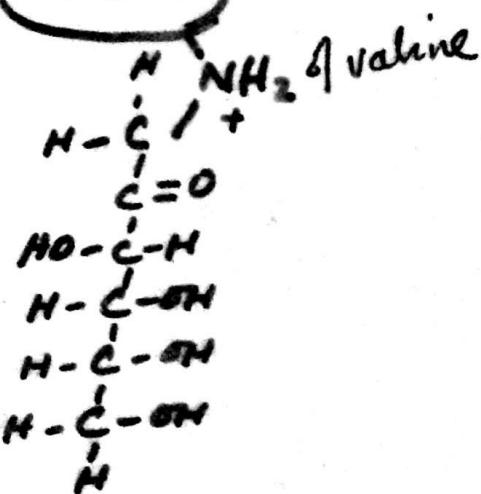
Glucose + N-terminal amino groups
of the β -chain (Val)

↓ Non-enzymatic

Hb A_{1c}



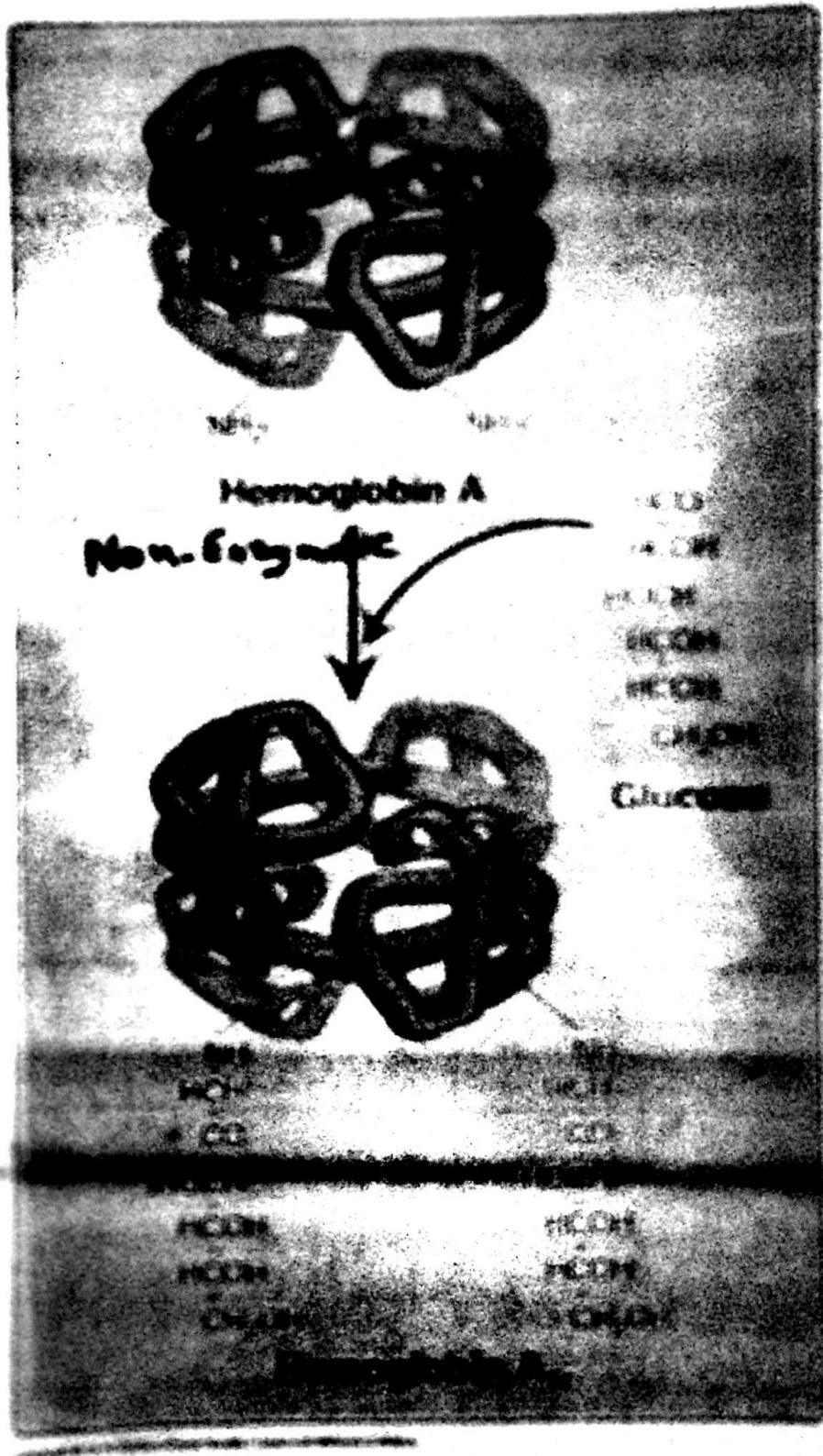
Hb A



- Hb A_{1c} conc. is
proportional to glucose conc.
in blood.

- Hb A_{1g} } G-6-P + Fru-1,6-diP
Hb A_{1b} } ~ 1%

Glycosylated (Glycated) Hb HbA_{1c}



Minor Hemoglobins

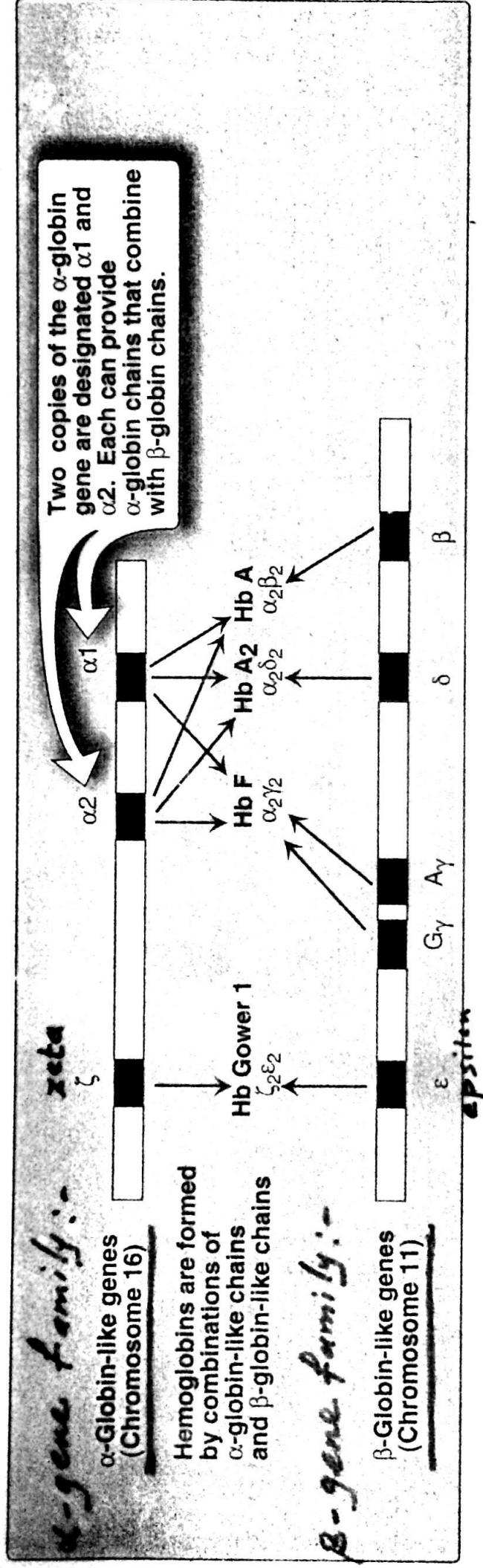
3a

Normal Adult Human Hemoglobin Variants

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA2	$\alpha_2\delta_2$	2%–5%
HbA1C	$\alpha_2\beta_2$ -glucose	3% 6%

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-Organization of the Globin genes



- Hemoglobinopathies
 - 1- Abnormal Hb structure
 - 2- Synthesis of insufficient quantities of normal hemoglobins

Molecular Pathology of Hemoglobin^{56,}

- Structural changes may lead to a change in any of the following:-

- Solubility

e.g. HbS, HbC

- Methemoglobinemia

- Unstable Hb

- O₂- affinity

- The Regional changes

I. Altered Exterior

Nearly all are harmless

Exceptions:-

HbS, HbC ; HbE
Africa Ceylon & Malaysia

; Hb Punjab
India & Pakistan

II Altered Active Site

Substitution allowing $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$

Methemoglobinemia
e.g. $\text{HbM}_{\text{Iwata}}$ $\alpha 87 \text{ His} \rightarrow \text{tyr}$; $\text{HbM}_{\text{Hyde Park}}$ $\beta 92 \text{ His} \rightarrow \text{tyr}$
Proximal His

also distal His
 $\text{HbM}_{\text{Boston}}$ $\alpha 58 \text{ His} \rightarrow \text{tyr}$; $\text{HbM}_{\text{saskatoon}}$ $\beta 63 \text{ His} \rightarrow \text{tyr}$

III Unstable Hb

Altered tertiary structures
 ↳ dematured \rightarrow Ppt \rightarrow Heinz bodies

e.g. substitution of Pro to an amino acid within α -helical segment
 or: substitution by a large a.a. or small to make a contact
 or: charged or polar within domain

IV Altered Affinity

Altered quaternary structure

Decreased Affinity Increased Affinity
 (Lower P₅₀)

- e.g.
- Point of contacts between subunits
- BPG binding sites
- H⁺ binding site
 e.g. Hb Cowtown $\beta^{146} \text{His} \rightarrow \text{Leu}$
 which is responsible for Bohr effect
 (50%)
 which destabilize T state $\rightarrow \text{TR} + \uparrow \text{affinity}$

Table 4.1
Amino acid substitutions in mutant hemoglobins

α Chain	Mutant Hemoglobin ^a	Position Number ^b	Normal Residue	Substitution
G Honolulu		30	Glu	Gln
G Philadelphia		68	Asn	Lys
I		16	Lys	Glu
M Boston		58	His	Tyr
Norfolk		57	Gly	Asp
O Indonesia	α^A Glu \rightarrow Lys	116	Glu	Lys
β Chain	$\alpha_2^A \beta_2$			
C		6	Glu	Lys
D Punjab		121	Glu	Gln
G San Jose		7	Glu	Gly
E	α^A Glu \rightarrow Val	26	Glu	Lys
S \rightarrow $\alpha_2^A \beta_2$		6	Glu	Val
Zurich		63	His	Arg

^aThe hemoglobins are often named for the cities where they were first discovered.

^bThe numbering for an amino acid position begins at the N-terminus.

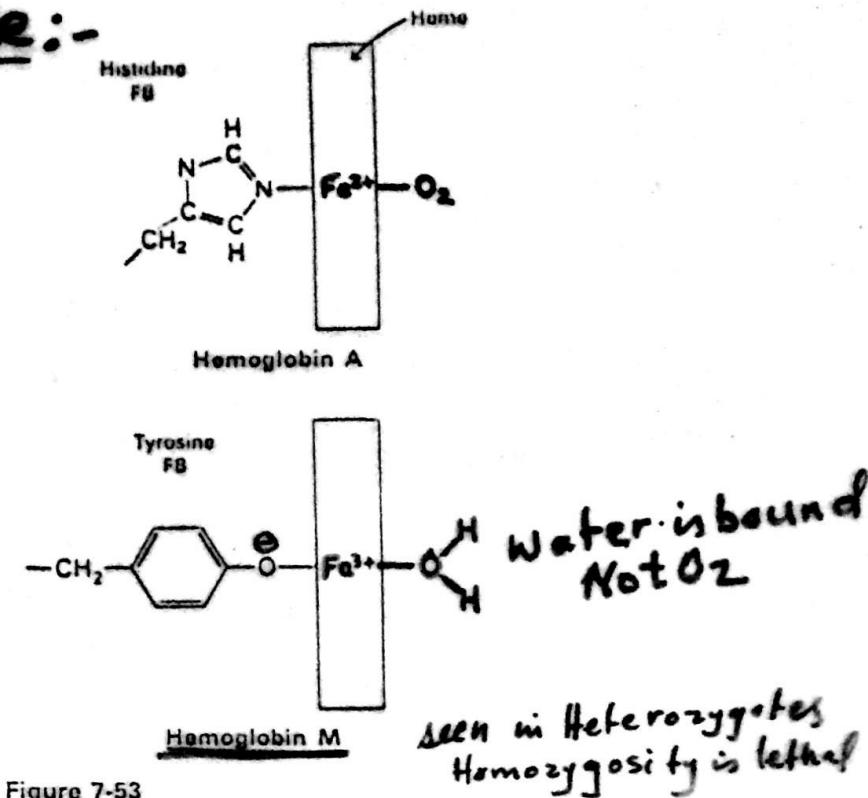
Altered Active Site:-

Figure 7-53

Substitution of tyrosine for the proximal histidine (F8) results in the formation of a hemoglobin M. The negatively charged oxygen atom of tyrosine is coordinated to the iron atom, which is in the ferric state. Water rather than O_2 is bound at the sixth coordination position.

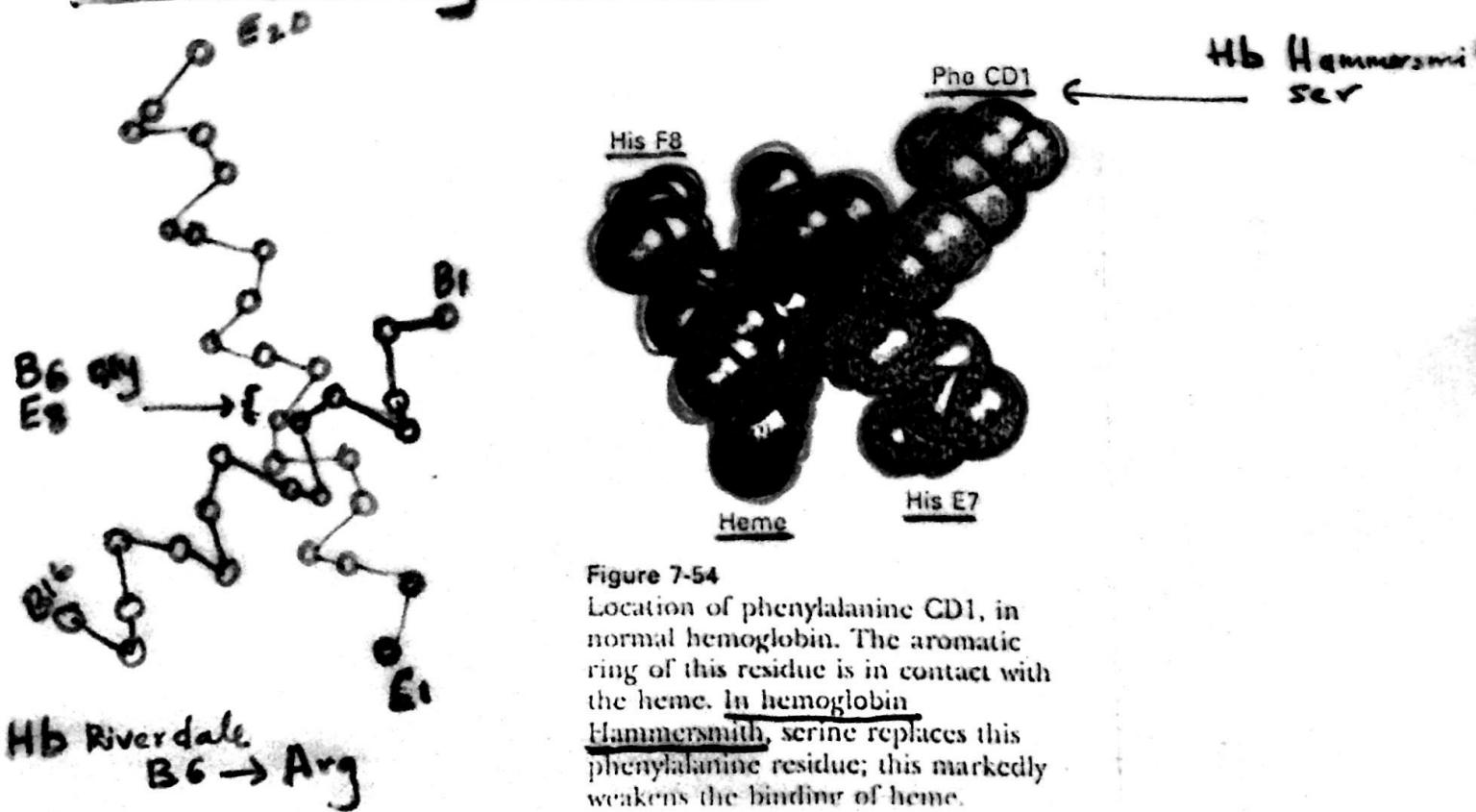
Altered Tertiary Structure:-

Figure 7-54

Location of phenylalanine CD1, in normal hemoglobin. The aromatic ring of this residue is in contact with the heme. In hemoglobin Hammersmith, serine replaces this phenylalanine residue; this markedly weakens the binding of heme.

Sickle Cell Anemia — sickle cell disease.

- most common disorder caused by Hb variant
homozygous recessive disorder
1 in 500 newborn infants is affected

- Heterozygotes — sickle cell trait
one of ten American black

- electrophoresis at alkaline pH

$\alpha_2^A \beta_2$ $\text{Glu} \rightarrow \text{Val}$

- Formation of aggregates + fibers

- Extent of sickling is increased by increasing proportion of deoxy HbS

- decreased O₂ tension by high altitude or flying in non-pressurized plane

- increased CO₂ conc.

- decreased pH

- increased 2,3-BPG

- selective advantage against Malaria — parasite plasmodium falciparum

Hemoglobin C disease (HbC) $\alpha_2^A \beta_2$ $\text{Glu} \rightarrow \text{Lys}$

Hb CS disease

double Heterozygote

No sickling
HbC crystals
mild anemia

Characteristic Features of Sickle Cell Anemia

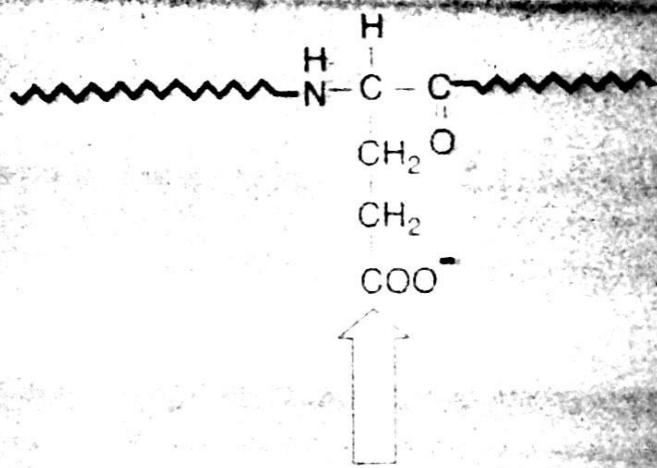
- Sickled cells lose water - becomes fragile → life span 17d instead of 120
→ Anemia
- More serious - small blood capillaries in different organs become blocked by long abnormally shaped red cell → anoxia, causing pain
→ death of cells
- People with sickle cell trait live normal if they avoid vigorous exercise, high altitude, anaesthesia, air travel in unpressurised plane
- People with sickle cell trait have increased resistance to malaria, specifically plasmodium falciparum

Management

Hydration, analgesics, antibiotic, intermittent transfusion.
Hydroxyurea → ↑ HbF

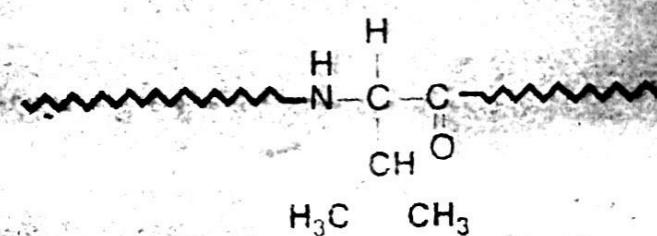
HbC : mild chronic anemia, no infarctive crisis, no sp. therapy required

Hb SC disease : double or compound heterozygote milder anemia than HbS, Painful crisis are less frequent



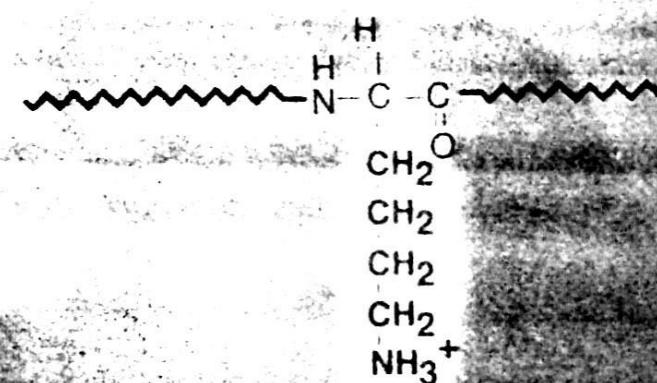
Val · His · Leu · Thr · Pro · **Glu** · Glu · Lys ~~~~
1 2 3 4 5 6 7 8

HbA



Val · His · Leu · Thr · Pro · **Vall** · Glu · Lys ~~~~
1 2 3 4 5 6 7 8

HbS



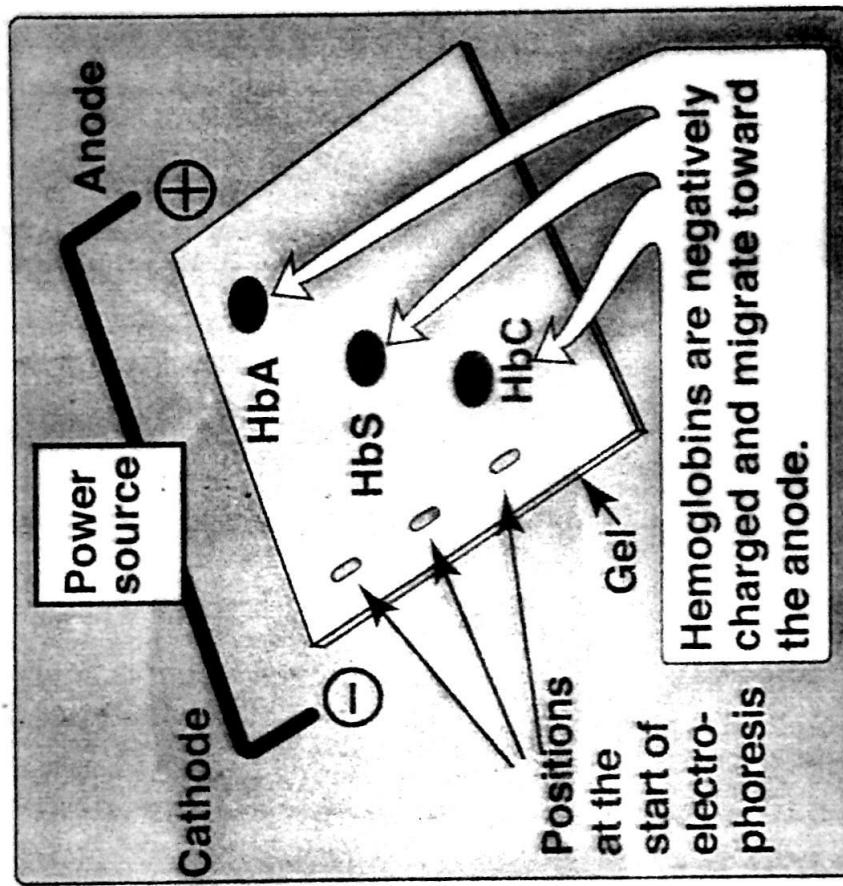
Val · His · Leu · Thr · Pro · **Lys** · Glu · Lys ~~~~
1 2 3 4 5 6 7 8

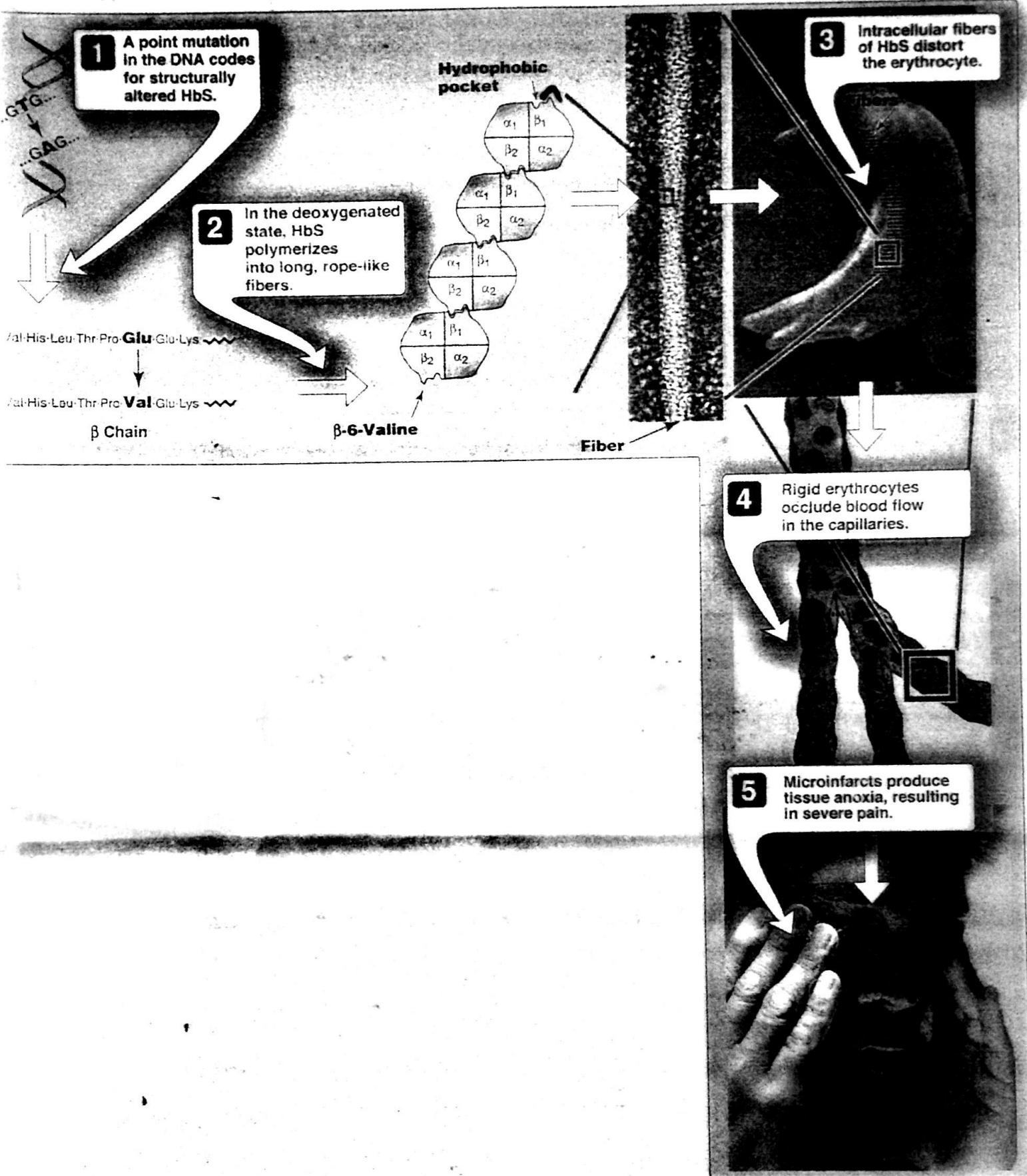
HbC

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Amino acid substitution in HbS and HbC

Gel electrophoresis of Hemoglobins Hb A, Hb S, Hb C





Polymerization of Deoxy HbS

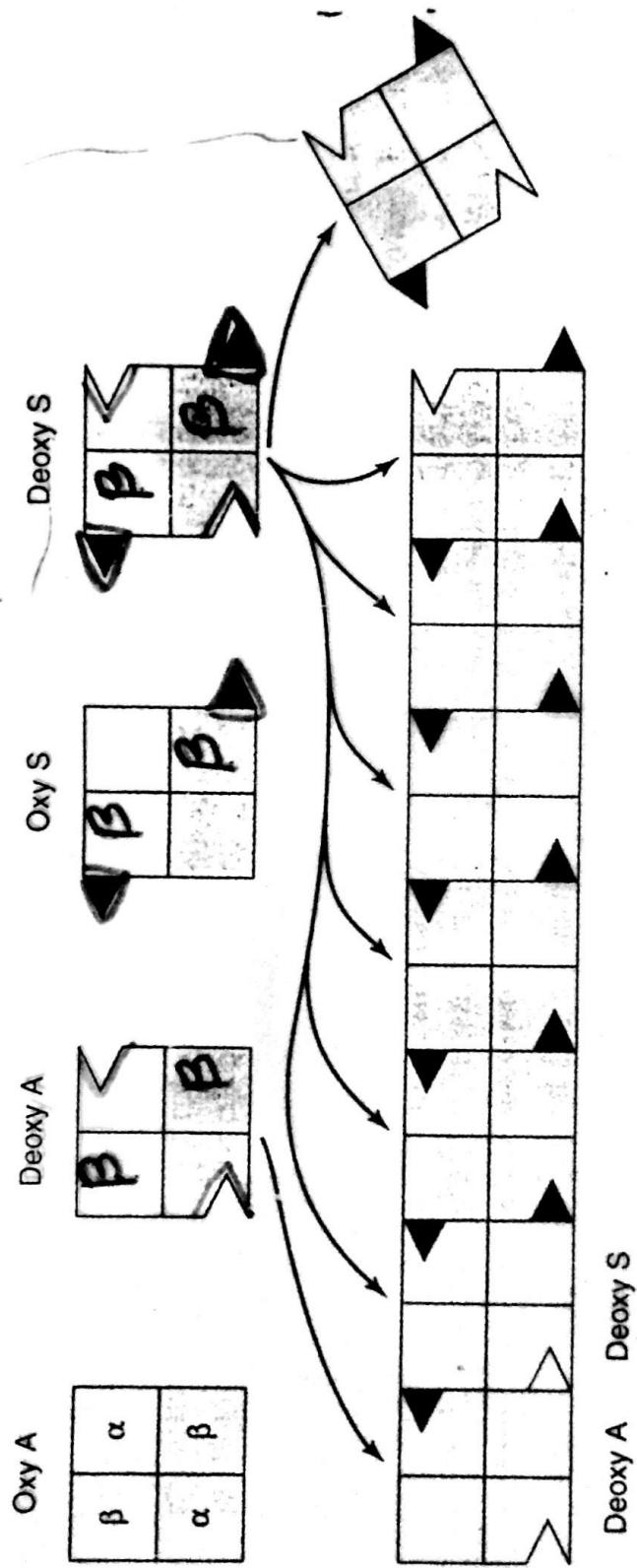
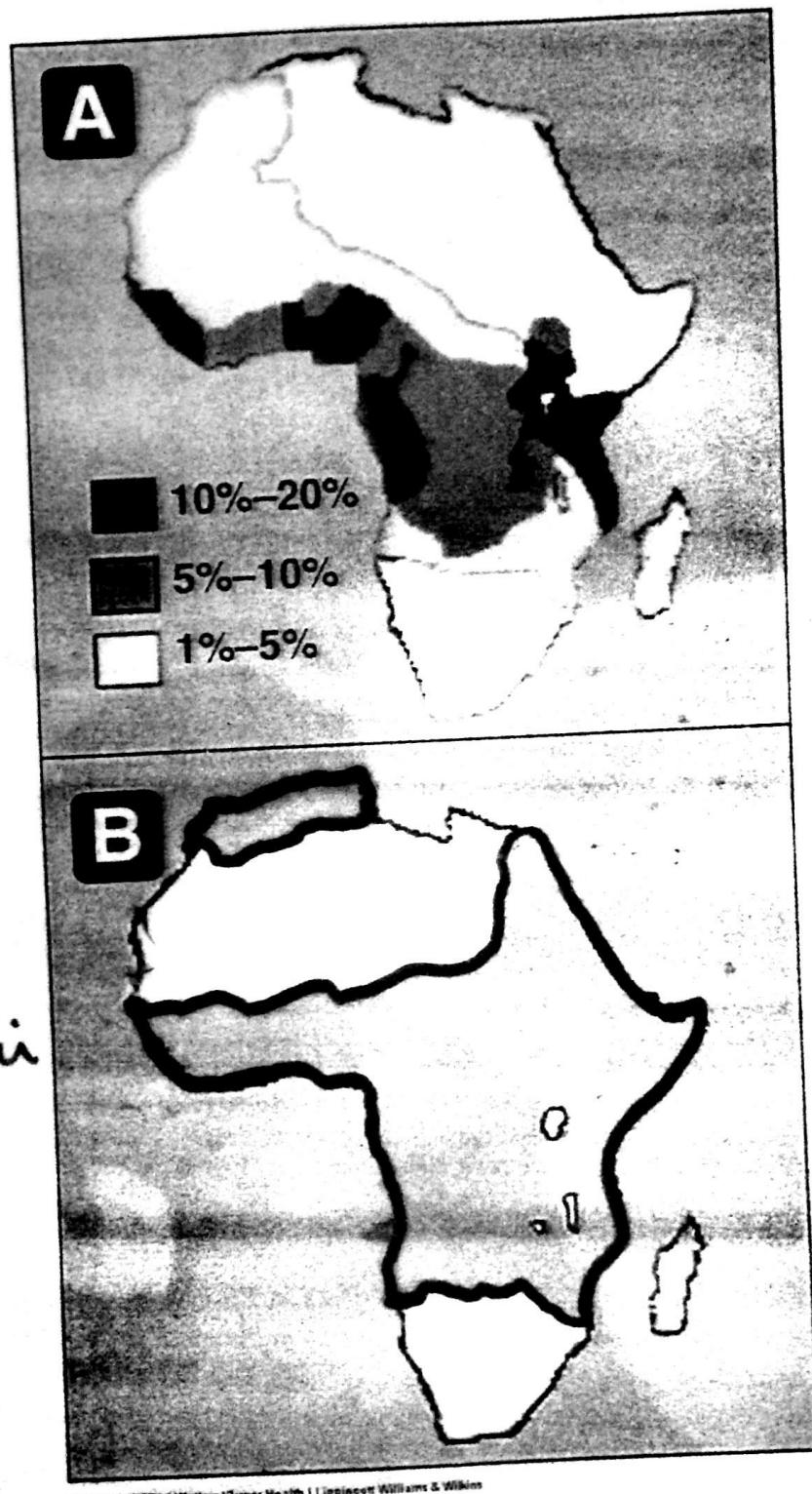


Figure 6-11. Representation of the sticky patch (\blacktriangle) on hemoglobin S and its "receptor" (\curvearrowleft) on deoxyhemoglobin A and deoxyhemoglobin S. The complementary surfaces allow deoxyhemoglobin S to polymerize into a fibrous structure, but the presence of deoxyhemoglobin A will terminate the polymerization by failing to provide sticky patches. (Modified and reproduced, with permission, from Stryer L: *Biochemistry*, 4th ed. Freeman, 1995. Copyright © 1995 W. H. Freeman and Company.)

Sickle
Cell
disease



Malaria in
Africa