Hematology

BIOCHEMSITRY

☐ Sheet ☑ Slide ☐ Handout

Number: **3**

Subject: **Heterogeneity of Hemoglobin**

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Date: **00/9/2016**

Price:

HETROGENITY OF HEMOGLOBIN

I. DEVELOPMENTAL

II MINOR-COMPONENT

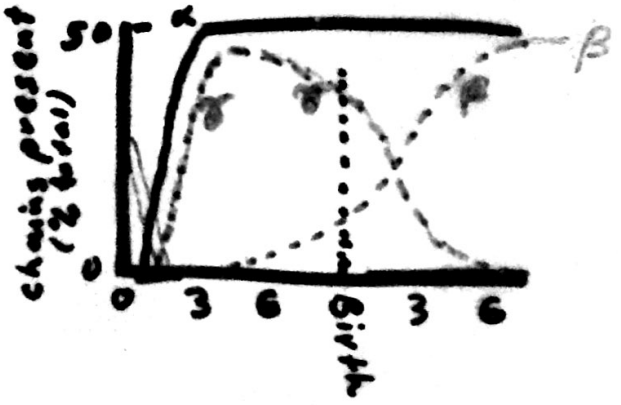
III GENETIC

DEVELOPMENTAL

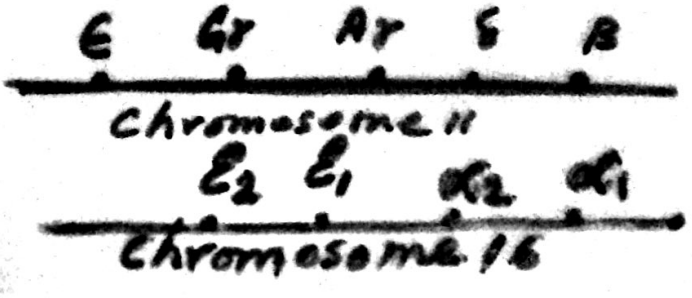
- Embryonic $\zeta_2 \epsilon_2$
  $\alpha_2 \epsilon_2$

- Fetal Hb .... HbF
  $\alpha_2 \gamma_2$

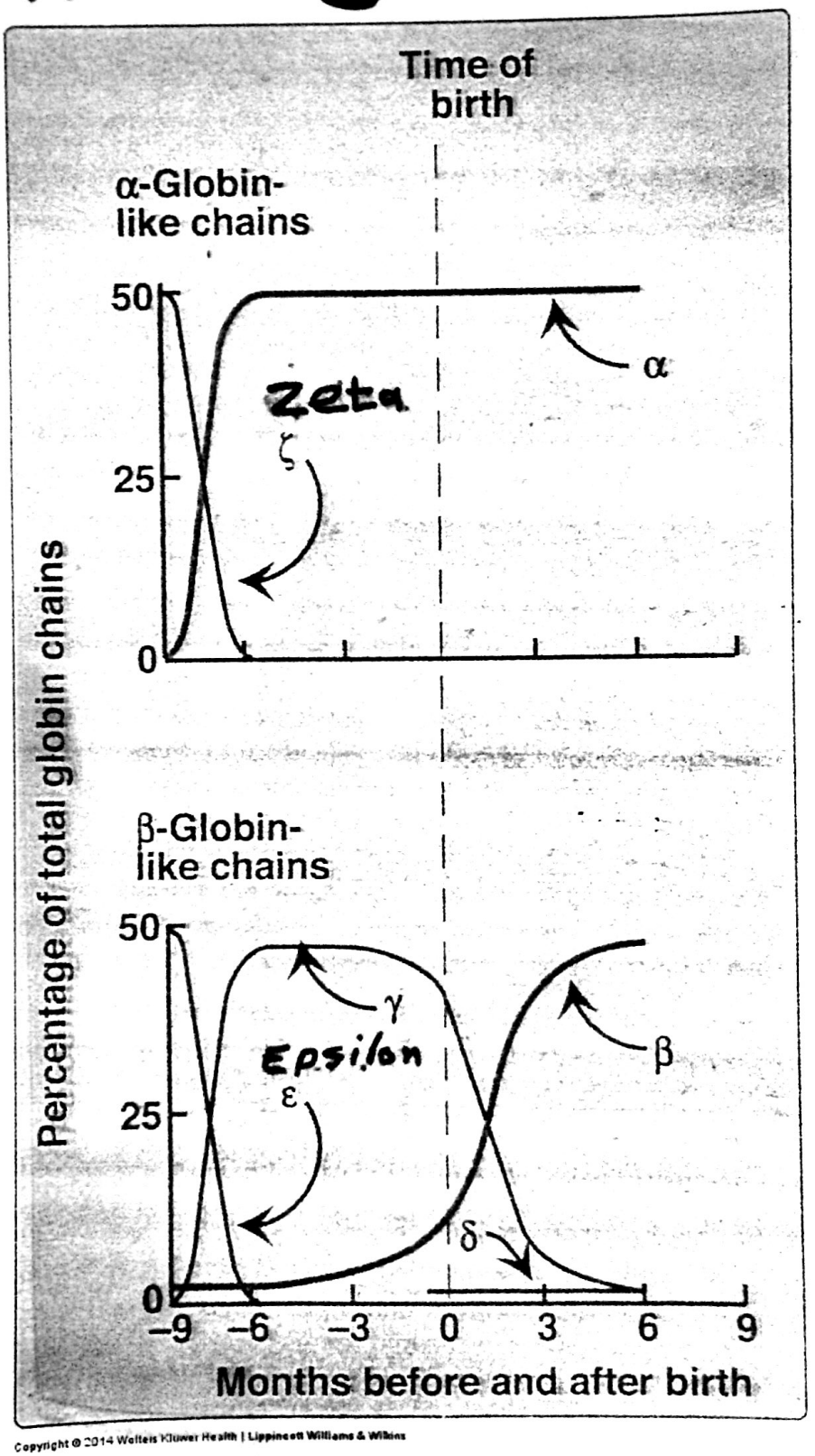
- HbF differ from HbA a.a. sequence in 37 a.a.
- 2,3-BPG binding
- Affinity for O_2



- Adult Hb — HbA > 90% $\alpha_2^A \beta_2^A$



Developmental Changes in Hemoglobin

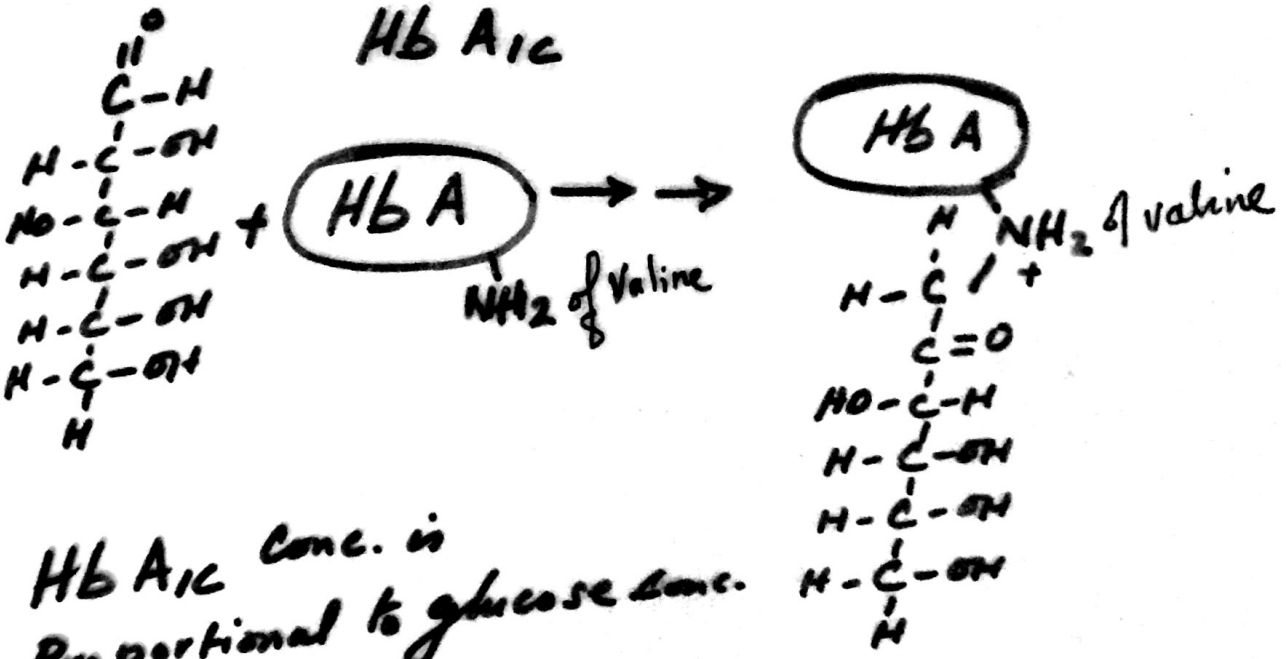


II Minor Component Hemoglobins

- $Hb\ A_2$ $\alpha_2 \delta_2$ 2.5%
  appears about 12 weeks after birth

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

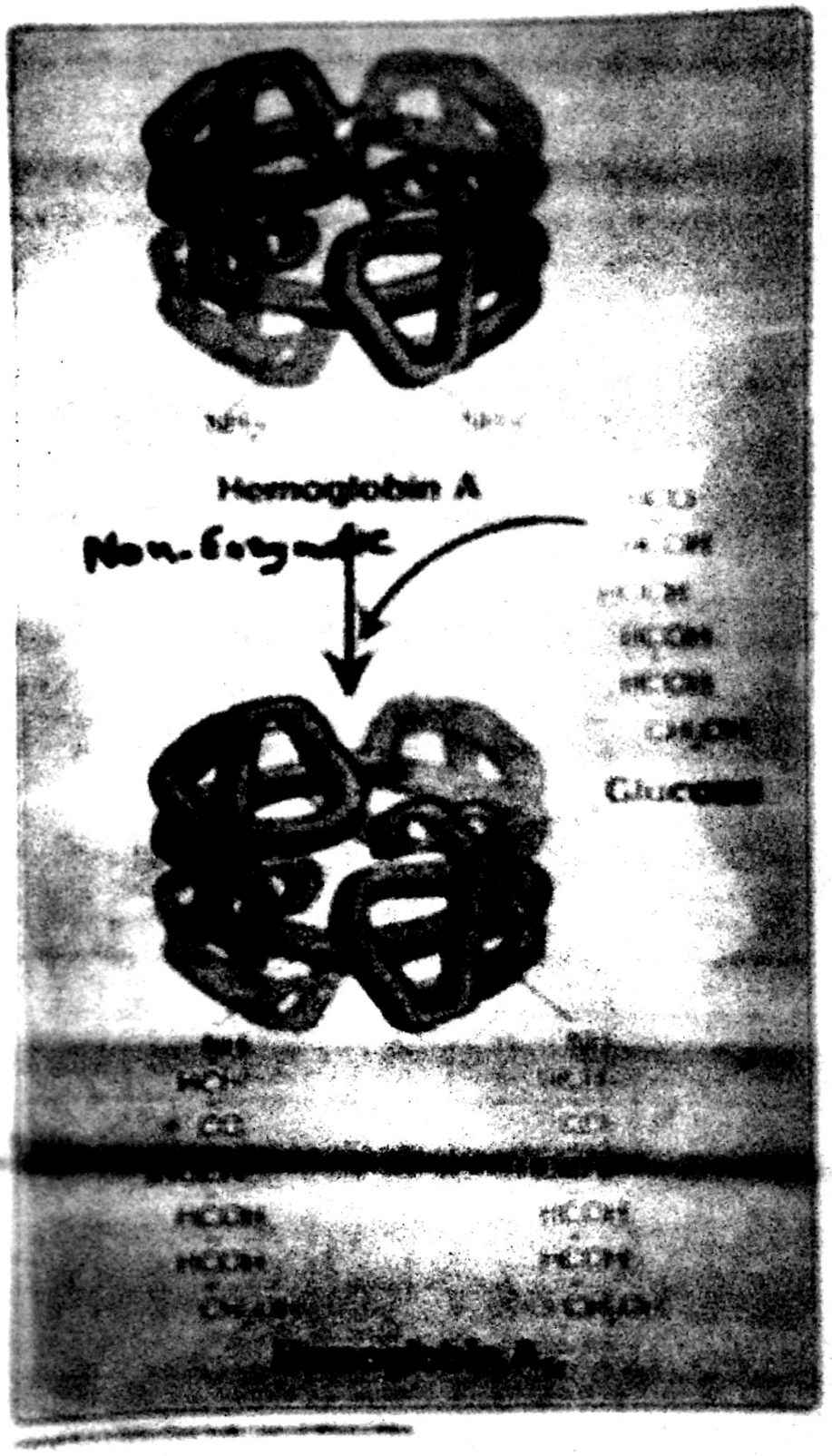
Glucose + N-terminal amino groups of the β-chain (Val)
↓ Non-enzymatic
$Hb\ A_{1c}$



- $Hb\ A_{1c}$ Conc. is Proportional to glucose conc. in blood.

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

Glycosylated (Glycated) Hb
HbA1c

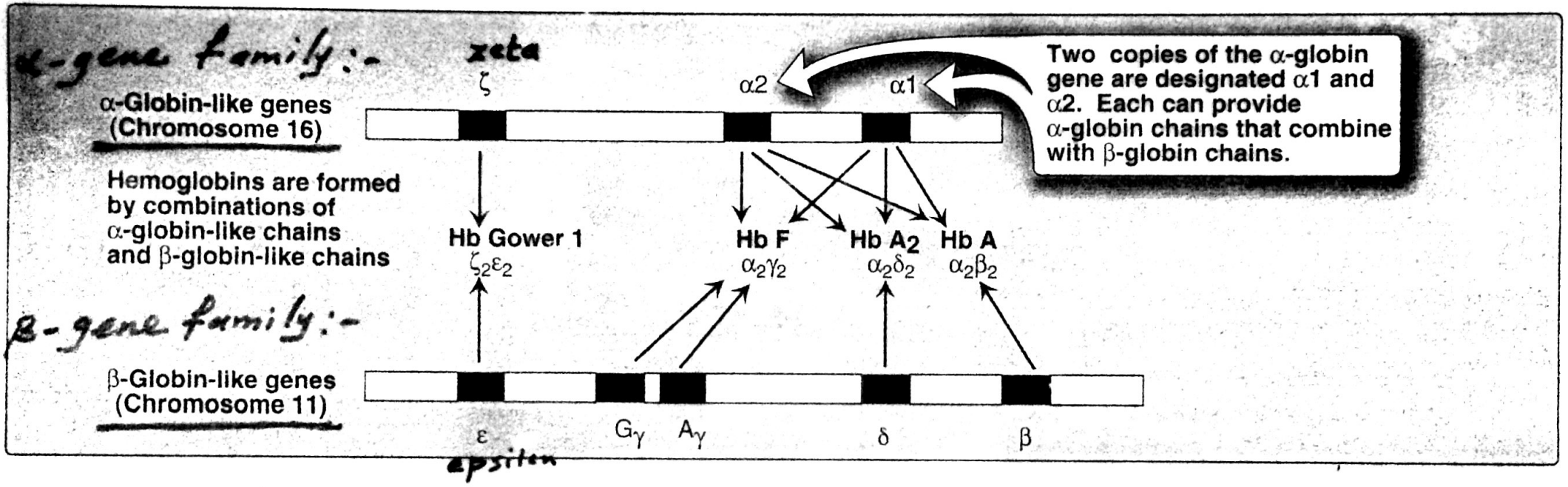


Minor Hemoglobins

Normal Adult Human Hemoglobin Variants

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2%–5%
HbA1c	$\alpha_2\beta_2$-glucose	3%–6%

-Organization of the Globin genes



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

Molecular Pathology of Hemoglobin

- Structural changes may lead to a change in any of the following:-
  - Solubility e.g. HbS, HbC
  - Methemoglobinemia
  - Unstable Hb
  - O_2- affinity

- The Regional changes
  I.. Altered Exterior
  Nearly all are harmless
  Exceptions:-
  HbS, HbC (Africa) ; HbE (Ceylon & malysia) ; Hb Punjab (India & Pakistan)

II Altered Active Site

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

Methemoglobinemia

e.g $Hb\,M_{Iwate}^{\alpha 87\ His \rightarrow tyr}$; $Hb\,M_{Hyde\ Park}^{\beta 92\ His \rightarrow tyr}$

Proximal His

also distal His

$Hb\,M_{Boston}^{\alpha 58\ His \rightarrow tyr}$; $Hb\,M_{Saskatoon}^{\beta 63\ His \rightarrow tyr}$

III Unstable Hb

Altered tertiary structure

↳ denatured → ppt → 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)

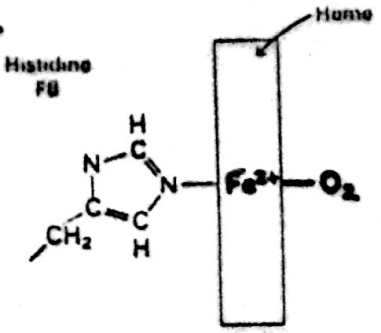
e.g.
- Point of contacts between subunits
- BPG binding sites
- H^+ binding site
  e.g. Hb Cowtown $\beta^{146 His \rightarrow Leu}$
  which is responsible for Bohr. effect (50%)
  which destabilize T state → ↑R + ↑affinity

Table 4.1
Amino acid substitutions in mutant hemoglobins

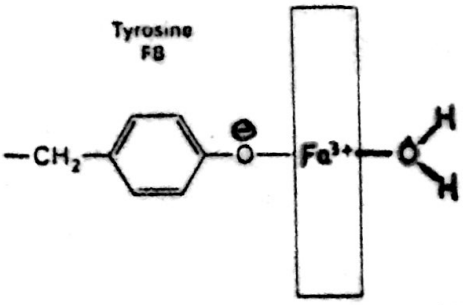
Mutant Hemoglobin[a]	Position Number[b]	Normal Residue	Substitution
α Chain			
$G_{Honolulu}$	30	Glu	Gln
$G_{Philadelphia}$	68	Asn	Lys
I	16	Lys	Glu
M_{Boston}	58	His	Tyr
Norfolk	57	Gly	Asp
$O_{Indonesia}$	116	Glu	Lys
β Chain			
C	6	Glu	Lys
D_{Punjab}	121	Glu	Gln
$G_{San\ Jose}$	7	Glu	Gly
E	26	Glu	Lys
S	6	Glu	Val
Zurich	63	His	Arg

[a] The hemoglobins are often named for the cities where they were first discovered.
[b] The numbering for an amino acid position begins at the N-terminus.

Altered Active Site :-



Hemoglobin A



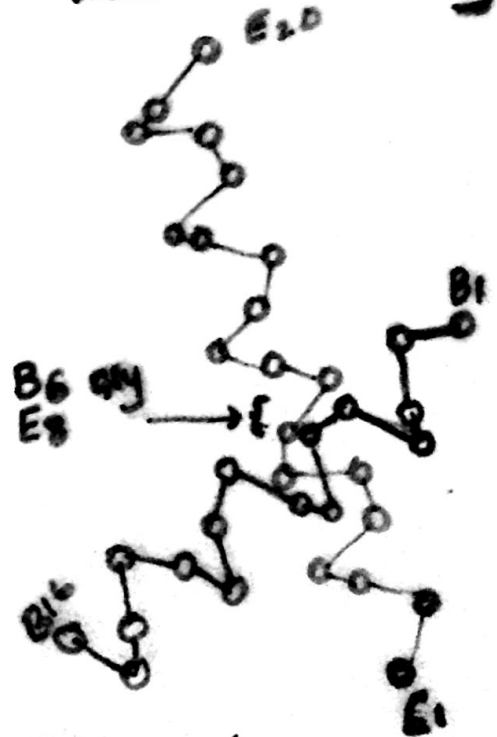
Water is bound Not O2

Hemoglobin M

seen in Heterozygotes
Homozygosity is lethal

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 :-



Hb Riverdale
B6 → Arg

Hb Hammersmith
ser

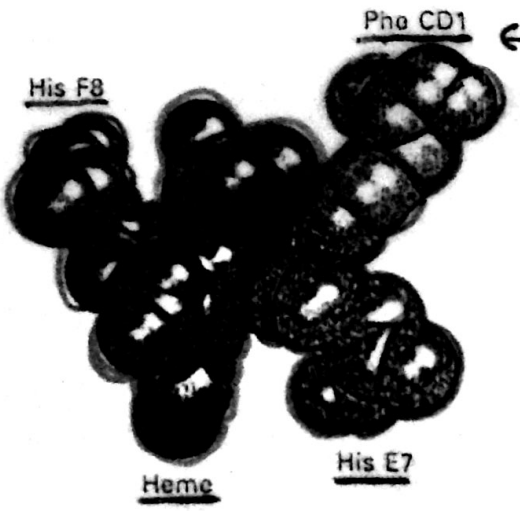


Figure 7-54
Location of phenylalanine CD1, in normal hemoglobin. The aromatic ring of this residue is in contact with the heme. In hemoglobin Hammersmith, serine replaces this phenylalanine residue; this markedly weakens the binding of heme.

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

- $\alpha_2^A \beta_2^{6\,Glu \rightarrow Val}$

- Formation of aggregates → fibers

- Extent of sickling is increased by increasing proportion of deoxy HbS
  - decreased O_2 tension by high altitude → flying in non-pressurized plane
  - increased CO_2 conc.
  - decreased pH
  - increased 2,3-BPG

- selective advantage against Malaria — parasite plasmodium falciparum

Hemoglobin C disease (HbC) $\alpha_2^A \beta_2^{6\,Glu \rightarrow lys}$
No sickling
HbC crystals
mild anemia

HbCS disease
double Heterozygote

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 excercise, high altitude, anaesthesia, air travel in unpressurised plane

- People with sickle cell trait have incred resistance to malaria, specifically plasmodium falciparum

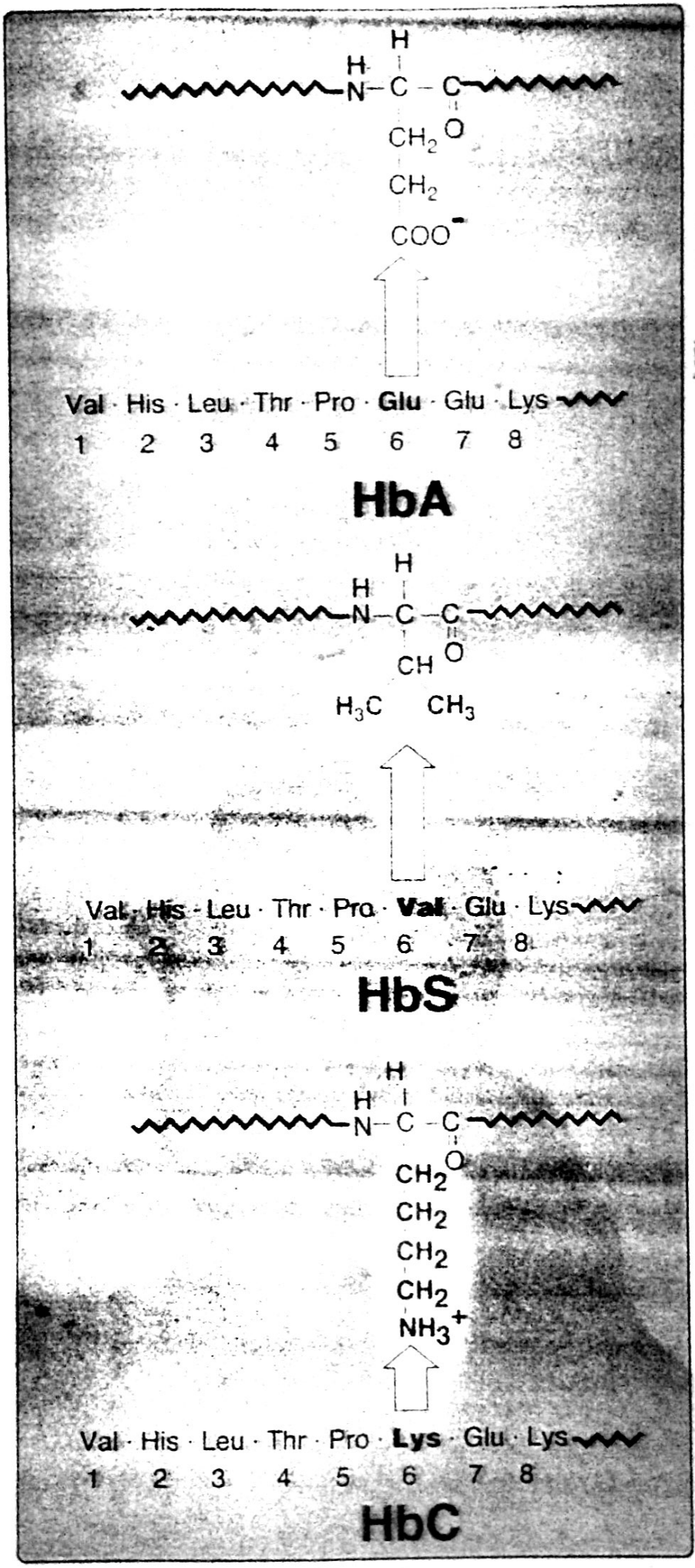
Management

Hydration, analgesics, antibiotic, Intermittent transfusion.

Hydroxyurea → ↑ HbF

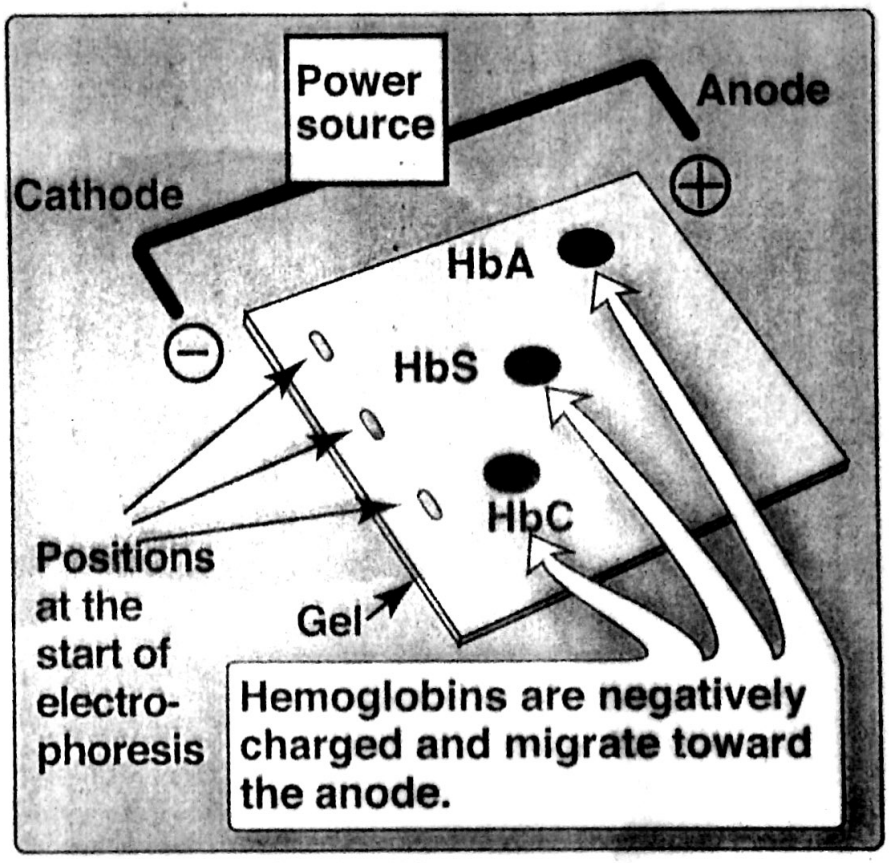
HbC: mild chronic anemia, no infarctive crisis, no sp. therapy required

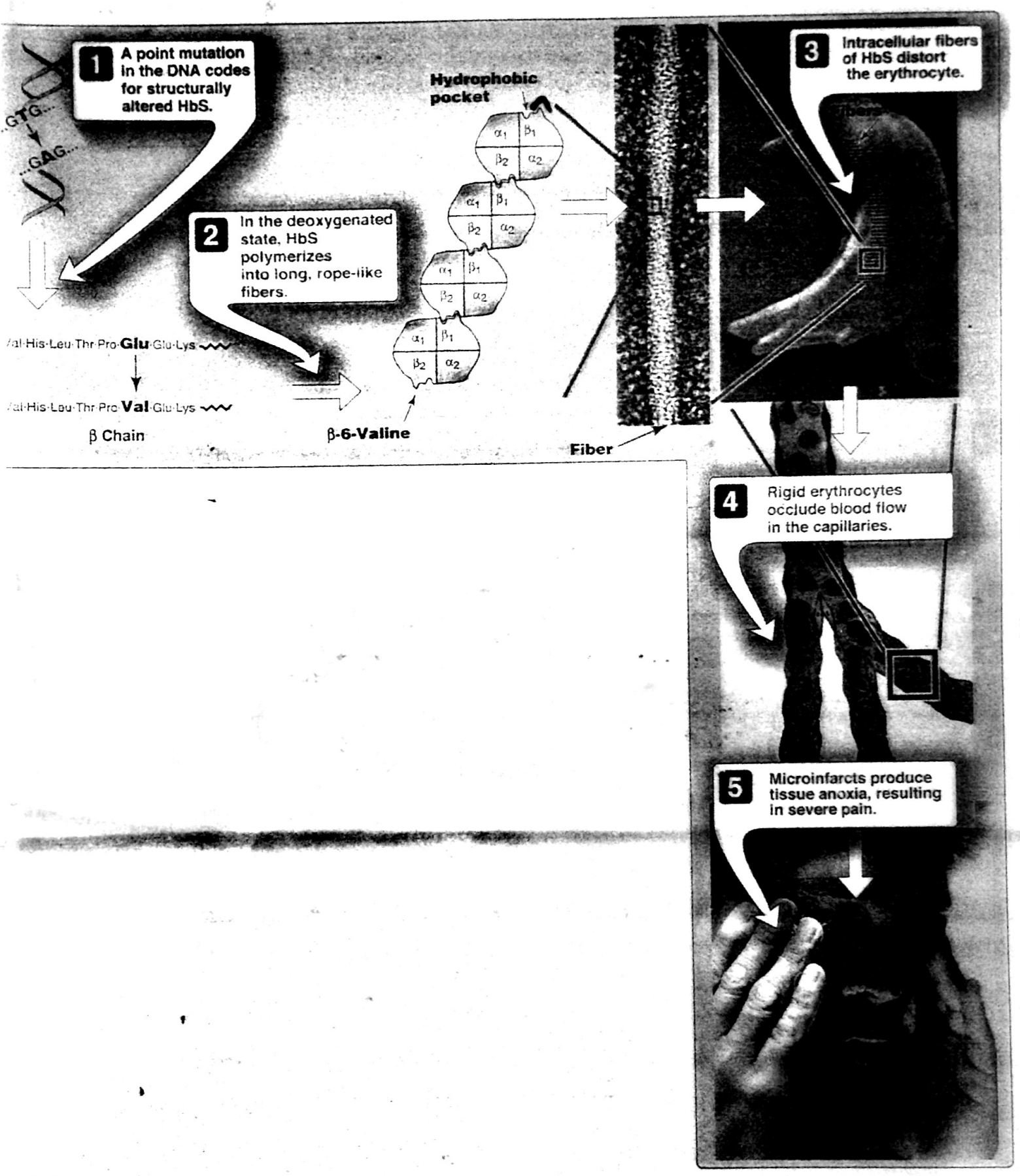
Hb SC disease: double or compound heterozygote milder anemia than HbS, Painful crisis are less frequent



Amino acid substitution in HbS and HbC

Gel electrophoresis of Hemoglobins HbA, HbS, HbC




1 A point mutation in the DNA codes for structurally altered HbS.
GTG...
...GAG...
2 In the deoxygenated state, HbS polymerizes into long, rope-like fibers.
Val·His·Leu·Thr·Pro·Glu·Glu·Lys
Val·His·Leu·Thr·Pro·Val·Glu·Lys
β Chain
β-6-Valine
Hydrophobic pocket
Fiber
3 Intracellular fibers of HbS distort the erythrocyte.
Fibers
4 Rigid erythrocytes occlude blood flow in the capillaries.
5 Microinfarcts produce tissue anoxia, resulting in severe pain.

Polymerization of Deoxy HbS

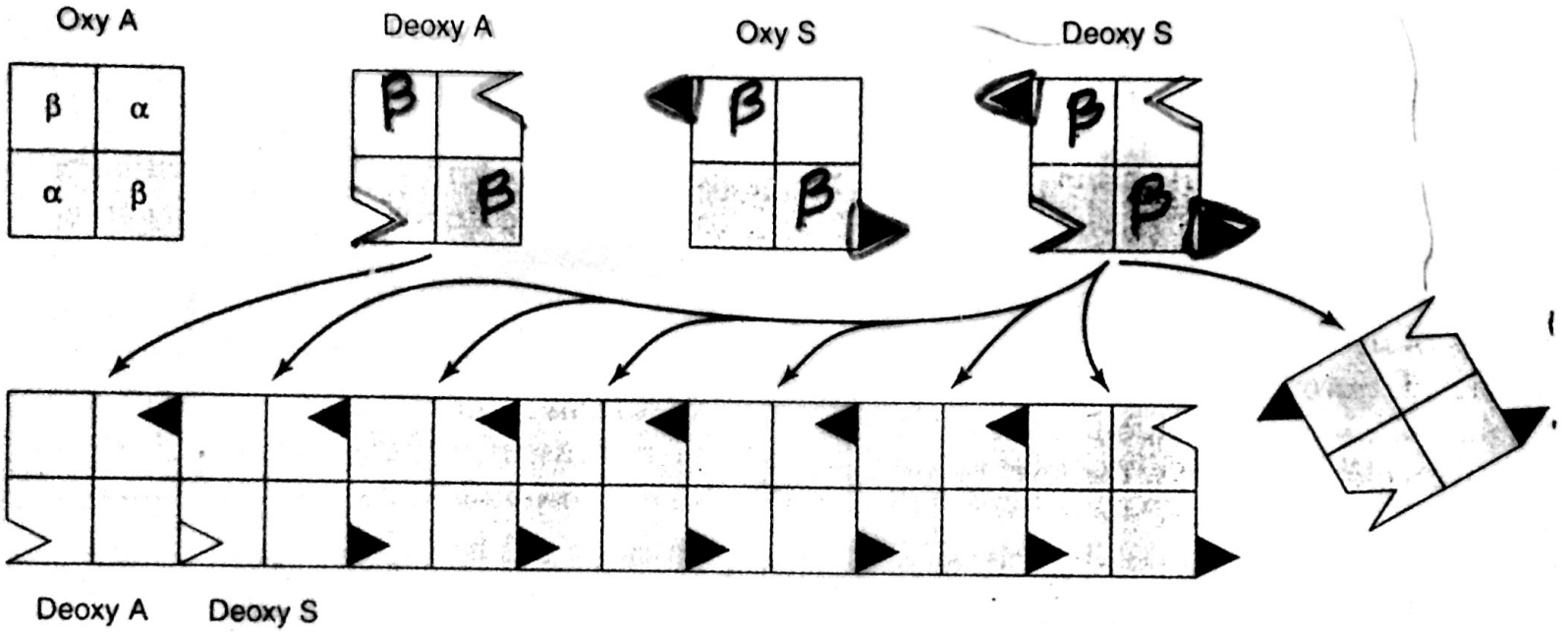
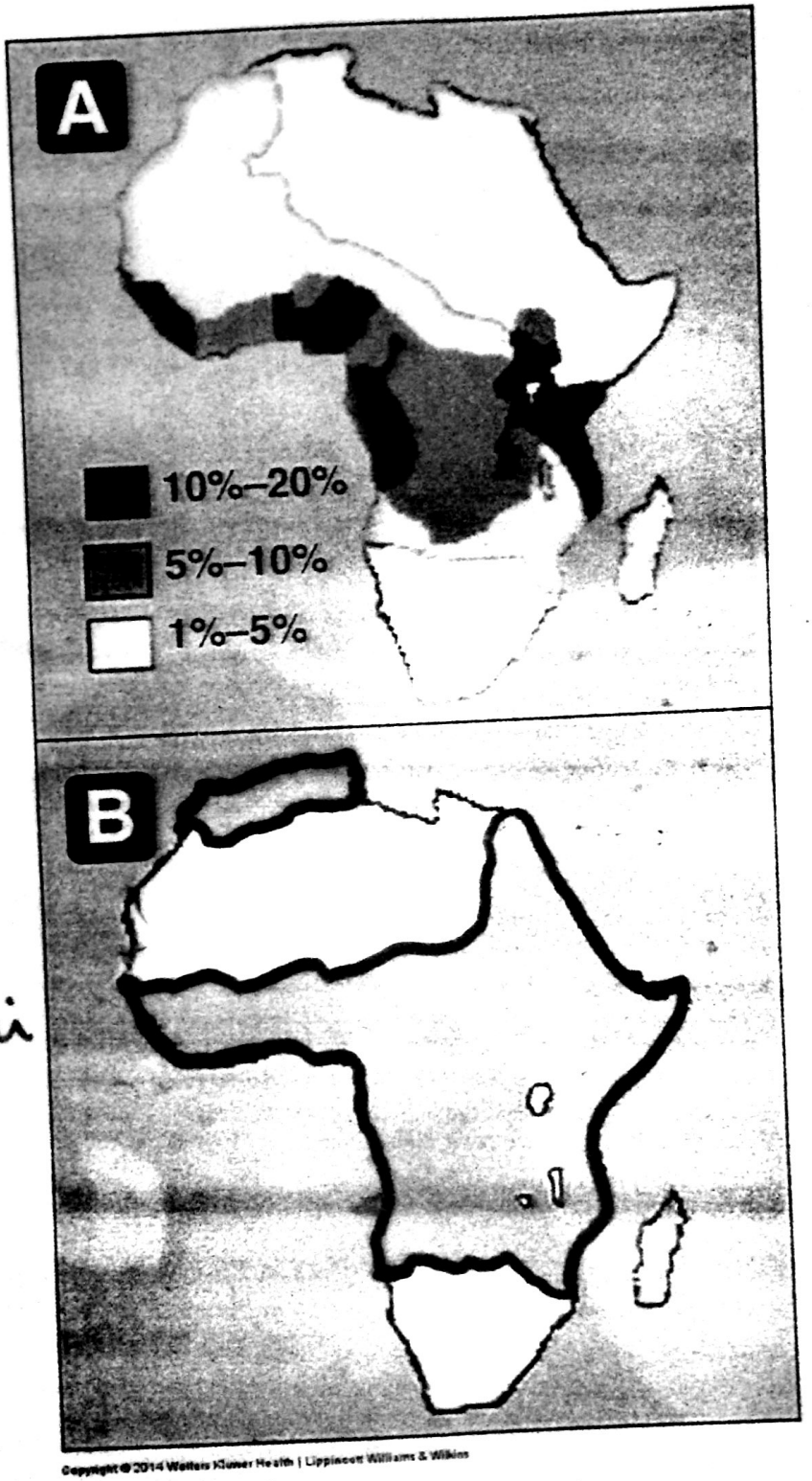


Figure 6–11. Representation of the sticky patch (▲) on hemoglobin S and its "receptor" (△) on deoxyhemoglobin A and deoxyhemoglobin S. The complementary surfaces allow deoxyhemoglobin S to polymerize into a fibrous structure, but the presence of deoxyhemoglobin A will terminate the polymerization by 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