Haemoglobin
Haemoglobin Derivatives
&
Haemoglobinopathy

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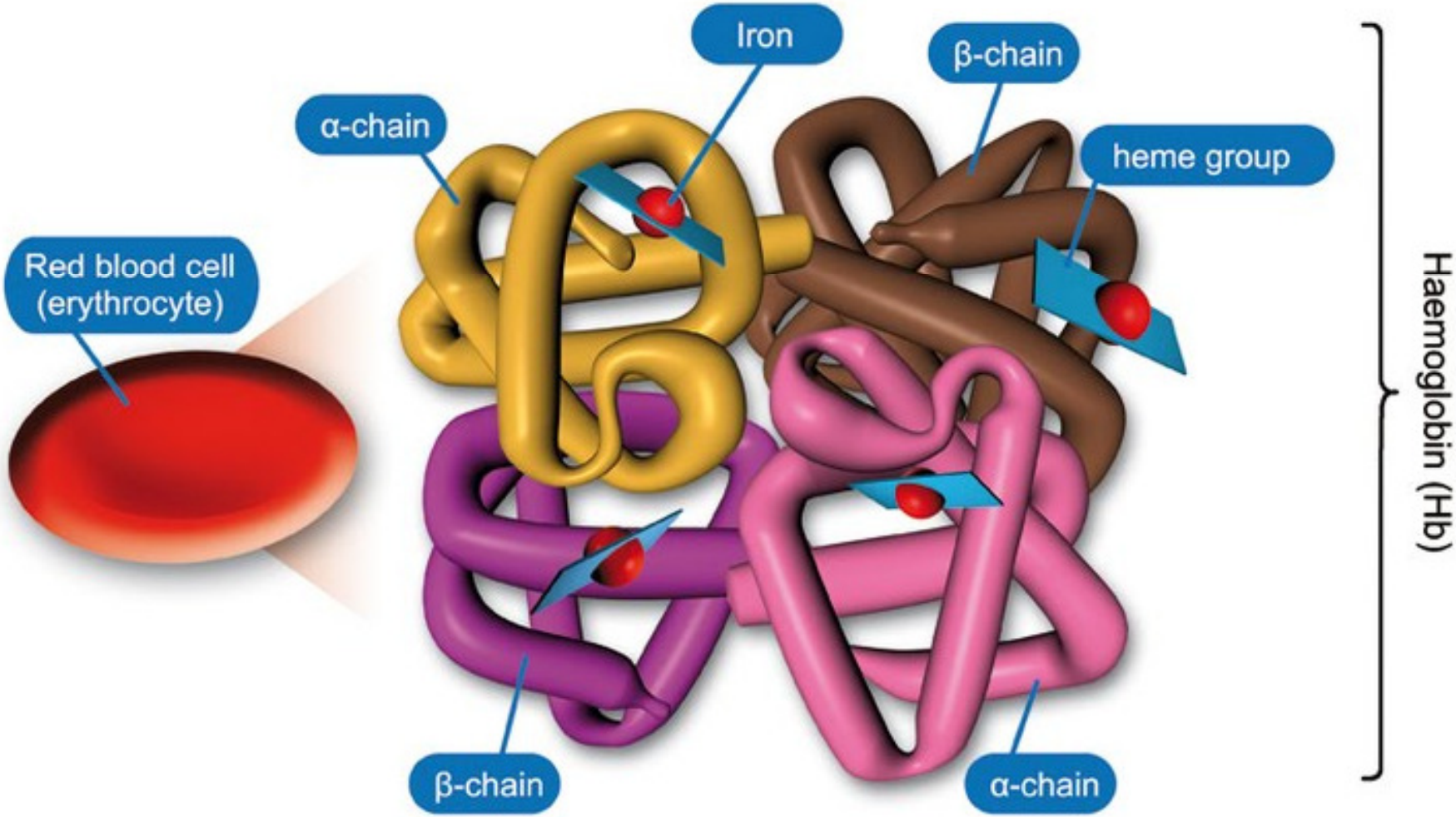
STRUCTURE

- Normal level of hemoglobin (Hb) in blood :

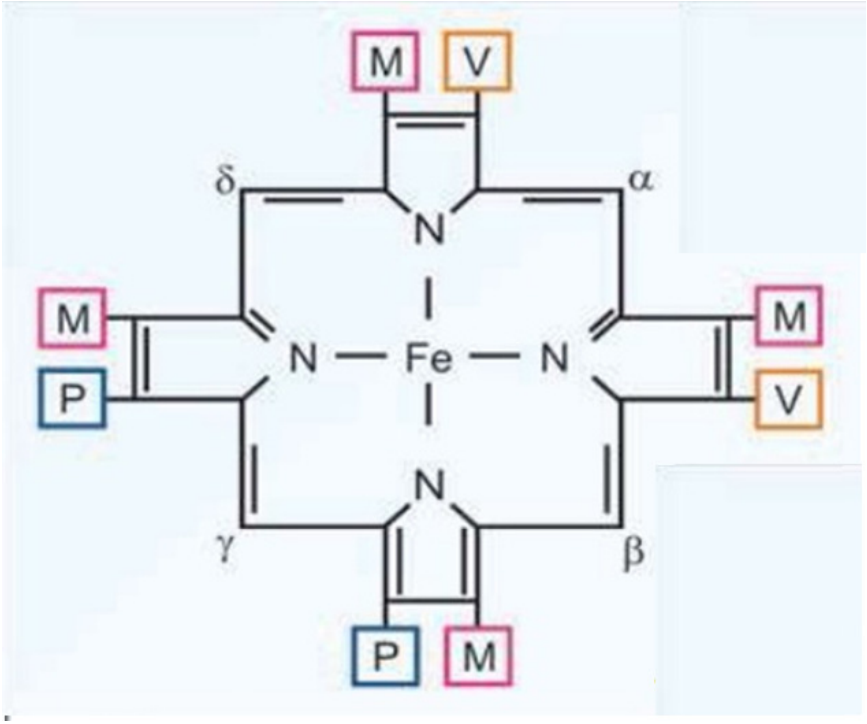
  In males : 14-16 g/dl

  In females : 13-15g/dl
- Adult Hb (HbA) = 2 α + 2 β chains.
- Fetal Hb (HbF) = 2α + 2γ chains.
- HbA2 = 2α + 2δ chains.
- Normal adult blood
  - 97% HbA
  - 2% HbA2
  - 1% HbF.

Haemoglobin Structure



Componant of Haemoglobin



- 4 Globin Chain
  - 2 alpha
  - 2 beta
- 4 Heme
  - 4 Porphyrin ring
    - 16 pyrrole ring
    - 4 pyrrole ring in each Porphyrin ring
  - 4 Iron
    - Reduced state = Ferrous(Fe++)
    - One Fe^{+2} in middle of each Porphyrin ring

Partial Pressure of Oxygen

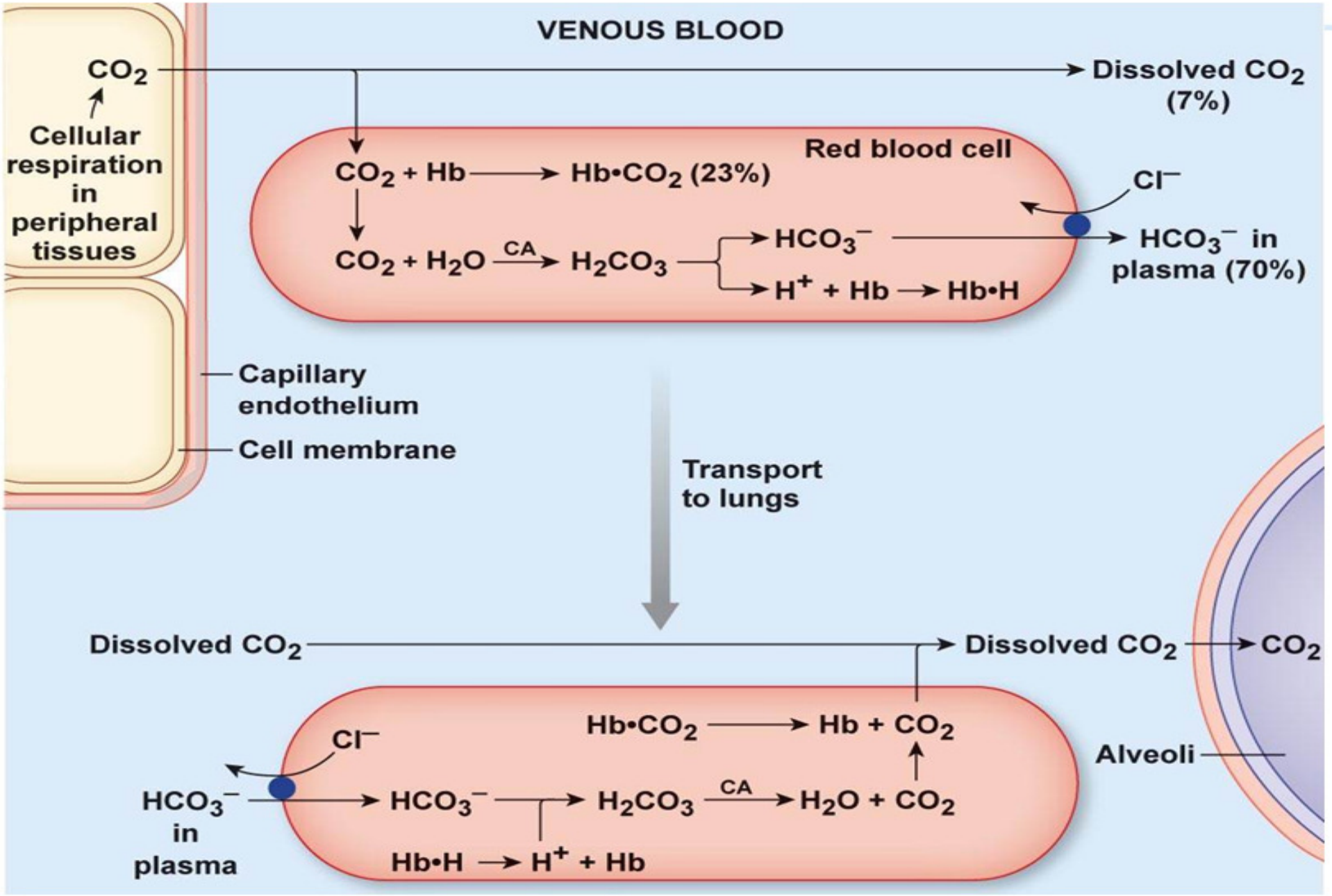
- pO_2 in Inspired air = 158 mmHg ;
- pO_2 in alveolar air = 100mmHg ;
- pO_2 in the blood in lungs = 90mmHg ;
- pO_2 in capillary bed = 40mmHg.
- In lung capillaries, oxygen is taken up by Hb.
- In tissues , oxygen is liberated from Hb.

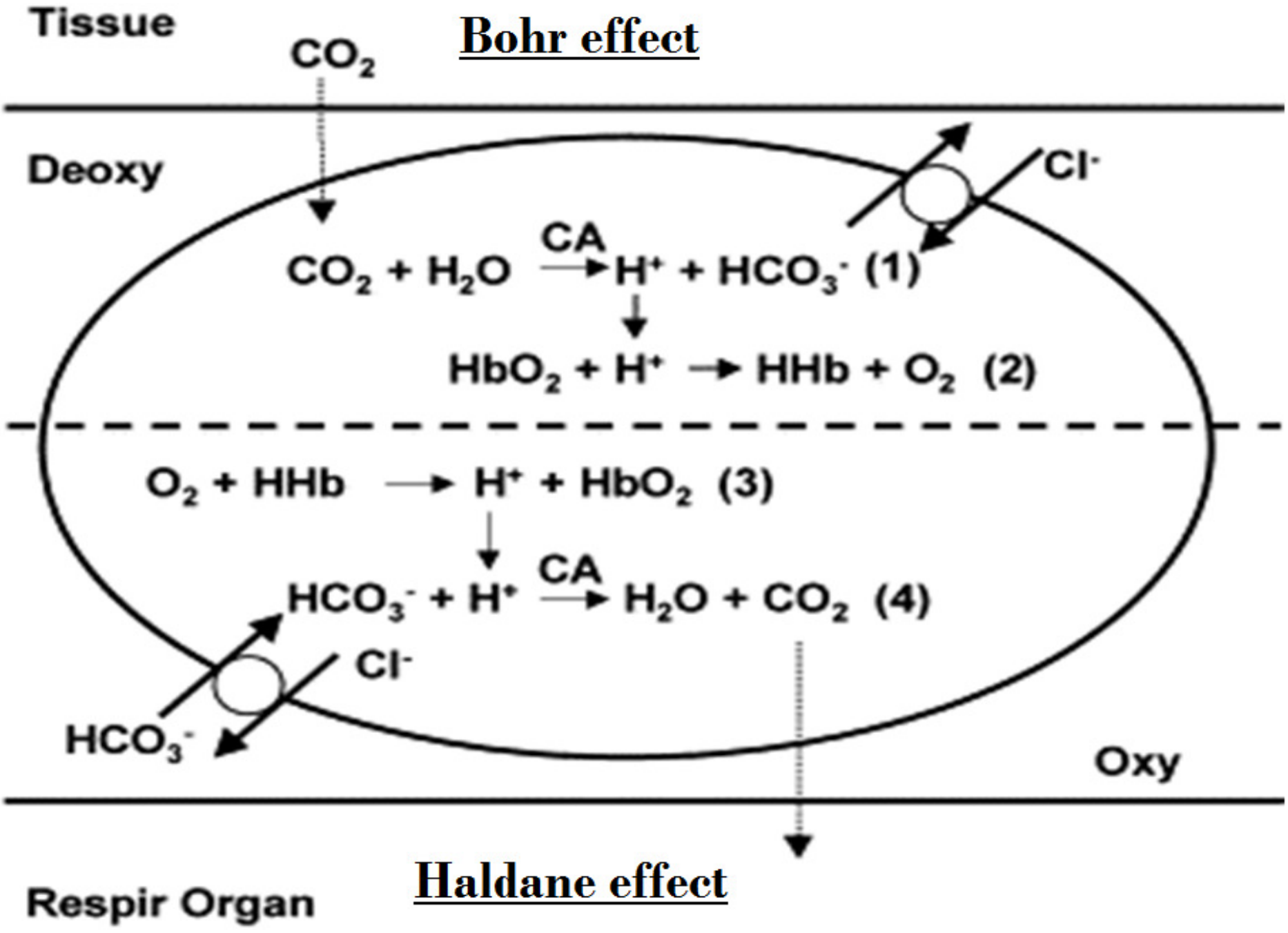
pO2 at Different Attitude

Table 1. **Barometric Pressure and Inspired Po_2 at Various Altitudes**

Altitude, *m (ft)*	Barometric Pressure, *mm Hg*	Inspired Po_2, *mm Hg (% of sea level)*
0 (0)	760	149 (100)
1000 (3281)	679	132 (89)
2000 (6562)	604	117 (79)
3000 (9843)	537	103 (69)
4000 (13 123)	475	90 (60)
5000 (16 404)	420	78 (52)
8848 (29 028)	253	43 (29)

Bohr & Haldane Effect

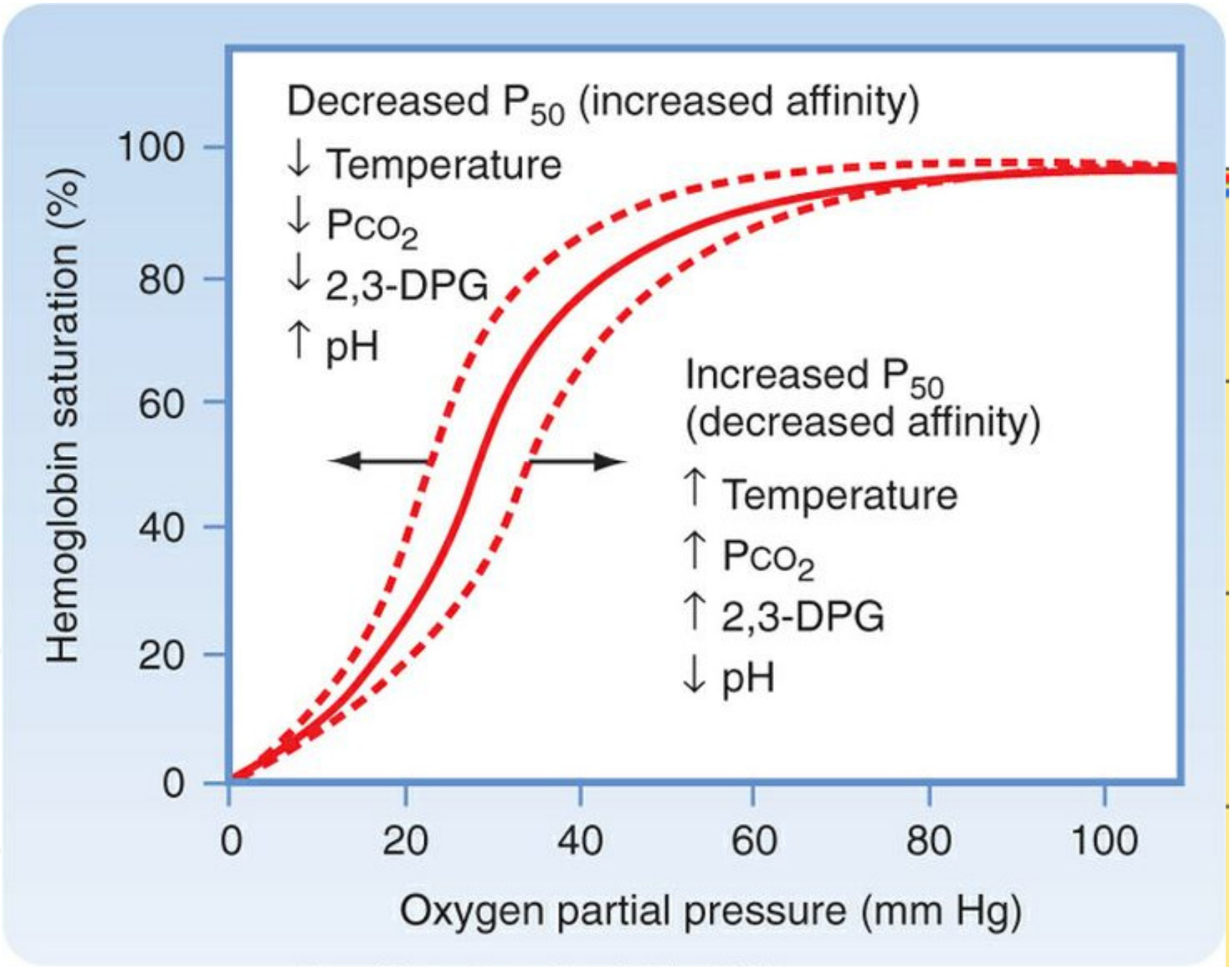



Tissue
CO_2
Bohr effect
Deoxy
Cl^-
$CO_2 + H_2O \xrightarrow{CA} H^+ + HCO_3^-$ (1)
$HbO_2 + H^+ \rightarrow HHb + O_2$ (2)
$O_2 + HHb \rightarrow H^+ + HbO_2$ (3)
$HCO_3^- + H^+ \xrightarrow{CA} H_2O + CO_2$ (4)
Cl^-
HCO_3^-
Oxy
Haldane effect
Respir Organ

Transport of CO2

Three ways:

1. As Carbamino-haemoglobin = 30 %
2. Free CO2 - In Plasma - Dissolved form = 10 %
3. As Bicarbonate form = 60%


Decreased P_{50} (increased affinity)
↓ Temperature
↓ P_{CO_2}
↓ 2,3-DPG
↑ pH
Increased P_{50}
(decreased affinity)
↑ Temperature
↑ P_{CO_2}
↑ 2,3-DPG
↓ pH
Hemoglobin saturation (%)
100
80
60
40
20
0
0
20
40
60
80
100
Oxygen partial pressure (mm Hg)

Clinical applications of 2 3 BPG & O2 Dissociattion Curve

- In hypoxic condition
  - O2 affinity is decreased with a shift in ODC to right
  - Increase in 2,3-BPG inside RBC.
  - Facilitate unloading of O2
- At high altitude
  - pO2 is low
  - Increased pulmonary ventilation
  - Polycythemia and increase in 2,3-BPG level
  - Increase O2 transport and unloading at tissue

Clinical applications of 2 3 BPG & O2 Dissociattion Curve

- In chronic pulmonary diseases and cyanotic cardiac diseases
  - Increase 2,3-BPG level
  - Ensuring maximum unloading of O2 to tissues.
- Transfusion of Large volumes of stored blood
  - Which has low level of 2,3-BPG
  - Lead to sudden hypoxia.

Fetal Hemoglobin (HbF)

- 2 alpha chains = 141 amino acids
- 2 gamma chains = 146 amino acids.
- Synthesis of HbF starts at 7th week of gestation.
- At birth 80% Hb is HbF.
- During the first 6 months of life it decreases to about 5% of total.
- **Physicochemical properties compare to HbA**
  - More solubility of deoxy-HbF
  - Slower electrophoretic mobility
  - Less interaction with 2,3-BPG.
  - More affinity to O2
- Remain elevated in children with
  - Anemia
  - Thalassemia

Haemoglobin Derivatives

- Carbaminohaemoglobin (CO2 + Hb)
- Carboxy Haemoglobin (CO + Hb)
- Met-Haemoglobin (Fe^{+2} converted to Fe^{+3})
- Sulf – Haemoglobin (Sulfur + Hb)

Colour of Different Haemoglobin Derivatives

- ✓ **Oxy-Hb = Dark red**
- ✓ **Deoxy-Hb = Purple**
- ✓ **Met-Hb = Dark brown**
- ✓ **CO-Hb = Cherry red**
- ✓ **Sulph-Hb = Green**

Carboxy-Hb (Carbon monoxy Hb) (CO-Hb):

- Hb binds with carbon monoxide(CO)
- Affinity of CO to Hb is 200 times more than for O2.
- Unsuitable for O2 transport. = O2 bind but it can not unloaded.
- CO poisoning is a major occupational hazard
  - workers in mines.
  - Breathing the automobile exhaust
- Normal people = 0.16%.
- Smoker = Additional 4%
- Clinical symptoms manifest when carboxy-Hb levels exceed 20%.
  - Breathlessness , Headache, Chest pain
  - At 40-60% saturation, death can result.
- Treatment = O2 under high pressure(hyperbaric O2)

Met-hemoglobin (Met-Hb)

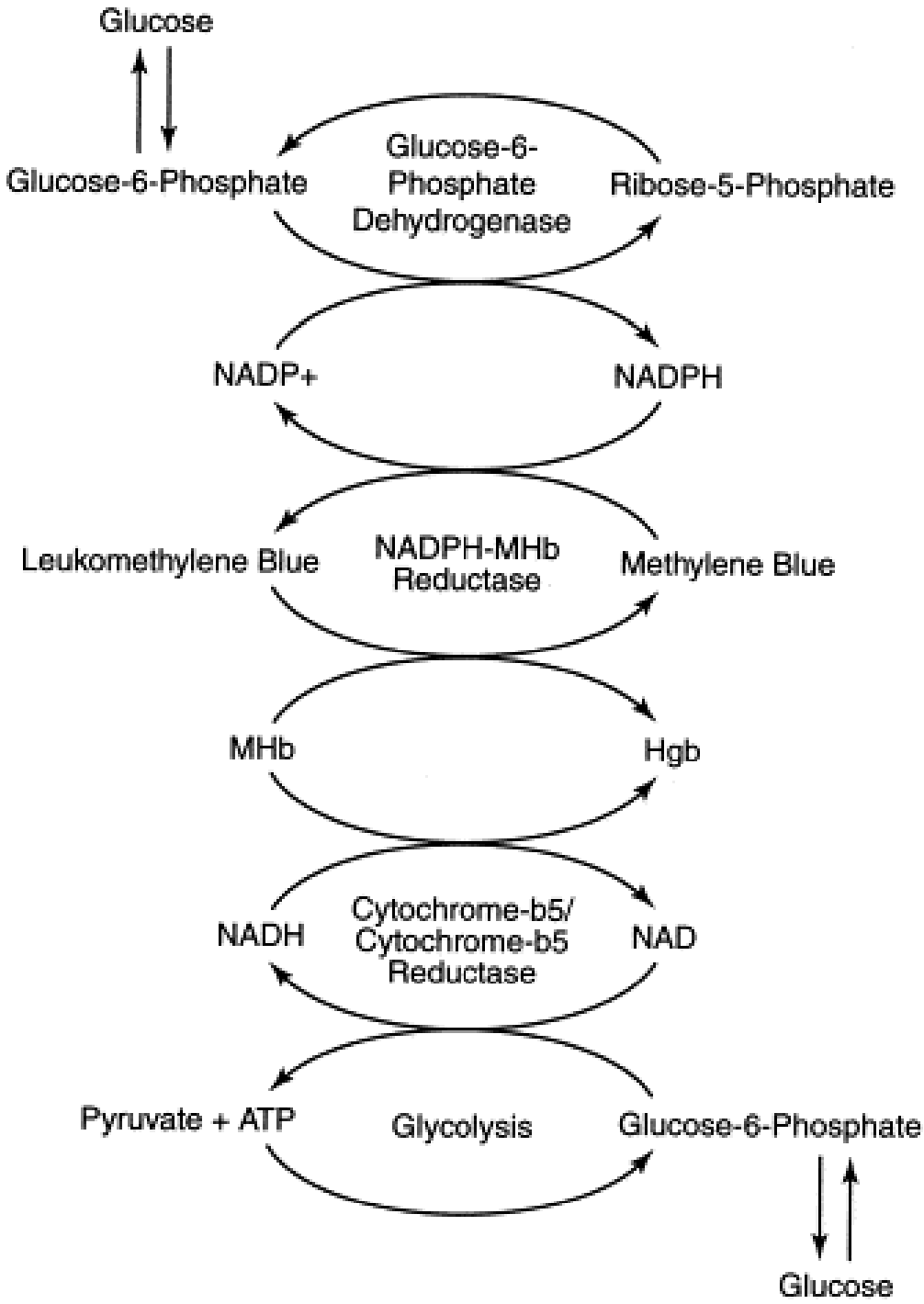
- Fe^{+2} (reduce)converted to Fe^{+3} (oxidized)
- Markedly decreased capacity for O2 binding and transport.
- Normal blood = 1% of met-Hb .
- Reducing activity is due to
  - Cytochrome b5
  - NADH (75%)
  - NADPH (20%)
  - Glutathione dependent Met-Hb reductase (5%)

Methemoglobinemia

Met-Haemoglobin = 10 – 15 %
Manifested as Cyanosis .

Methemoglobinemia

- Causes
  - Congenital
    - Cytochrome b5 reductase deficiency
  - Acquired.
    - Intake of water containing nitrites
    - Absorption of aniline dyes.
    - Drugs
      - Acetaminophen, Amyl nitrite , Sodium Nitroprusside.
    - G-6-PD deficiency with small doses of oxidizing drugs.
- Treatment
  - Methylene blue
  - Ascorbic acid

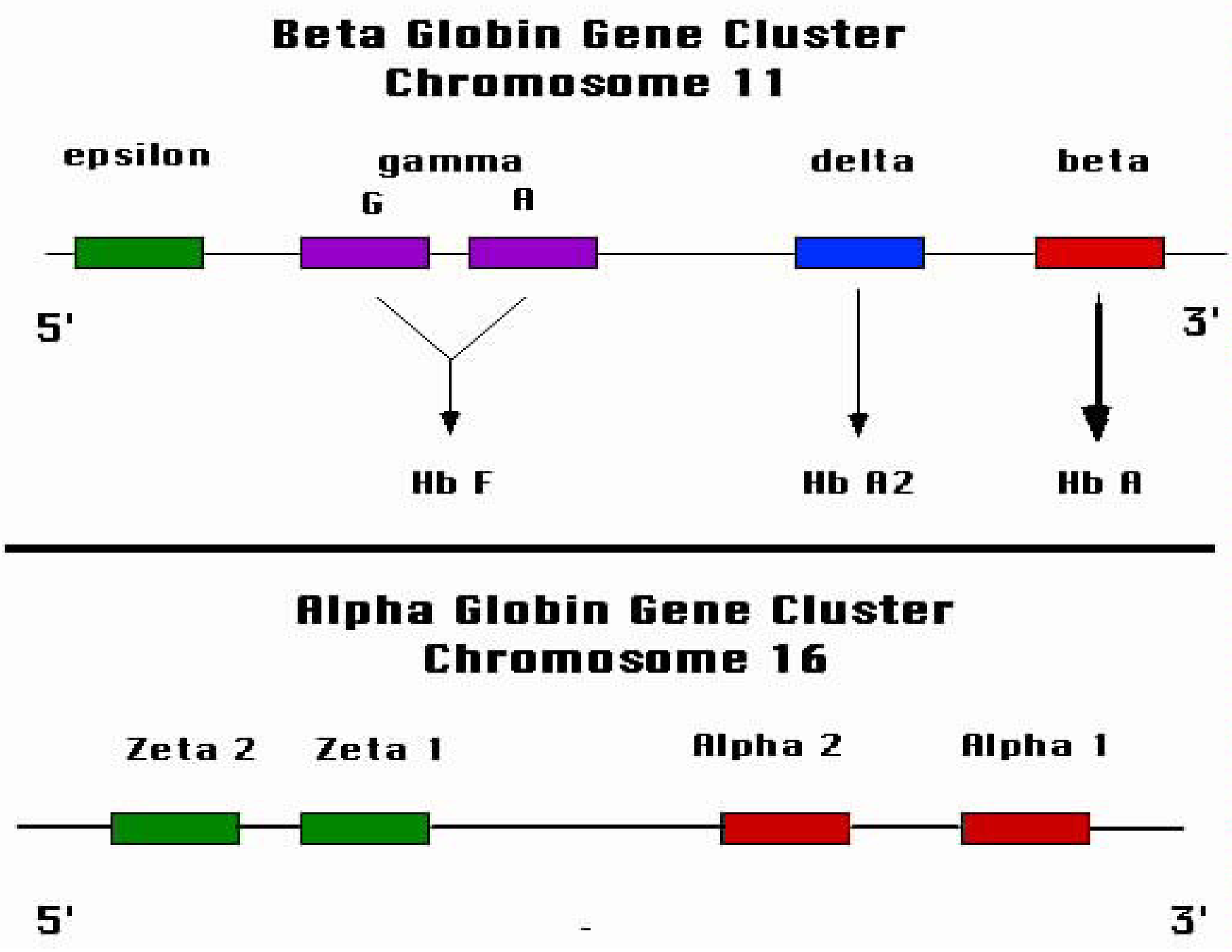

Glucose
Glucose-6-Phosphate
Glucose-6-Phosphate Dehydrogenase
Ribose-5-Phosphate
NADP+
NADPH
Leukomethylene Blue
NADPH-MHb Reductase
Methylene Blue
MHb
Hgb
NADH
Cytochrome-b5/ Cytochrome-b5 Reductase
NAD
Pyruvate + ATP
Glycolysis
Glucose-6-Phosphate
Glucose

Sulf-hemoglobinemia

- When hydrogen sulfide acts on oxy-Hb, sulf-hemoglobin is produced.
- Cause
  - Drugs
    - Sulphonamides, Dapson
- Treatment
  - No Specific treament require
  - RBC turn over reduce conc. Of sulf-Hb

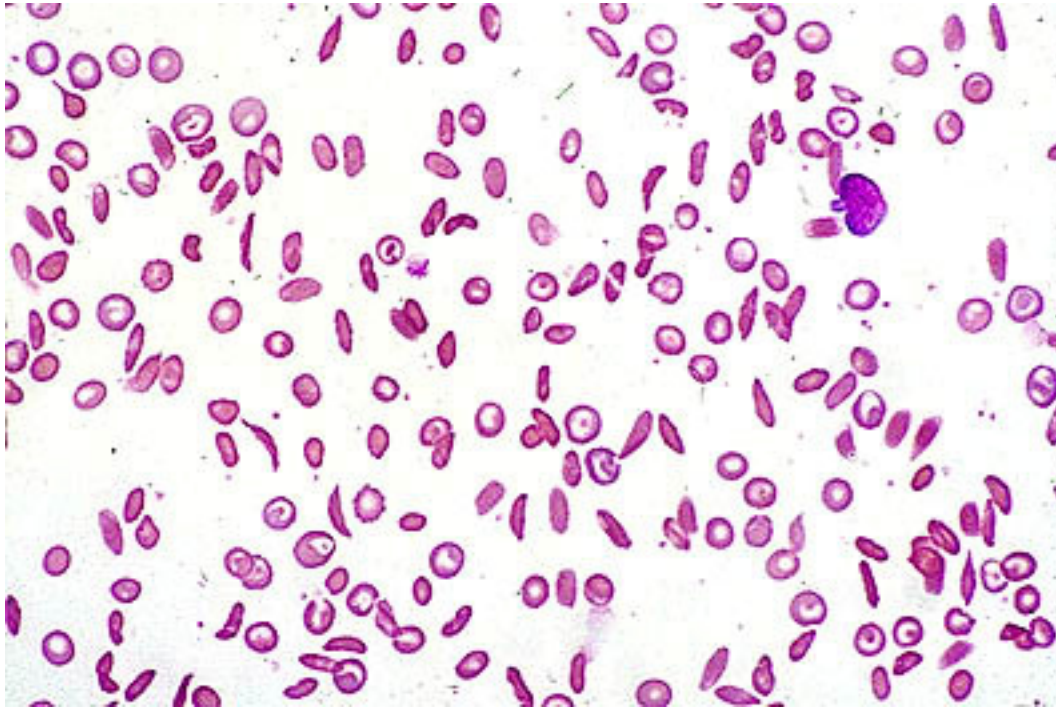
HEMOGLOBINOPATHIES

- Alpha chain genes = 2 Alletes = 16 no. chromosome
- Beta chain genes = 1 Alletes = 11 no. chromosome
- **Haemoglobinopathy = Chain Varient**

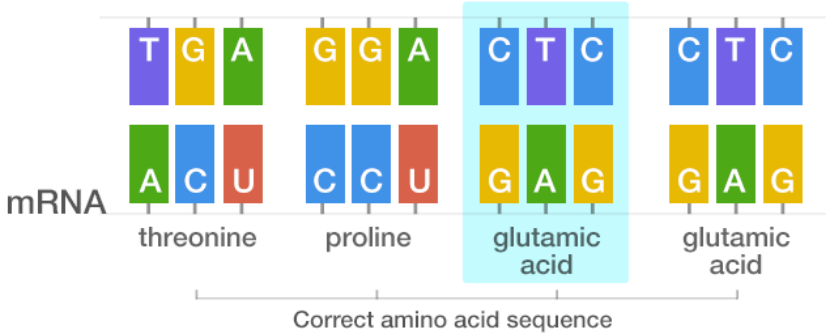

Beta Globin Gene Cluster
Chromosome 11
epsilon
gamma
G
A
delta
beta
5'
3'
Hb F
Hb A2
Hb A
Alpha Globin Gene Cluster
Chromosome 16
Zeta 2
Zeta 1
Alpha 2
Alpha 1
5'
3'

Sickle Cell Disease

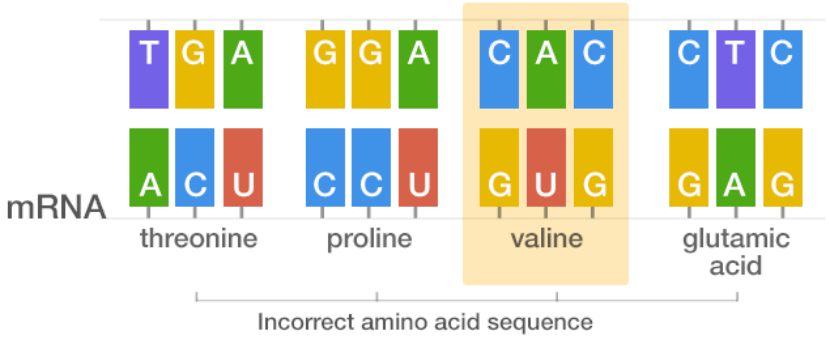
- 6th Position Glutamic acid of Beta Chain is replace by Valine
- Glutamic acid = Hydrophilic & Negative Charge
- Valine = Hydrophobic & Neutral Charge
- HbS can bind and transport O2.
- The sickling occur under deoxygenated state.
- The sickled cells form small plugs in capillaries and occlude of major vessels,lead to infarction in organs.



Normal DNA sequence (HbA)



Mutated DNA sequence (HbS)



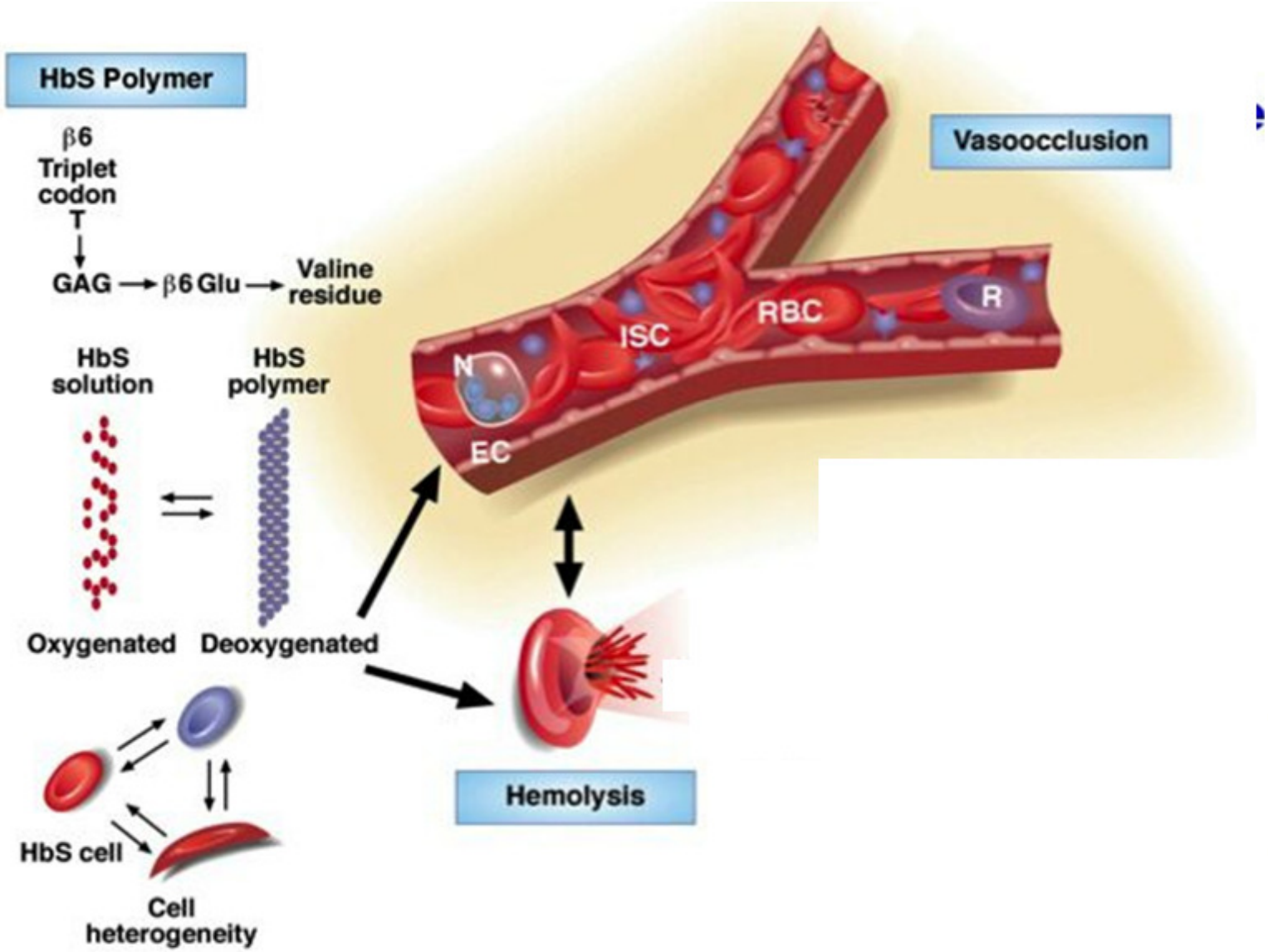
Sickle Cell Disease

- **Sickle cell trait - In heterozygous (AS)**
  - **50% of Hb in the RBC is abnormal.**
  - **50% of Hb in the RBC is normal.**
- Does not produce clinical symptoms.
- Hypoxia causes manifestration.
  - At higher altitudes
  - Chronic lung disorder

Sickle Cell Disease - Pathogenesis

- Hypoxia induce formation of deoxy –HbS
- Make polymerization of Hb
- Sickle Shape of RBC
- Turbulence & Occlusion of blood flow
- Small Capillary & End Arteries Affected
- Ischemia & Later Infarction to Distal Tissue
- Splenic Infarct & Avascular Necrosis of Femur Head

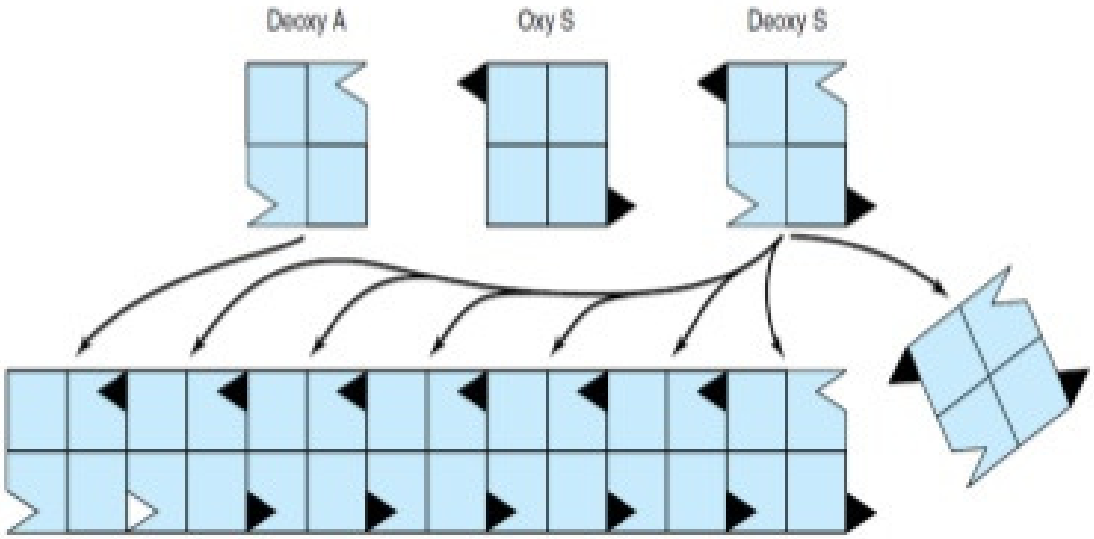
Sickle Cell Disease Pathogenesis



Sickle Cell Haemoglobin Polymerization

Polymerization of deoxy HbS

Sickling occurs under deoxygenated state



Sticky patch of 1 deoxyHbS binds with complementary site of another deoxy HbS leading to polymerization of deoxy HbS to form gelatinous network of long fibrous polymer– Distort shape of RBC – sickle shape.

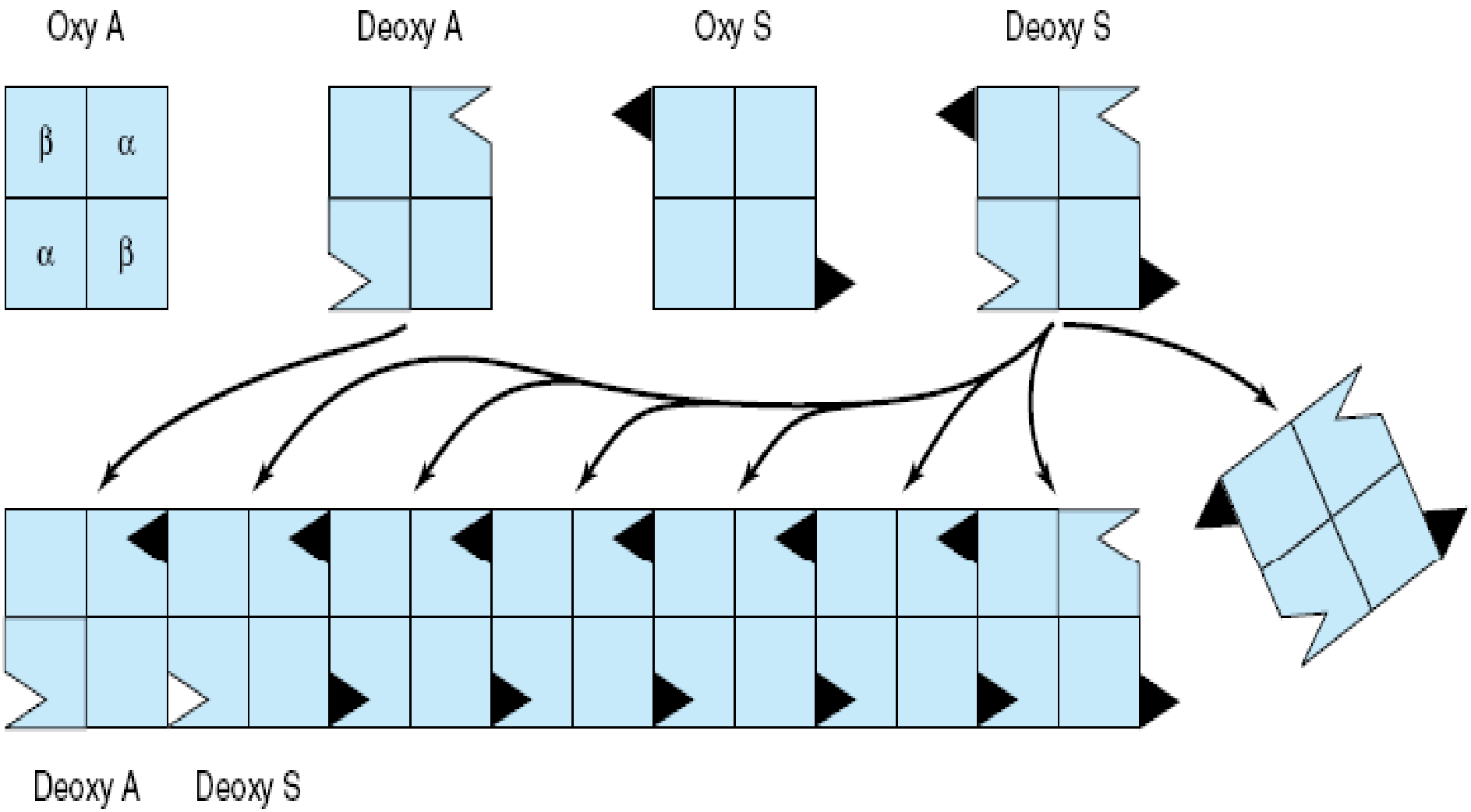


Figure 6–11. Representation of the sticky patch (▲) on hemoglobin S and its "receptor" (△) on deoxyhemoglobin A and deoxyhemoglobin S. The complementary surfaces allow deoxyhemoglobin S to polymerize into a fibrous structure, but the presence of deoxyhemoglobin A will terminate the polymerization by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

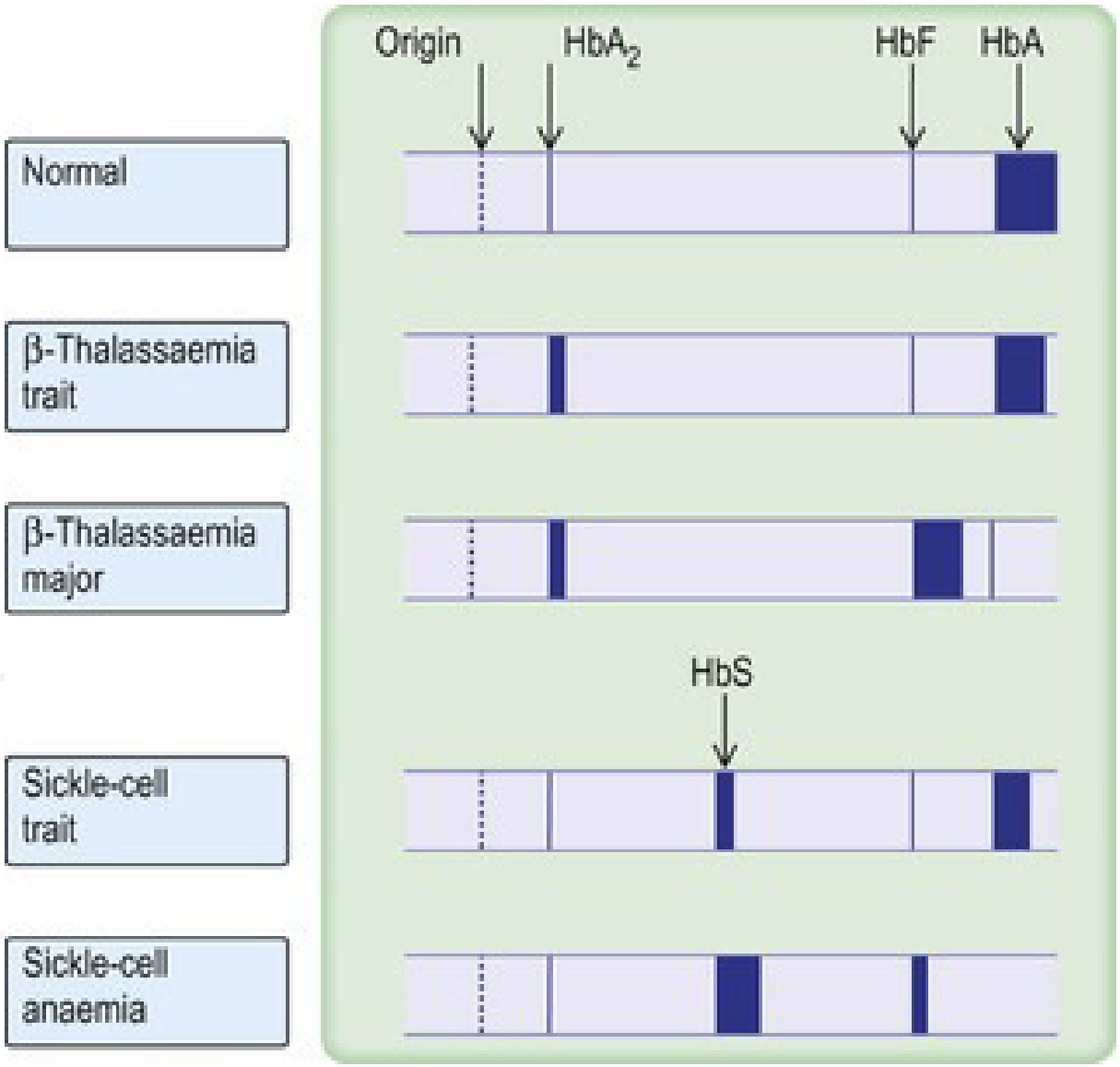
Sickle Cell Disease

Diagnosis

Electrophoresis:

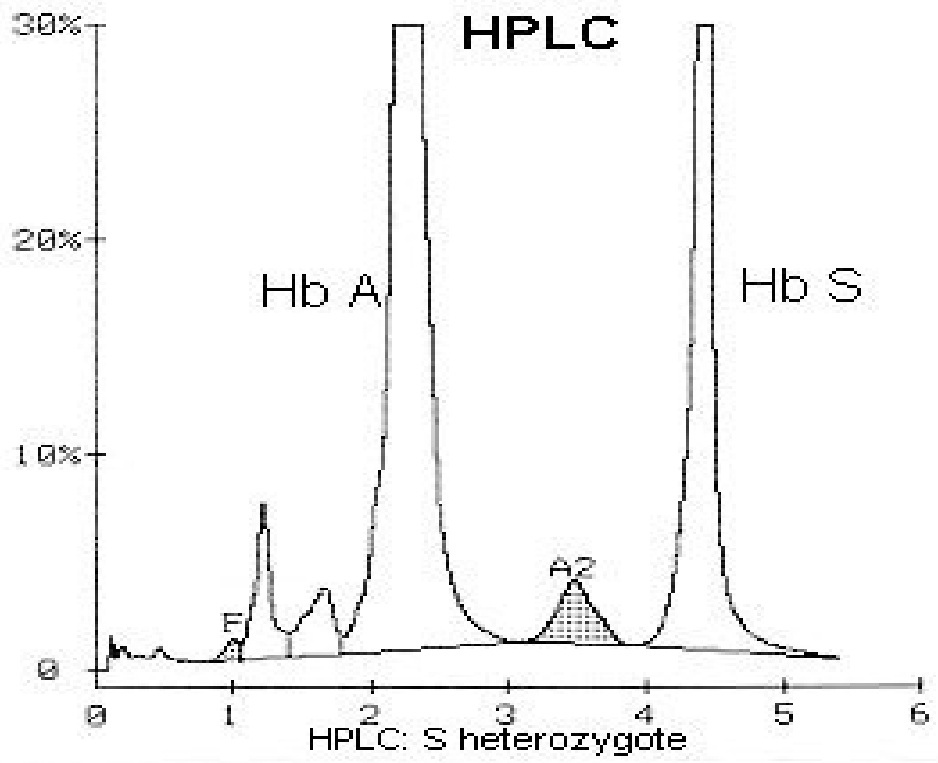
- Lack of Carboxyl group of Glutamic acid in HbS
- Lack of Negative charge Glutamic acid.
- HbS - less negatively charged
- Decreases electrophoretic mobility
- HbS move slower than HbA

Electrophoresis

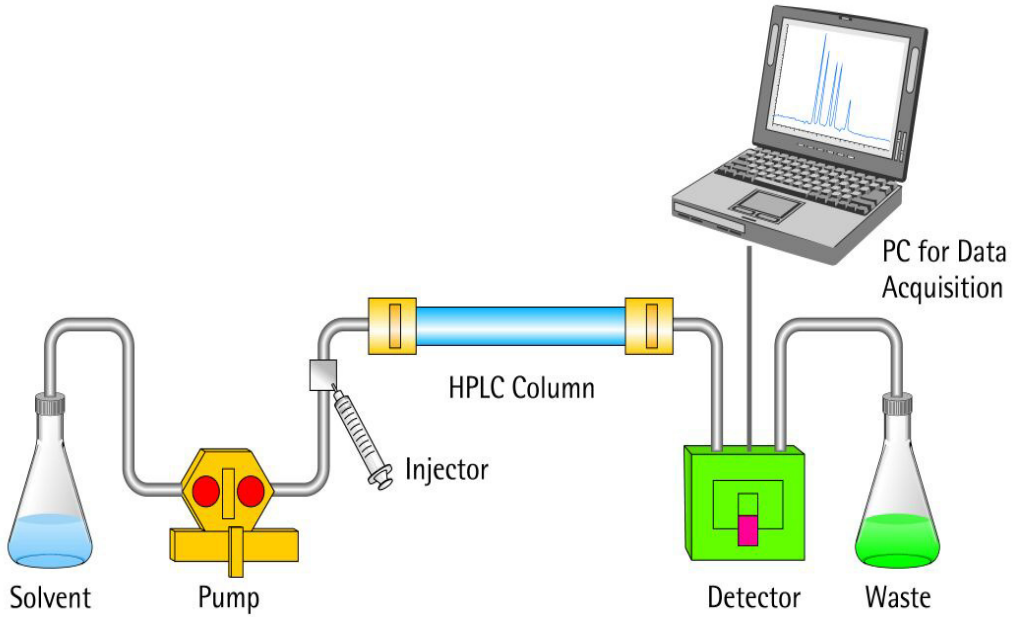


Dithionite test – Sickling Test

- Inexpensive & Rapid
- Use for Screening
- Less Sensitive
- The reagent consists
  - Saponin - Make RBC Haemolysis
  - Na-dithionite - Make Hb deoxygenates
- Principle :
- Reagent make Hb deoxygenated and causes polymerazition of HbS and Turbidity of Sample



High Performance Liquid Chromatography (HPLC)



Sickle Cell Disease - Treatment

- **Hydroxyurea**
  - Induce gene for gamma globin chain
  - 5 to 10 % fetal Hb synthesis
  - Interfere with polymerization of deoxy HbS
  - Prevent crisis and improve oxygenation
- **Oxygenation**
  - Decrease concentration of deoxygenated Hb
  - Decrease in polymerization & Decrease lysis of RBC
- **Hydration**
  - Increase in body fluid
  - Increase in circulation
  - Increase in oxygenation & Decrease polymerization
  - Decrease in lysis of RBC

HbS gives protection against malaria:

Important hemoglobinopathies

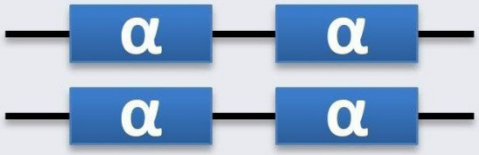
Hb	Point mutation position	Amino acid substitution	Codon and base substitution
HbS	Beta 6	Glu→Val	GAG→GUG
HbC	Beta 6	Glu→Lys	GAG→AAG
HbE	Beta 26	Glu→Lys	GAG →AAG
HbD	Beta 121	Glu→Gln	GAG →CAG
HbsM	Proximal or distal histidine in α or β chains	His→Tyr	CAC →UAC

THALASSEMIAS

- **α- thalassemia**
  - Deficiency of α chain
  - Increase synthesis of
    - **β chain – β tetramer = HbH (α thalassemia intermediate)**
    - **γ chain – γ tetramer = Hb Bart (α thalassemia major)**
- **β thalassemia**
  - Reduce function of β chain due to mutation in it's gene.
  - Deficiency of β chain
  - Increase synthesis of
    - **γ chain = α + γ tetramer = Increase Hb F**
    - **δ chain = α + δ tetramer = Increase Hb A2**

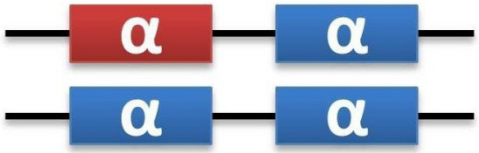
Alpha-thalassemia Genetics

Normal



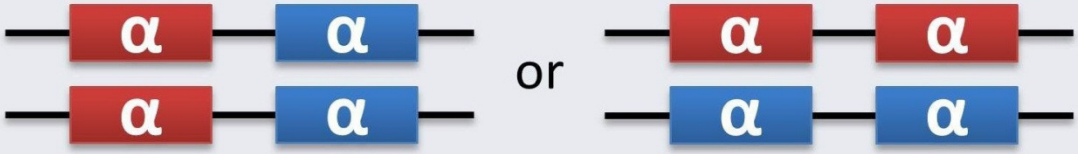
Alpha Thalassemia Carrier

Carrier: Asymptomatic
No abnormalities



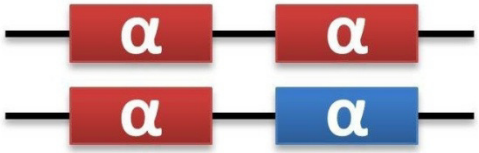
Alpha Thalassemia Minor / Trait

Asymptomatic



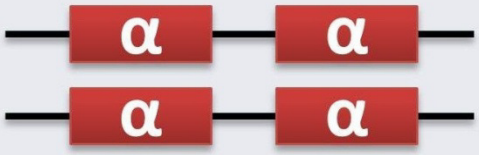
Alpha Thalassemia Intermideate

Hb H Disease: Symptomatic



Alpha Thalassemia Major

Incompatible with Life
Hydrops Fetalis = Hb Bart



β – Thalassemia Genotype

	Genotype	Clinical Feature
β – Thalassemia Minor	β / β+ or β / β0	Heterozygous Asymptomatic Mild Microcystic anaemia
β – Thalassemia Intermediate	β+ / β+ or β+ / β0	Mild Symptomatic Manage normal life
β – Thalassemia Major	β0 / β0	Homozygous Severely Symptomatic Severe Hypochromic Microcystic anaemia
β+	Mutation in β chain gene β chain synthesized But •Reduce function of β chain •Partial function of β chain is conserved	
β0	Mutation in β chain gene β chain does not synthesized	

Disease - Pathogenesis

- **Decrease amount of alpha / beta chain formation**

1. **Decrease Haemoglobin = Severe Anaemia**
   - ✓ **<u>More positive feedback to Bone marrow</u>**
     - Bone marrow Hypertrophy = Bulging of Facial bone
     - Cortical Thinning = Banding of weight bearing bone
   - ✓ **<u>Reticular Endothelial System-Organ hypertrophy</u>**
     - Hepatomegaly
2. **More amount of abnormal Hb (HbH & Hb Bart)**
   - ✓ **<u>More haemolysis of RBC</u>**
     - Spleenomegaly & Jaundice

Pathogenesis Due to Treatment

Frequent Blood Transfusion

- Increase Iron Overload
  - Hemosiderosis / Hemochromatosis
  - Liver cirrhosis
  - Cardiomyopathy – Severe Systolic Dysfuntion
    - Main cause of death of thalassemia patient
  - Chelating agent is given to prevent iron overload
- Increase chances of infection like - HIV,HBsAg

Bone marrow transplantation

Clinical Feature of Thalassemia due to Pathology

- Severe Anaemia
- Jaundice
- Stunted growth
- Frontal Bossing
- Maxillary hypertrophy
- Zygomatic process prominent
- Depression of nasal bridge
- Osteoporosis in all the bones
- Huge Hepato-Splenomegaly

MYOGLOBIN (Mb)

- It is seen in muscles.
- Single polypeptide chain
- One molecule of Mb combine with 1 O2.
- Mb has higher affinity for O2 than that of Hb.
- The pO2 in tissue is about 30 mmHg
  - Mb is 90% saturated.
  - Hb is 50% saturation.
- In severe physical exercise, pO2 in muscles lowers to 5 mmHg, when myoglobin releases all the bound O2.

Definition of Anemia

- Decrease in RBC mass
- Deficiency in the oxygen-carrying capacity of the blood due to a diminished erythrocyte mass.
- May be due to:
  1. **Erythrocyte loss**
  2. **Decreased Erythrocyte production**
  3. **Increased Erythrocyte destruction**

Type of Anaemia

	Men	**Women**
Normal	14 – 17.5 gm%	12.5 – 15.5 gm %
Mild Anaemia	Up to 11 gm %	
Moderate Anaemia	8 to 11 gm%	
Severe Anaemia	Less than 8 gm%	

Cause of Anaemia

1. Decrease Production
2. Increase destruction
3. Loss of Blood

Cause of Decrease Production of RBC

- **Nutritional deficiency**
  - Iron deficiency
  - Folic acid deficiency
  - Vitamin B12 deficiency
- **Genetic defect (defective chain synthesis)**
  - Thalalssemia
  - Sickle Cell anemia
- **Bone Marrow defect**
  - Aplastic anemia
  - Bone marrow depression
  - Myelodysplastic anemia
- **Renal Failure** – Decrease erythropoien production
- **Inhibiton of Heme Synthesis**
  - Lead Poisoning – Petrochemical Occuption
  - Congenital erythropoietic porphyria

Cause of Increase Destruction of RBC

- **Intrinsic abnormalities**
  - •paroxysmal nocturnal hemoglobinuria
  - •Hereditary spherocytosis
  - •Hereditary elliptocytosis
- **Enzyme deficiencies**
  - •Pyruvate kinase & hexokinase deficiencies
  - •G-6-PD deficiency
- **Hemoglobinopathies**
  - •Sickle cell anemia
  - •Thalassemia
- **Infections**
  - •Malaria
- **Extrinsic abnormalities**
  - •Blood Transfusion reaction
  - •Erythoblastic fetalis
  - •hemolytic disease of the newborn
  - •Autoimmune hemolytic
  - •Systemic Lupus Erythematosus
  - •Chronic lymphocytic leukemia
- **Drugs Induce**
  - • Aspirin
  - • Quinine

Cause of Increase Loss of RBC (Blood)

- **Polytrauma**
- **Post Major Surgery**
- **Internal Hemorrhage**
  - Haematemesis - Malena
    - Portal Hypertension – Cirrhosis of Liver
    - Peptic ulcer
    - Inflammatory Bowel Disease
  - Haemoptysis
    - Lung malignancy
    - Tuberculosis
  - Haematuria
    - Renal Malignancy
    - Renal Stone
- **Menorrhagia**