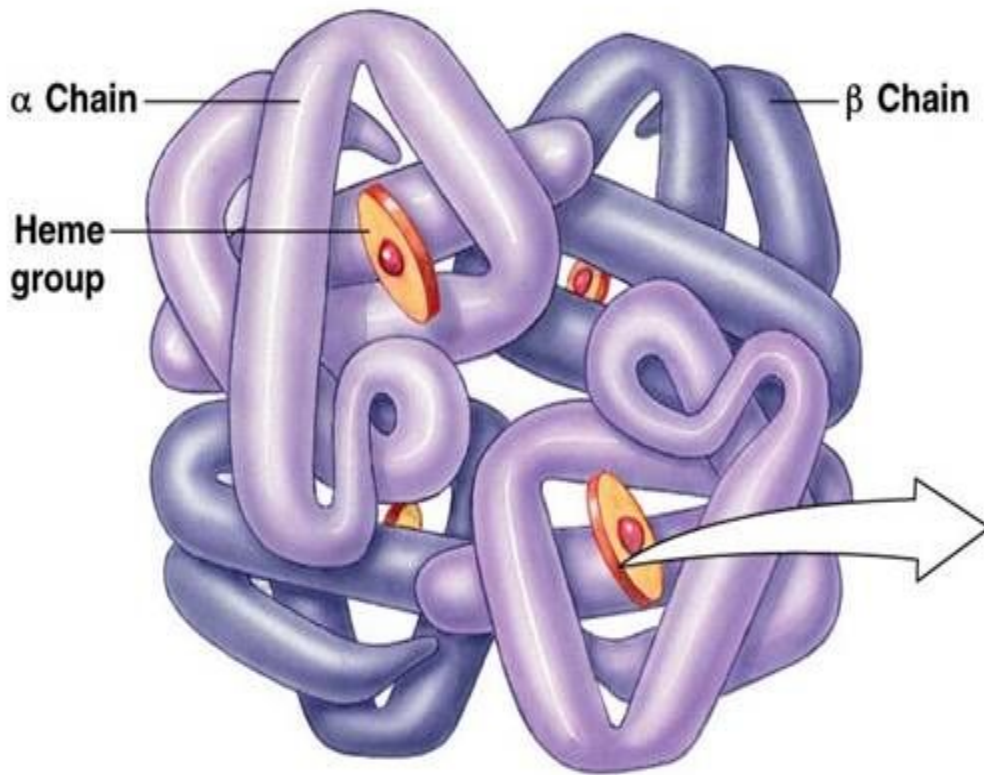


# Hemoglobinopathies

- Hemoglobinopathies
- Thalassemia genetics
- Hb synthesis
- Hb A, A<sub>2</sub>, F
- Hb ELP
- Hb Constant-Spring
- Hb Bart's
- Hb H
- Hb Lepore
- Hb E
- Hb S
- Hb C
- Hb SC disease
- HPFH

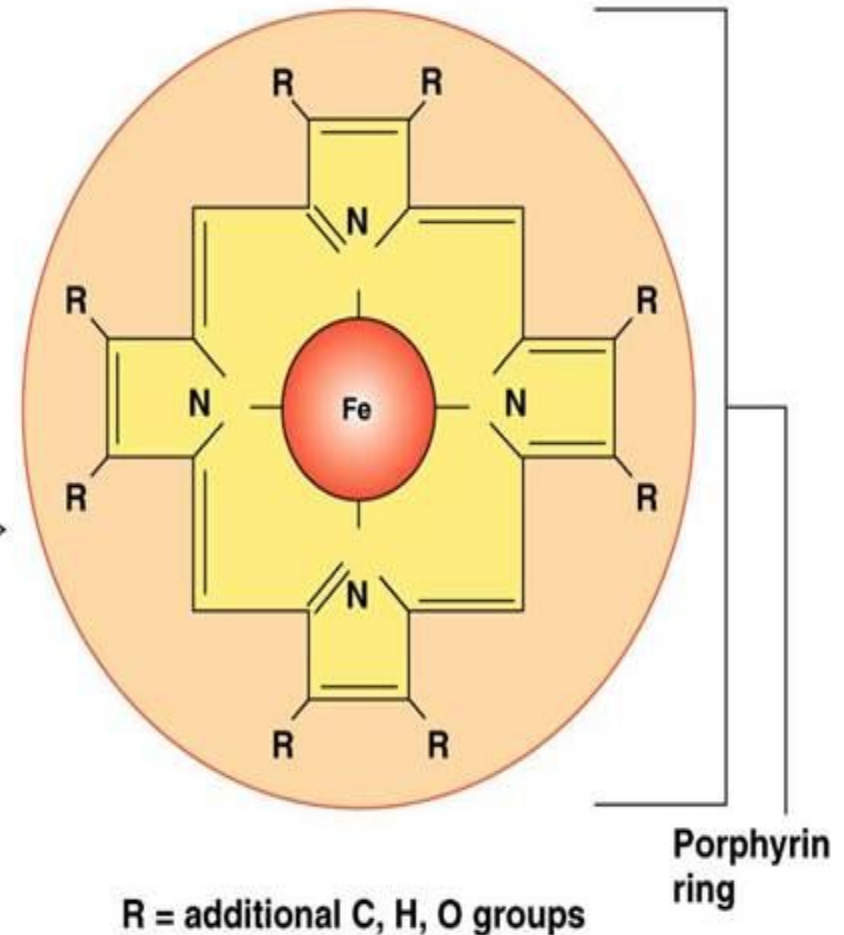
# Hb structure

**(a)** A hemoglobin molecule is composed of four protein globin chains, each surrounding a central heme group.



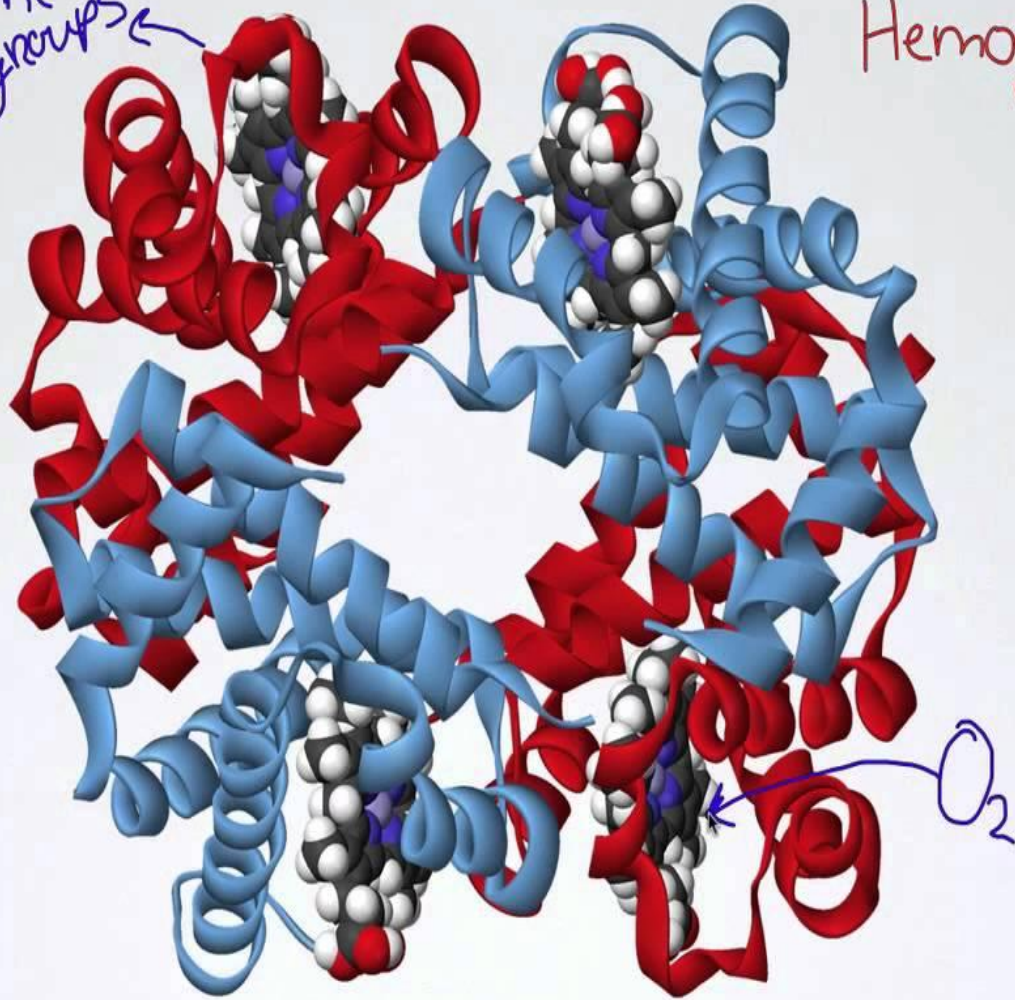
In most adult hemoglobin, there are two alpha chains and two beta chains as shown.

**(b)** Each heme group consists of a porphyrin ring with an iron atom in the center.

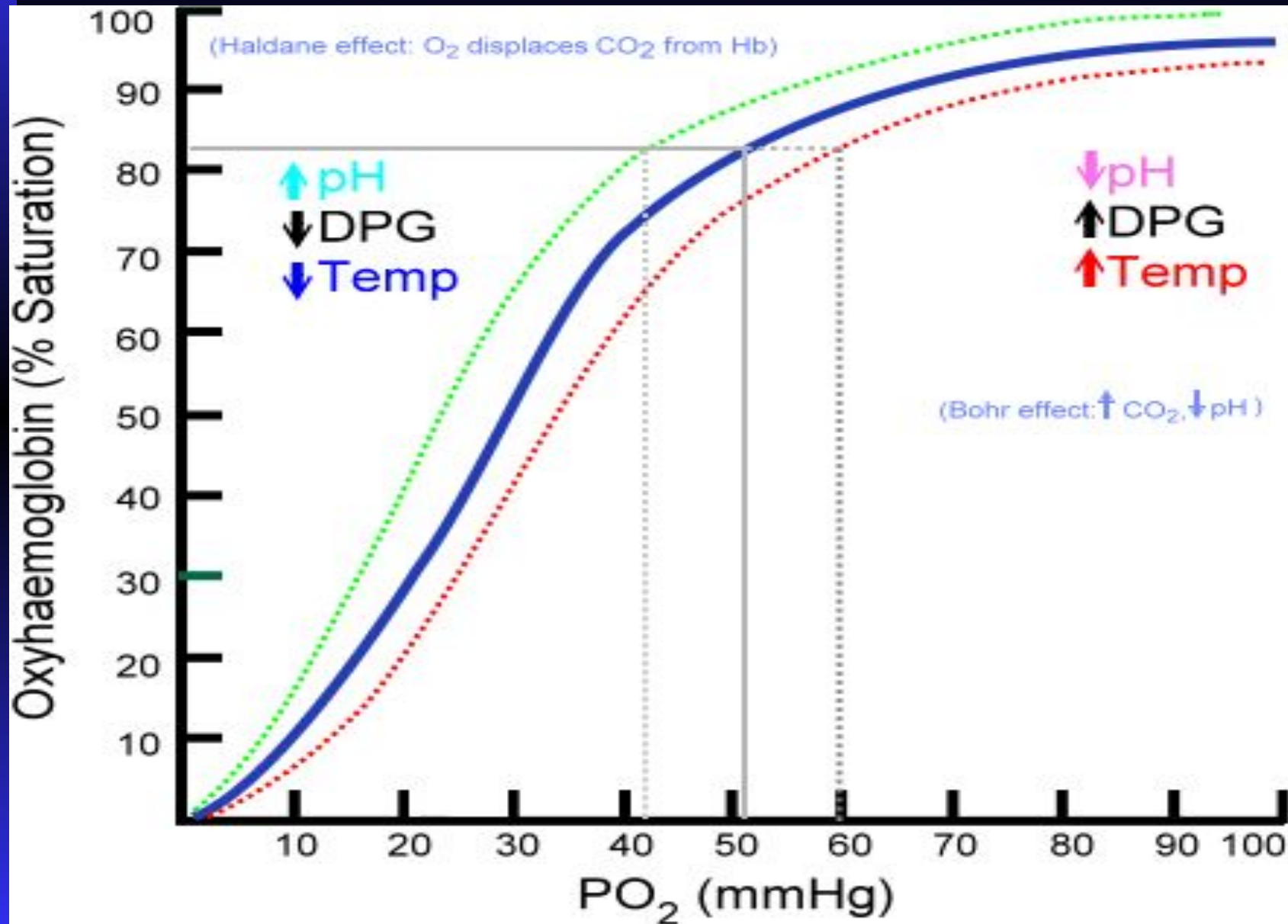


Heme  
Groups

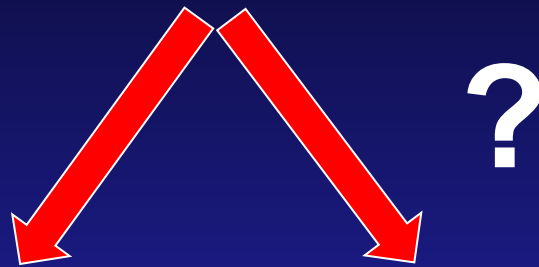
Hemoglobin



# Hb dissociation curve



# Anemia



**Production?**

**Survival/Destruction?**

**The key test is the .....**

# The reticulocyte count (kinetic approach)

- Increased reticulocytes (greater than 2-3% or  $100,000/\text{mm}^3$  total) are seen in blood loss and hemolytic processes, although up to 25% of hemolytic anemias will present with a normal reticulocyte count due to immune destruction of red cell precursors.
- Retic counts are most helpful if extremely low ( $<0.1\%$ ) or greater than 3% ( $100,000/\text{mm}^3$  total).

# Causes of Anemia

- Decreased erythrocyte production
  - Decreased erythropoietin production
  - Inadequate marrow response to erythropoietin
- Erythrocyte loss
  - Hemorrhage
  - Hemolysis



# Morphological Approach (big versus little)

## **First, measure the size of the RBCs:**

- Use of volume-sensitive automated blood cell counters, such as the Coulter counter. The RBC's pass through a small aperture and generate a signal directly proportional to their volume.
- Other automated counters measure red blood cell volume by means of techniques that measure refracted, diffracted, or scattered light
- By calculation



# Underproduction macrocytic

## MCV > 115

- B12, Folate
- Drugs that impair DNA synthesis (AZT, chemo)
- MDS

## MCV 100 - 115

- Endocrinopathy (hypothyroidism)
- Erythropoietin ↓
- Reticulocytosis

# Underproduction

## Normocytic

- Anemia of chronic disease
- Mixed deficiencies
- Renal failure
- MM, Lymphoma

## Microcytic

- Iron deficiency
- Thalassemia
- Anemia of chronic disease (30-40%)
- Sideroblastic anemias

# Review red blood cell disorders

## Marrow production

- Thalassemias
- Myelodysplasia
- Myelophthisic
- Aplastic anemia
- Nutritional deficiencies

## Red cell destruction

- Hemoglobinopathies
- Enzymopathies
- Membrane disorders
- Autoimmune

# Review red blood cell disorders

## *Marrow Production - Aplastic Anemia*

- Acquired
  - ◆ Immunological
  - ◆ Toxins – Benzene
  - ◆ Drugs – methotrexate, chloramphenicol
  - ◆ Viruses – EBV, hepatitis
- Hereditary
  - ◆ Fanconi,
  - ◆ Diamond-Shwachman

# Review red blood cell disorders

## *Marrow Production - Myelodysplasia*

- Preleukemia, most commonly in the elderly.
- Supportive care that involves transfusion therapy is an option.
- Poor response to growth factors

# Review red blood cell disorders

## *Marrow Production - Myelophthisic*

- Anemia associated with marrow infiltration
- “teardrops”
- Cancer, infections
- Myelofibrosis
- Treatment is aimed at the underlying disease
- Supportive transfusions as needed.

# Review red blood cell disorders

## *Red cell destruction*

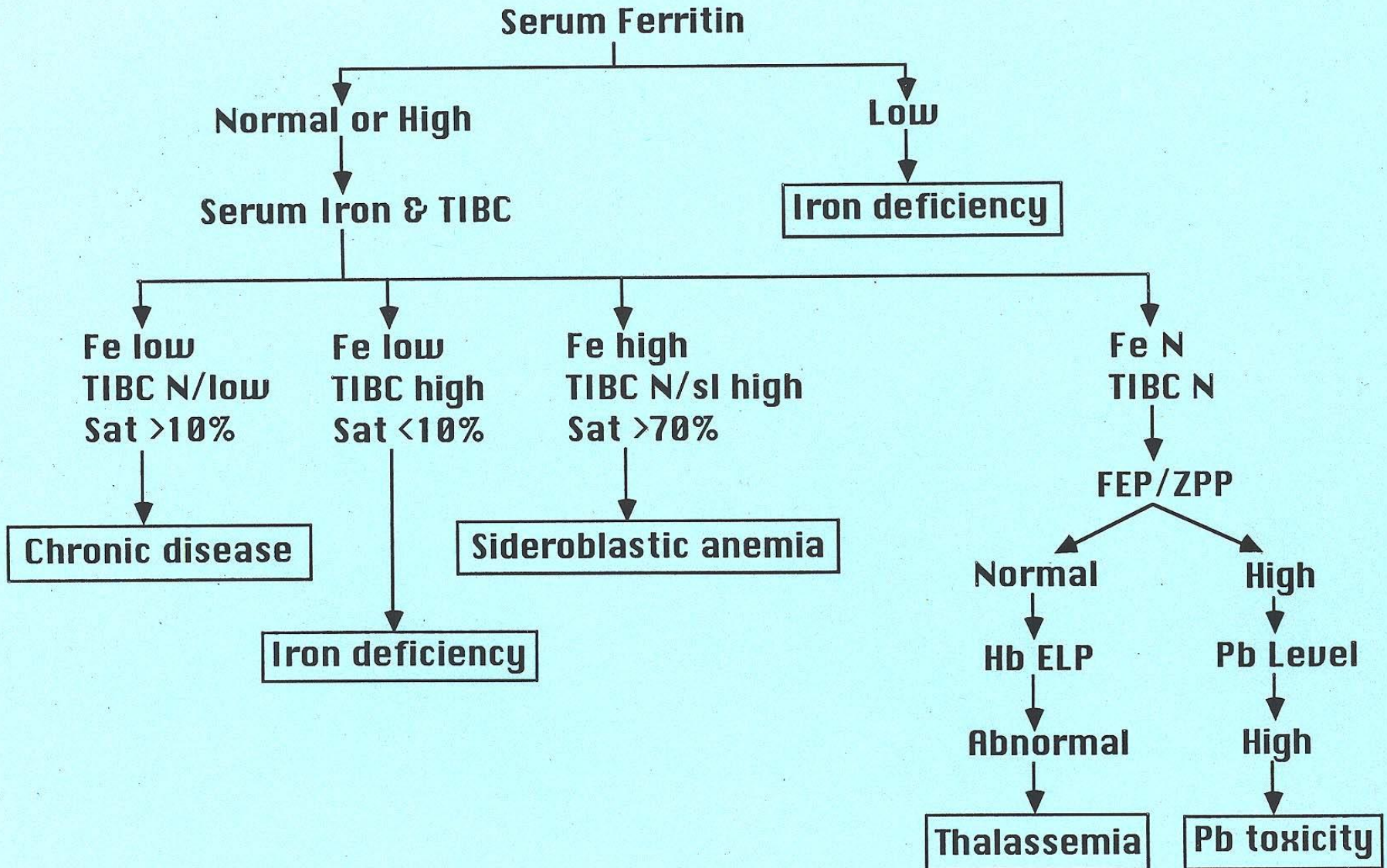
- Elevated reticulocyte count
- Mechanical
- Autoimmune
- Drug
- Congenital



# Hb Problems

- Heme production problem: porphyria
- Fe incorporation into Heme: Sideroblastic anemia
- Fe<sup>++</sup> problems: IDA, hemochromatosis
- Globin problem: sickle cell disease, thalassemia

# MICROCYTIC HYPOCHROMIC ANEMIA



# Hemoglobinopathies

- Decrease, lack of, or abnormal globin
- May be severe hemolytic anemia
- Abnormal Hb with low functionality
- Mutation may be deletion, substitution, elongation
- Hb electrophoresis may be helpful

# Hemoglobin

- Heme

- ◆ Porphyrin ring and Fe

- Globins

- ◆ Alpha family on chromosome 16

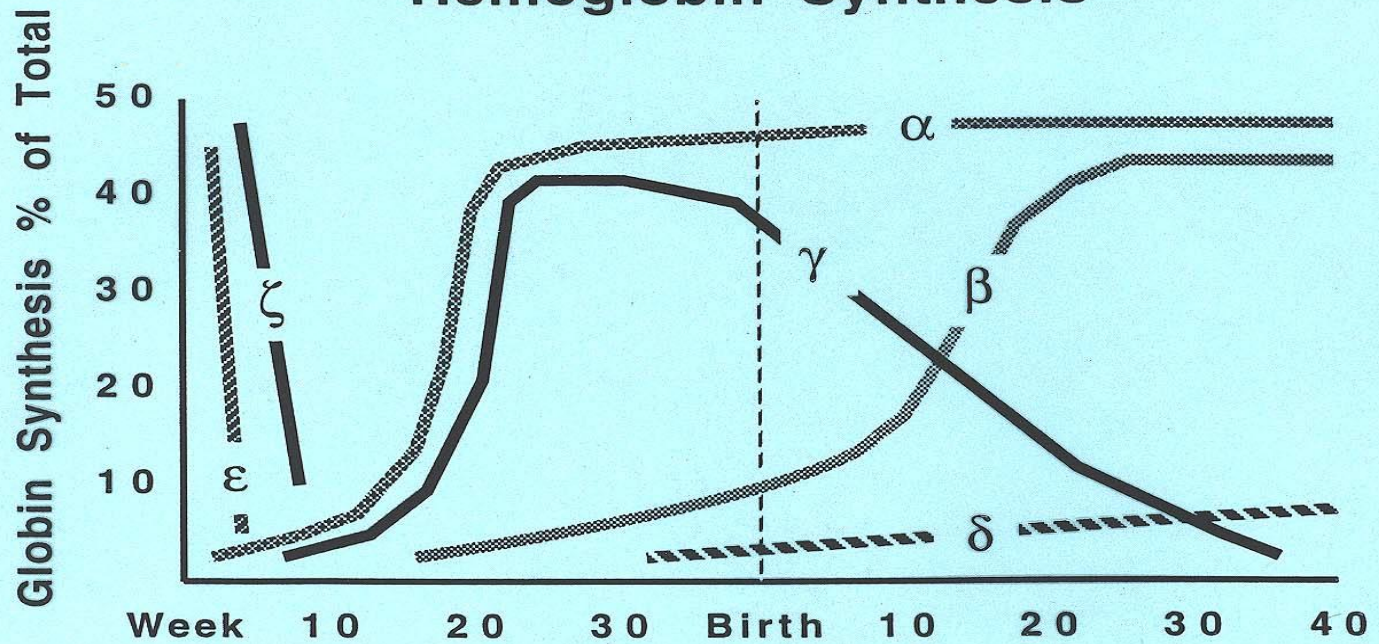
--[ζ]--//--[α2]--[α1]--

- ◆ Beta family on chromosome 11

--[ε]--//--[γ]--[γ]--[δ]--[β]--



# Hemoglobin Synthesis



		<u>Adult</u>	<u>Newborn</u>
$\alpha_2\beta_2$	Hb A	97 %	20 %
$\alpha_2\delta_2$	Hb A2	2.5	<0.5
$\alpha_2\gamma_2$	Hb F	<1	80

## Embryonic:

- $\zeta_2\varepsilon_2$  Gower-1
- $\alpha_2\varepsilon_2$  Gower-2
- $\zeta_2\gamma_2$  Portland

# Thalassemia

- Genetic defect in hemoglobin synthesis
  - ◆ ↓ synthesis of one of the 2 globin chains ( $\alpha$  or  $\beta$ )
  - ◆ Imbalance of globin chain synthesis leads to depression of hemoglobin production and precipitation of excess globin (toxic)
  - ◆ “Ineffective erythropoiesis”
  - ◆ Ranges in severity from asymptomatic to incompatible with life (hydrops fetalis)
  - ◆ Found in people of African, Asian, and Mediterranean heritage

# Thalassemia

- 1925: Described by Dr. Thomas Cooley and Dr. Pearl Lee of Detroit
- 1920's: Osmotic fragility test
- 1932: Dr. George Whipple of Rochester coined the name "thalassa anemia" from Greek story about Xenophon's army returning from Persia
- 1930's: Familial pattern recognized
- 1950's: Alkali denaturation test for Hb F, Hb ELP
- 1956: Coulter model A
- 1960's: RBC indices
- 1980's: Histogram, DNA analysis, PCR



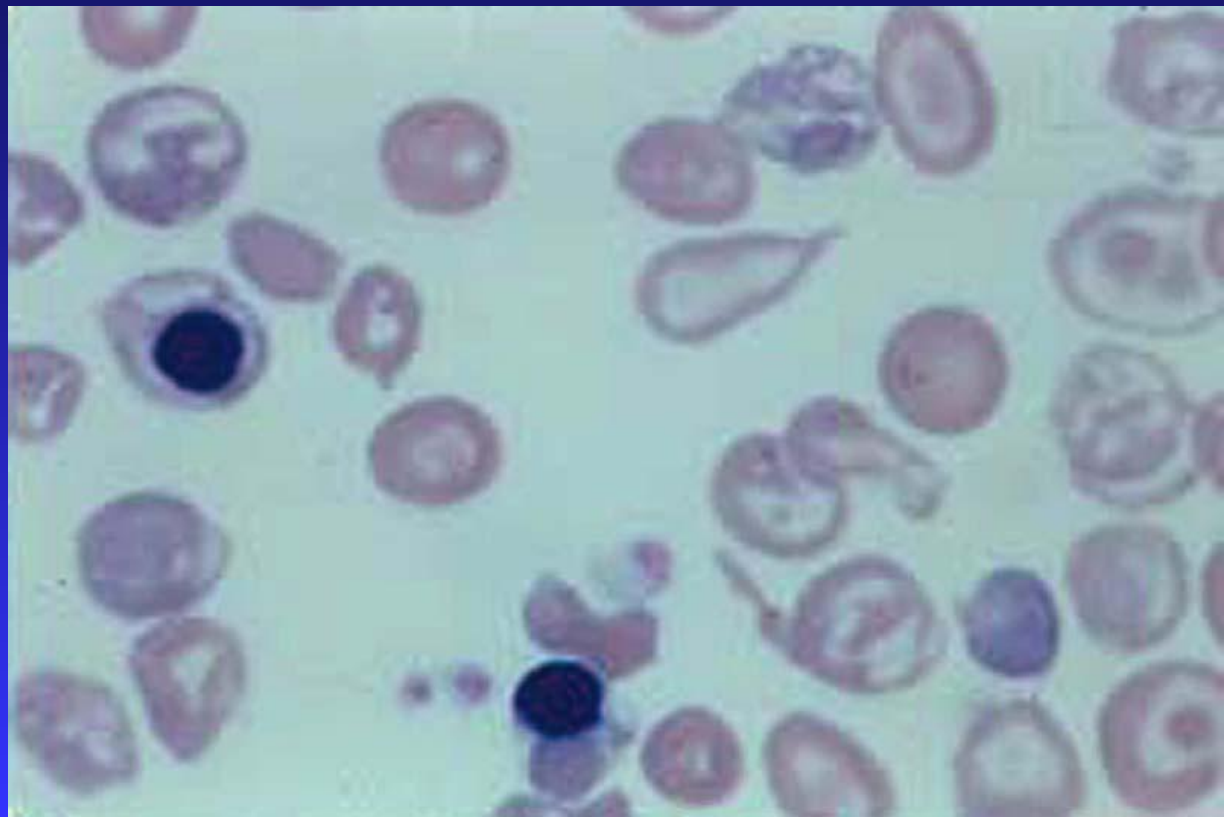
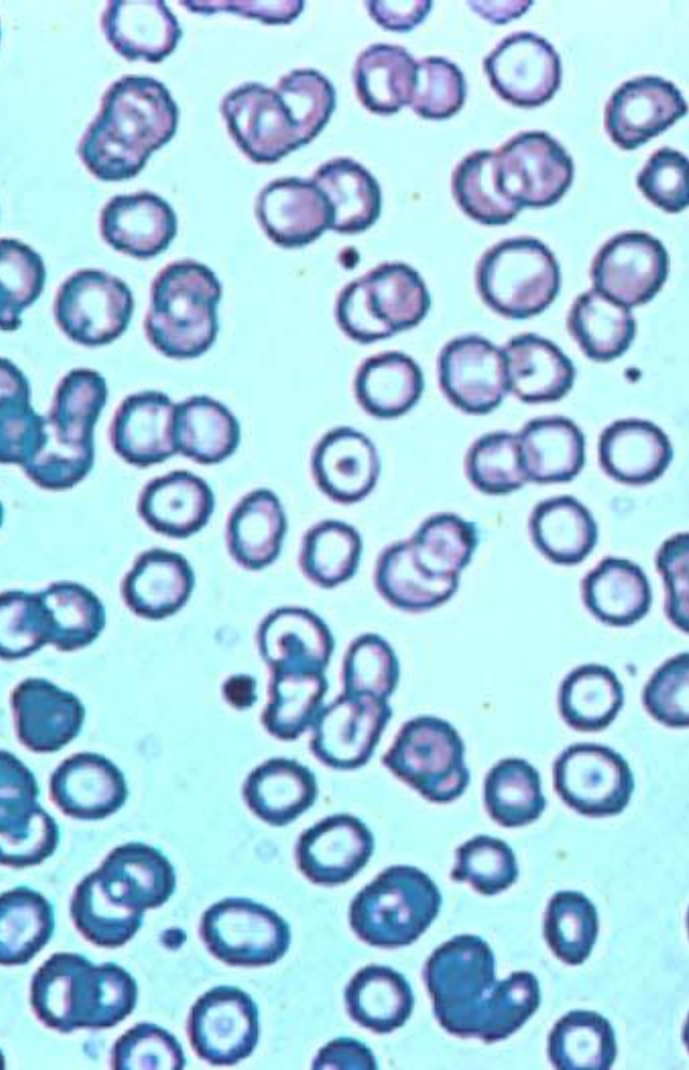
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# Signs and Symptoms

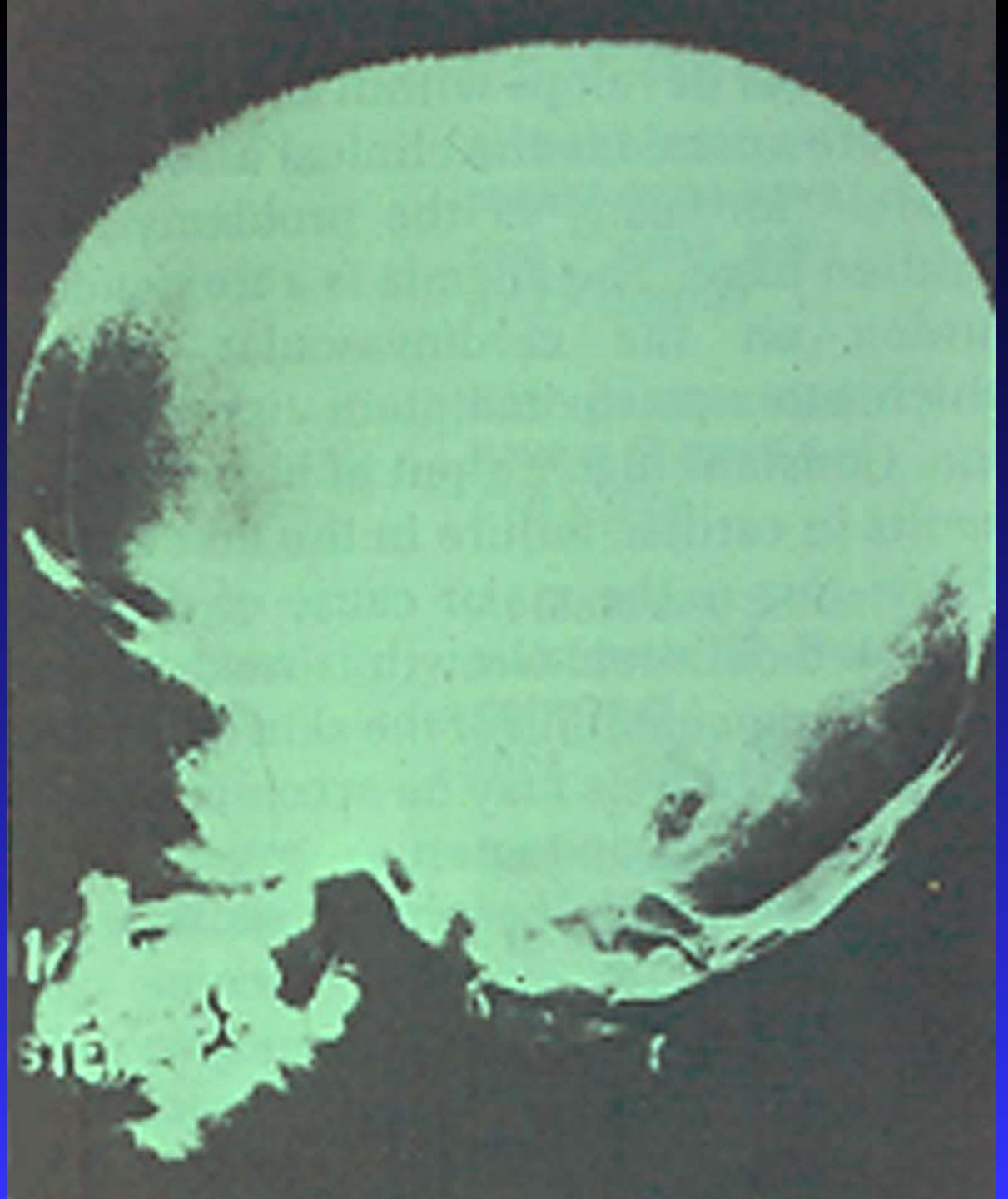
- Hemolytic
- Bone changes (hair on end)
- Ethnicity: Mediterranean, Africa, Southeast Asia
- Hypo-Micro, Poikilocytosis
- NRBC's, reticulocytosis, basophilic stippling
- Siderocytes (with repeated transfusions)

# Thalassemia Blood Smears



**X-ray of skull  
in Thalassemia:**

**“Hair-on-end”**

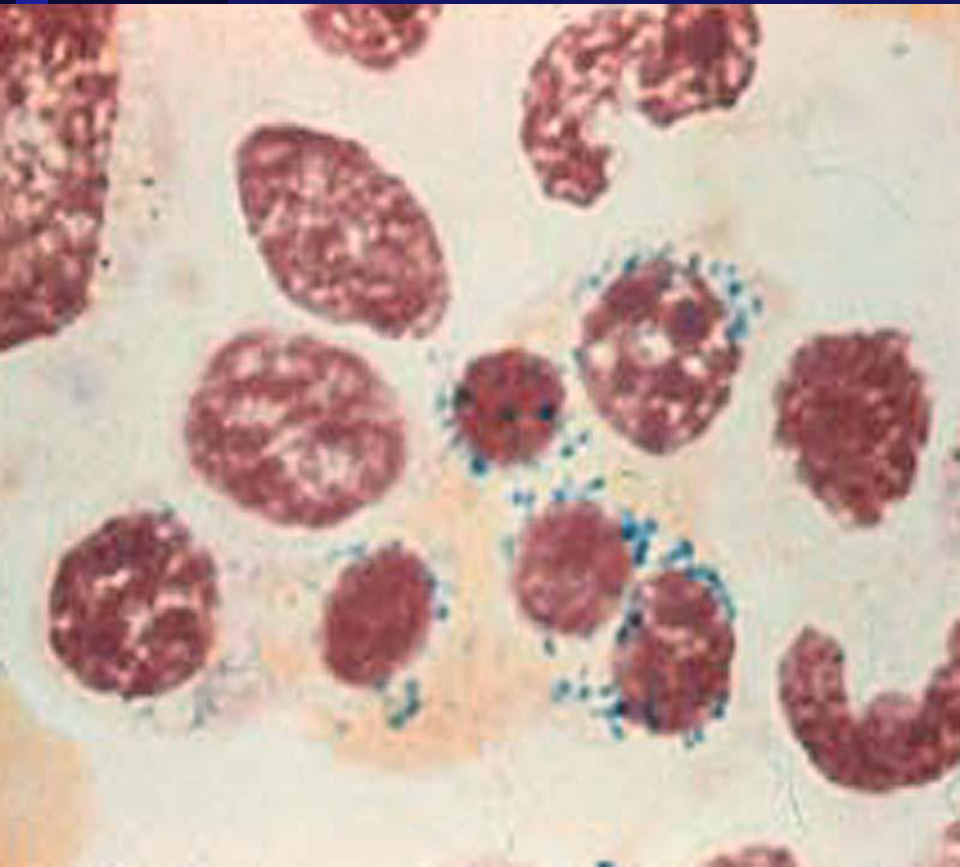


**Perl's iron stain (Prussian blue)**

**with potassium ferrocyanide**



**Siderocyte**



**Sideroblasts**



# $\alpha$ Thalassemia

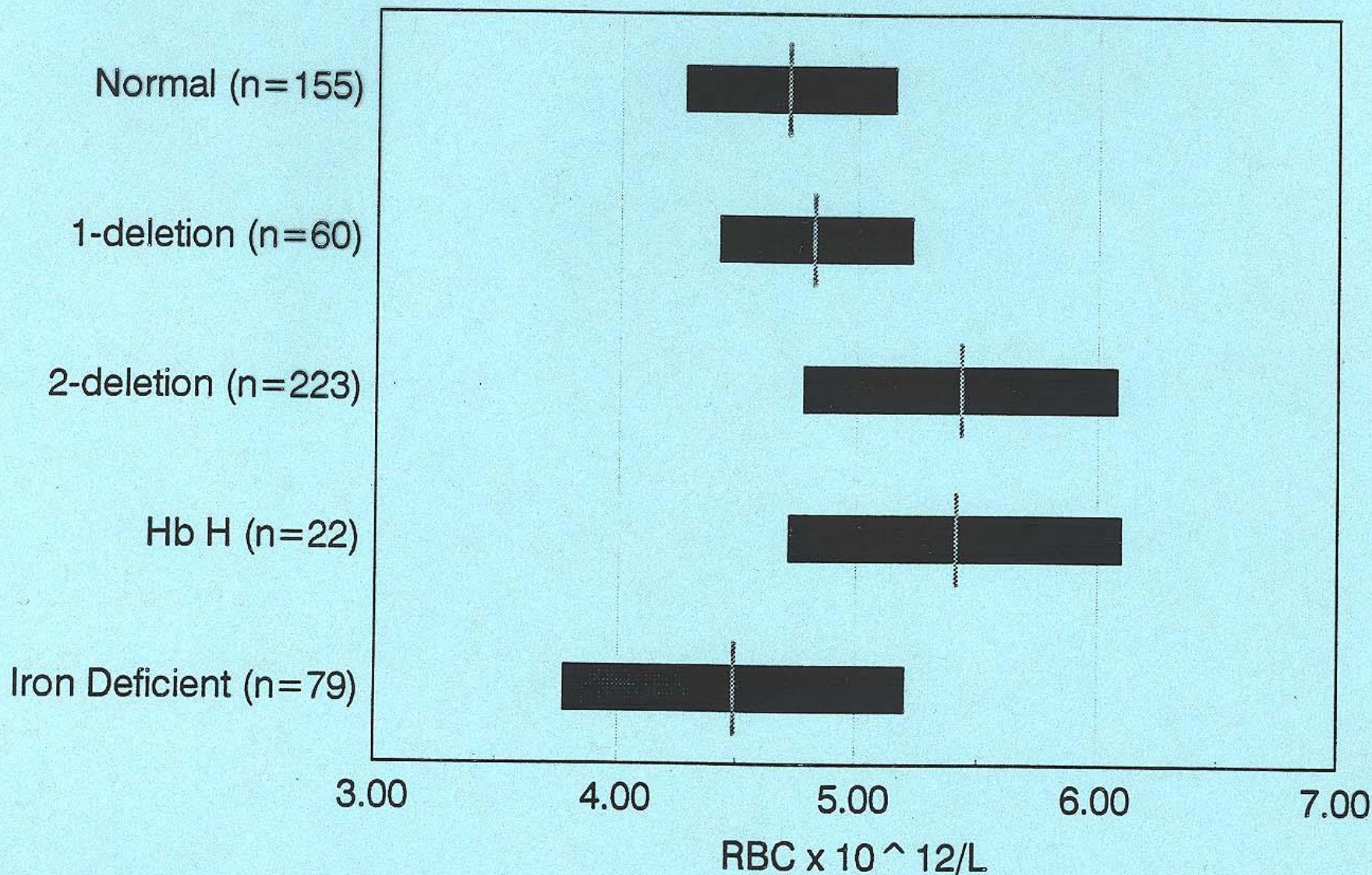
- Deletion of one or more alpha genes from chromosome 16
- $-\alpha/\alpha\alpha$ : silent carrier with little signs
- $--/\alpha\alpha$ : cis double deletion more common in SEA
- $-\alpha/-\alpha$ : trans double deletion
- $--/-\alpha$ : Hb H disease
- $--/--$ : Hb Bart's hydrops fetalis
- Hb Constant-Spring: elongation (discovered in Kingston, Jamaica; 2% of Thai have it)

# $\alpha$ Thalassemia Lab Changes

- High RBC
- Low H&H and indices
- High RDW
- May need to rule out IDA
- Hb ELP not useful except in Hb H
- BCB prep for Hb H

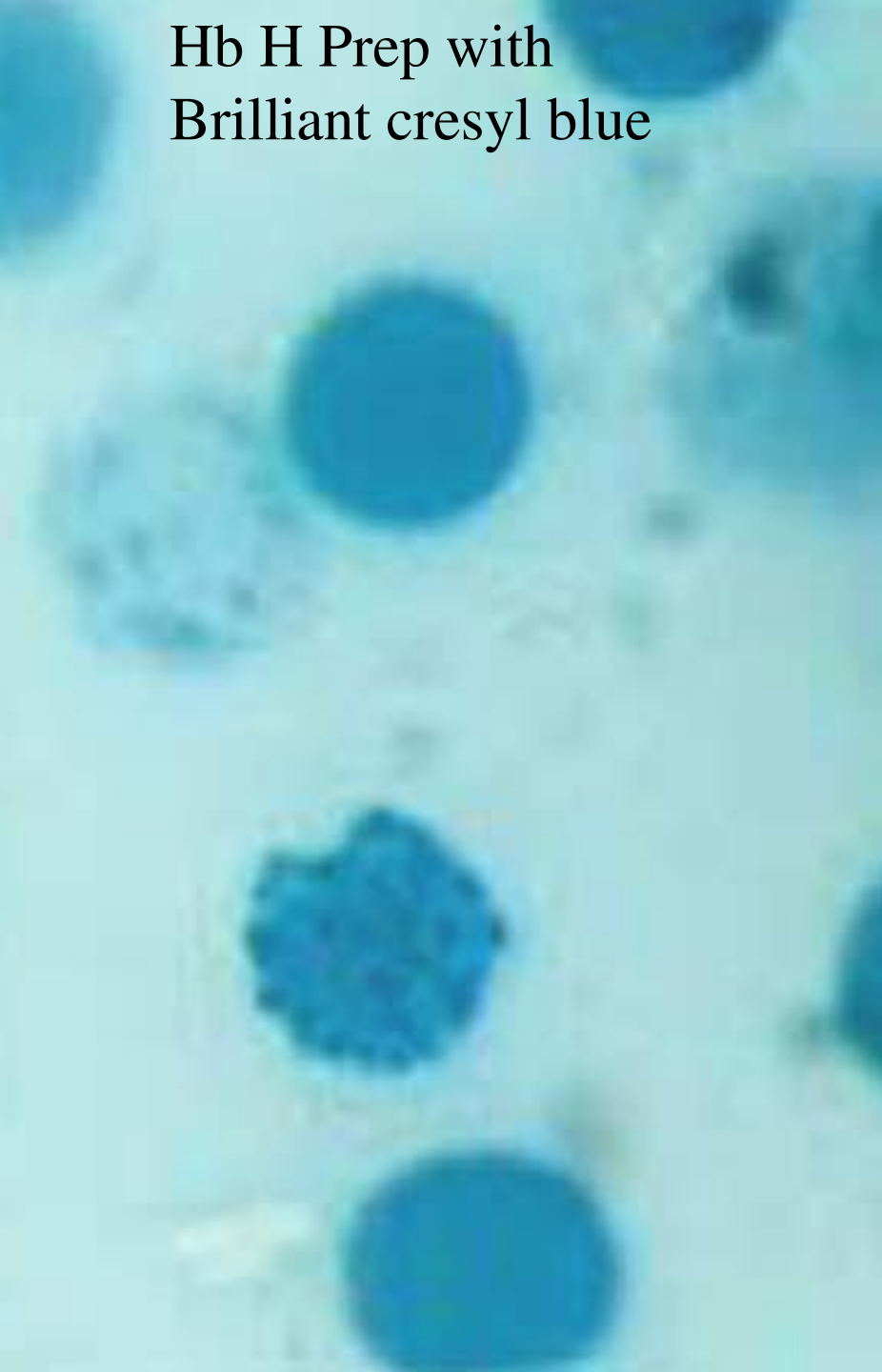


**CBC PARAMETERS (mean  $\pm$  1sd): Normal,  $\alpha$ -Thalassemia, Iron Deficiency**  
**Data from the Hawai`i Hereditary Anemia Project (1992)**

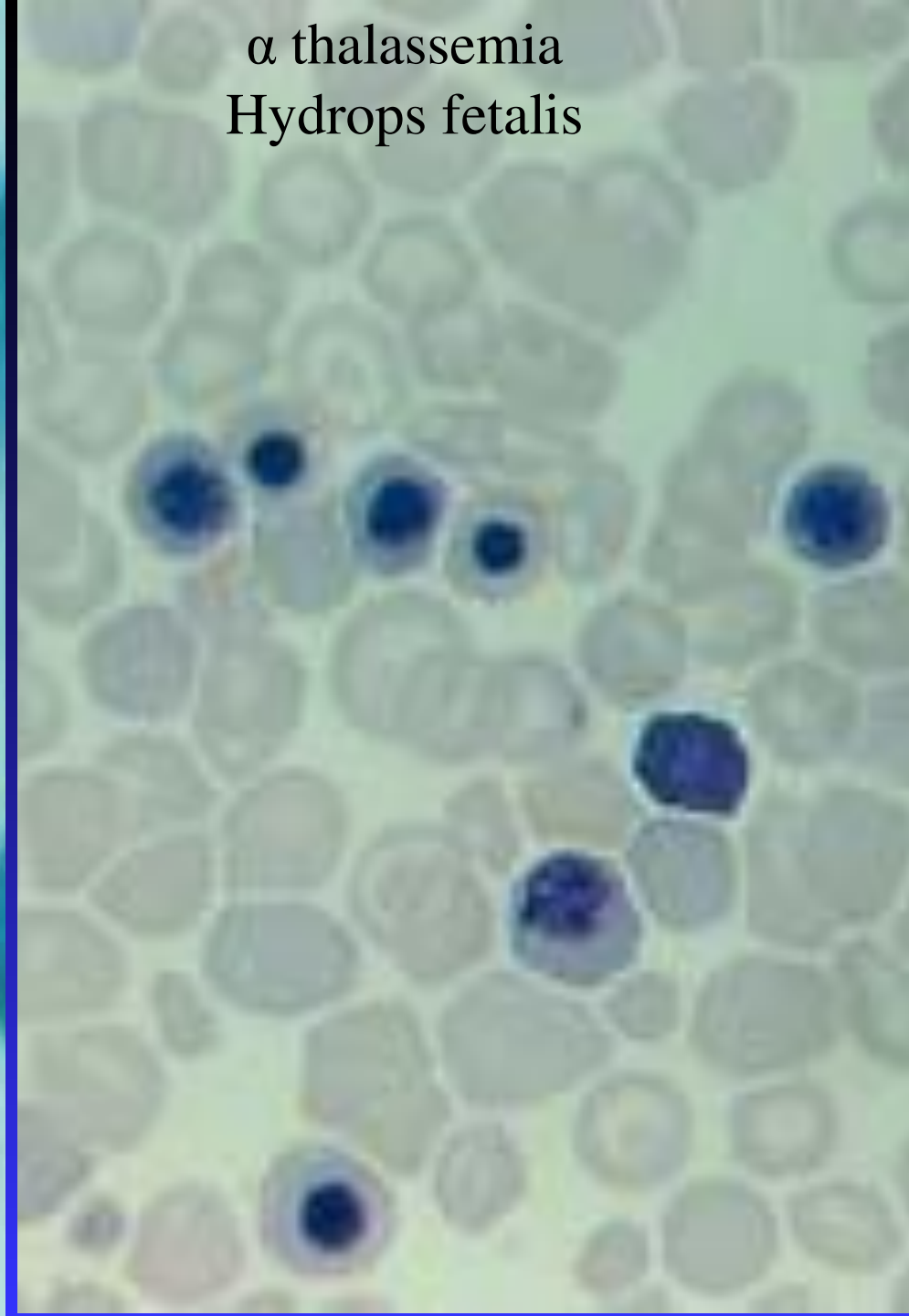


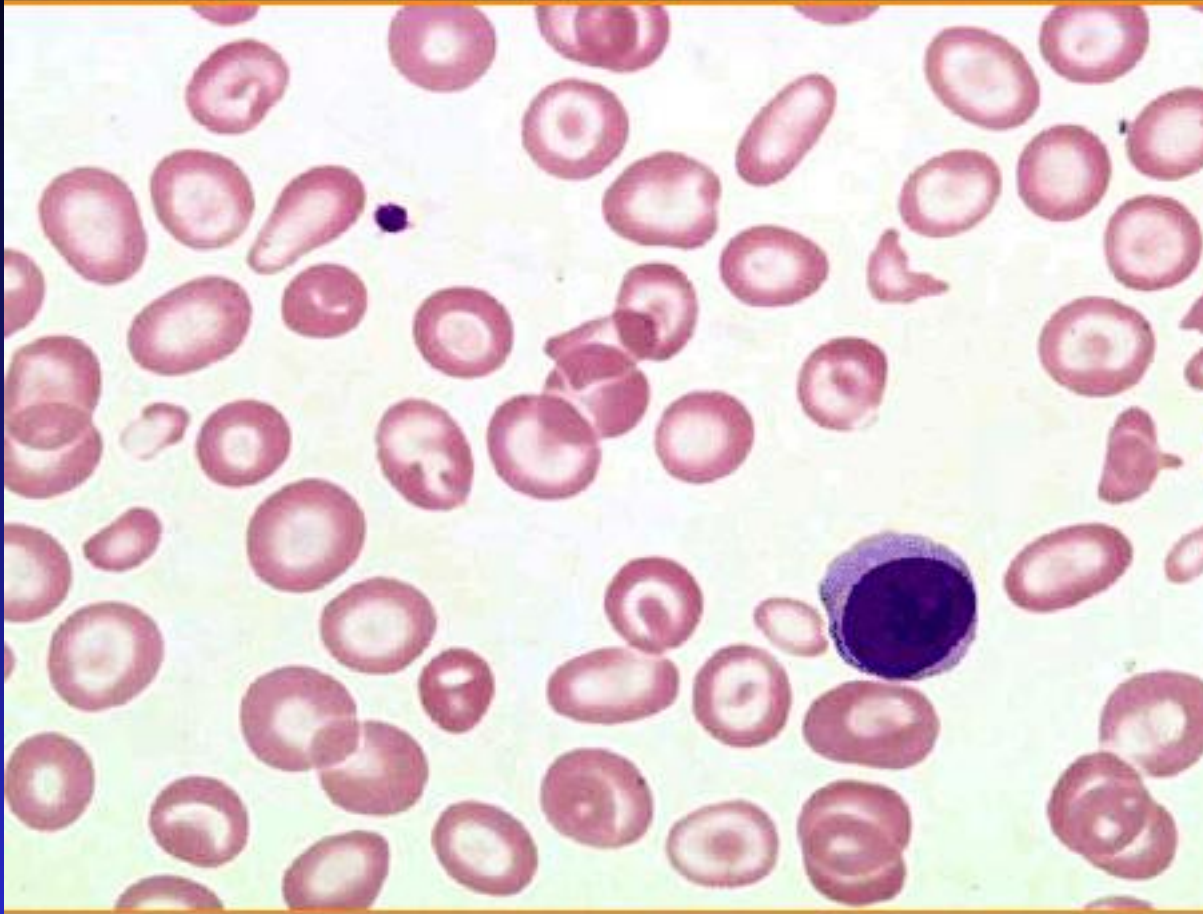


Hb H Prep with  
Brilliant cresyl blue



$\alpha$  thalassemia  
Hydrops fetalis



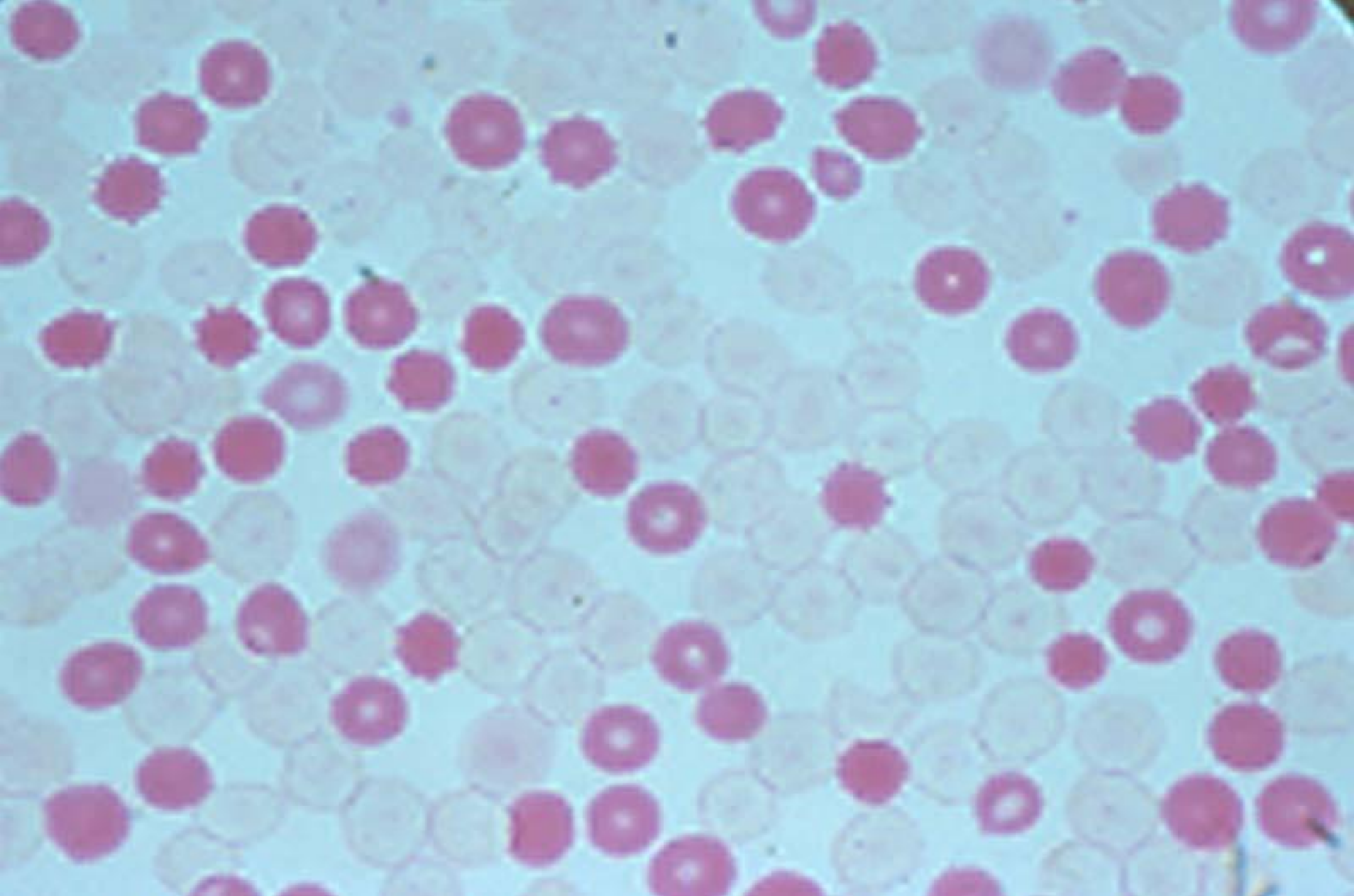


Source: Lab Med © 2007 American Society for Clinical Pathology

Peripheral blood smear: Hb H disease

# $\beta$ Thalassemia

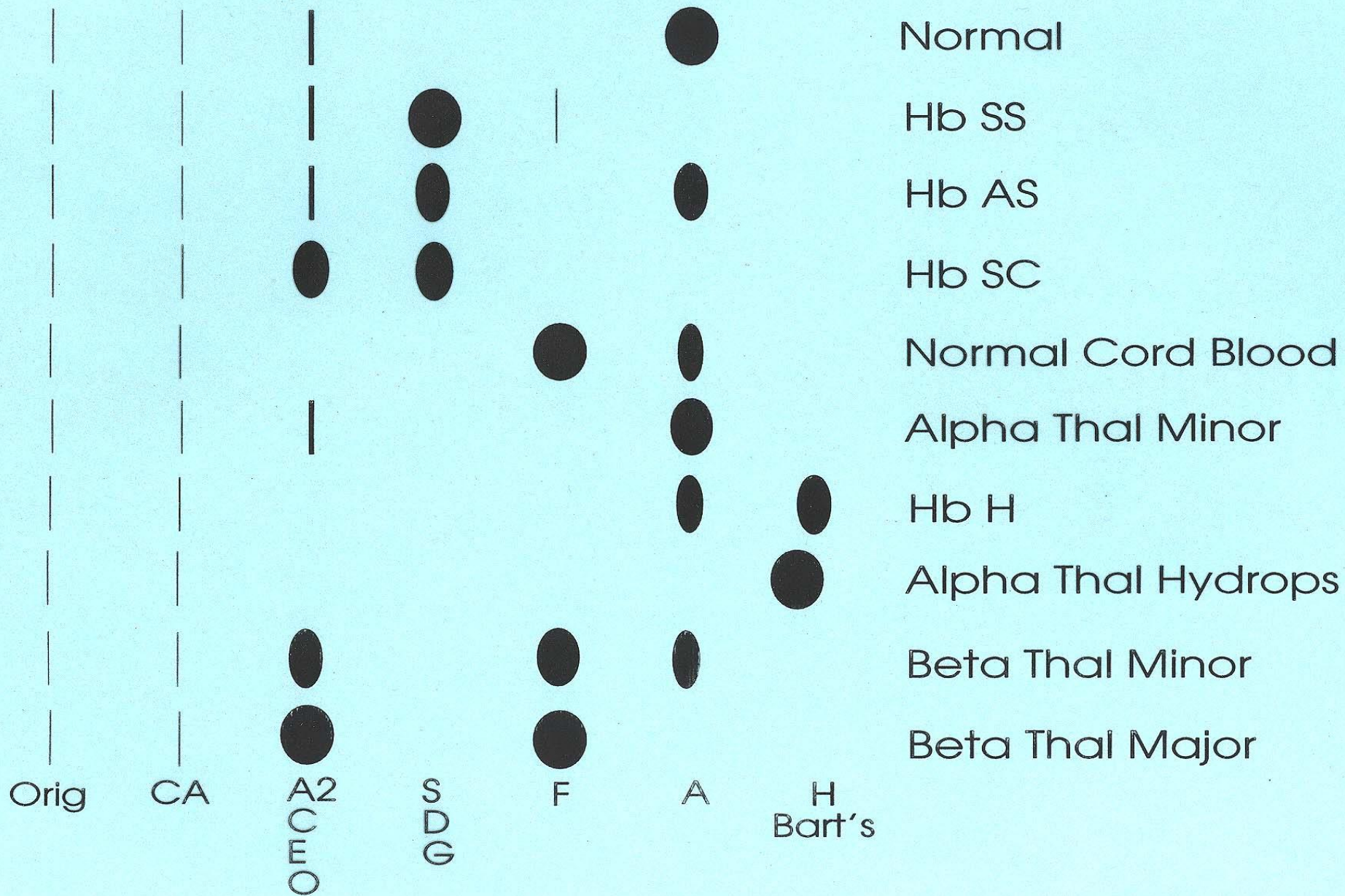
- Usually point mutation in the control region chr 11
- $\beta^+$  has minimal production
- $\beta^0$  has no production
- $\beta^+/\beta^+$  or  $\beta^0/\beta^0$  is  $\beta$  thal major or Cooley's anemia
- Often not apparent at birth until  $\beta$  chain takes over  $\gamma$  chain production
- High Hb A2, Hb F
- Related: Hb Lepore ( $\delta$ - $\beta$  fusion), HPFH



**Hb F preparation with Kleihauer-Betke  
Fetal Hb resists acid elution**



# Cellulose Acetate Hb ELP at pH 8.4





# IRON STUDIES: THALASSEMIA vs IRON DEFICIENCY

	Serum Fe	Ferritin	TIBC	% Sat	FEP/ZPP
Thalassemia	N to ↑	N to ↑	N to ↓	N to ↑	N
Iron Deficiency	↓	↓	↑	↓	↑

# CBC PARAMETERS: THALASSEMIA vs IRON DEFICIENCY

	RBC	MCV	MCH	MCHC	RDW
Thalassemia	N or ↑	↓↓	↓↓	↓	N
Iron Deficiency	↓↓	↓	↓	↓↓	↑



# SUMMARY OF HEMATOLOGIC PARAMETERS

	$\alpha$ -Thal Minor	Hb H	$\beta$ -Thal	Iron Def	Hb E
RBC	↑	↑	↑ ↑	↓	↑
Hb	↓	↓ ↓	↓	↓ ↓	↓
Hct	s1 ↓	↓ ↓	↓	↓ ↓	↓
MCV	↓	↓ ↓	↓ ↓	↓	↓
MCH	↓	↓ ↓	↓	↓	↓
MCHC	↓	↓ ↓	↓	↓	N
RDW	↑	↑ ↑	↑	↑ ↑	↑
ZPP/FEP	N	N	s1 ↑	↑	N - ↑ *
HbA2	N	↓	↑ ↑	N	↑ **

\* n was small; median close to control group, but upper range very high

\*\* due to Hb E eluting with Hb A2

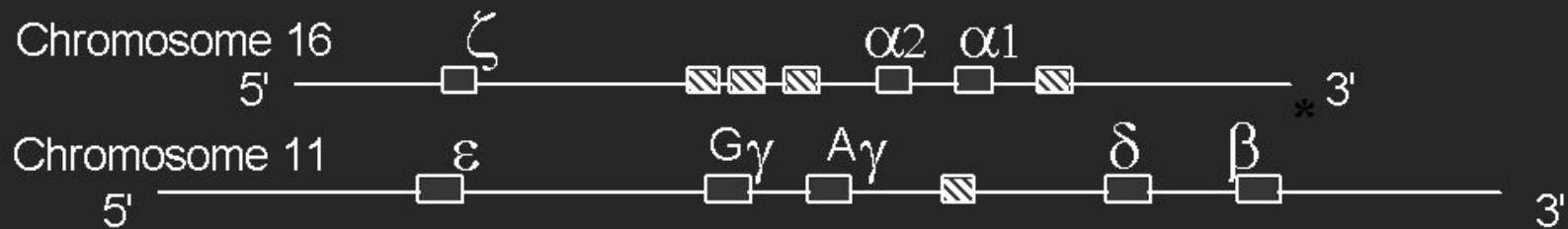
# Thalassemia

- The only treatments are stem cell transplant and simple transfusion.
- Chelation therapy to avoid iron overload has to be started early.

# Sickle Cell Anemia

- Single base pair mutation results in a single amino acid change.
- Under low oxygen, Hgb becomes insoluble forming long polymers
- This leads to membrane changes (“sickling”) and vasoocclusion

# Sickle Cell Mutation



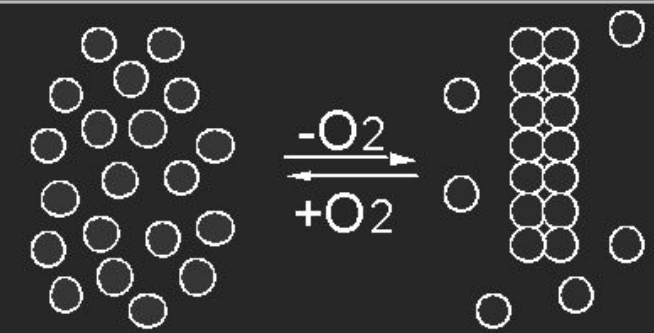
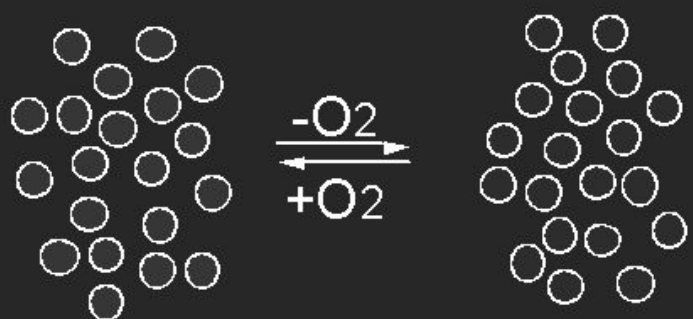
*Normal (HbA)*

CCT GAG GAG  
 -Pro-Glu-Glu-  
 5 6 7



*Abnormal (HbS)*

CCT GTG GAG  
 -Pro-Val-Glu-  
 5 6 7

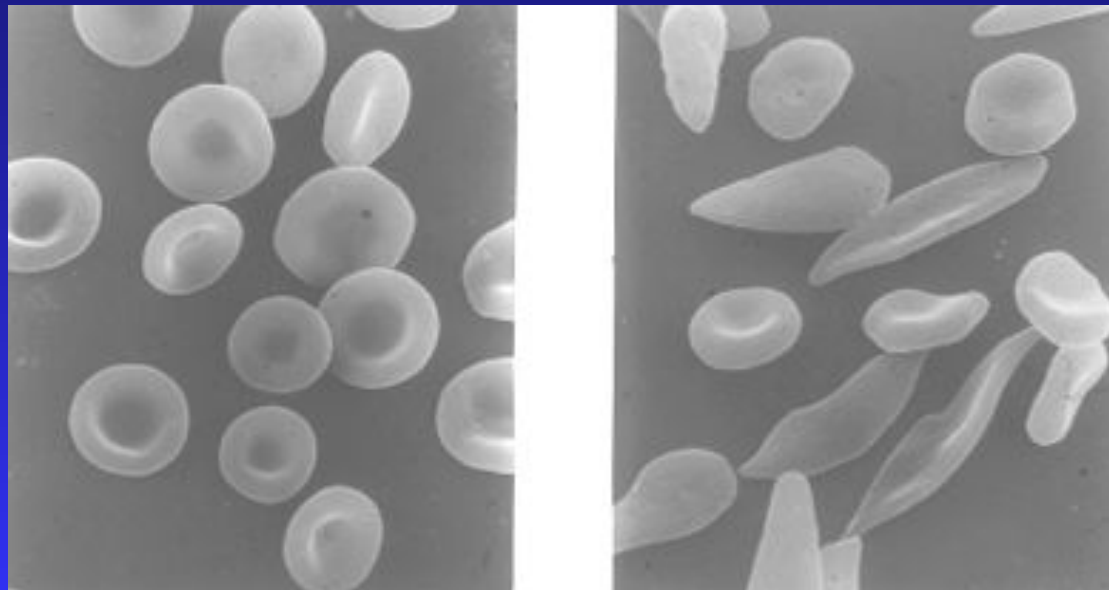


CTV

# Red Blood Cells from Sickle Cell Anemia

- Deoxygenation of SS erythrocytes leads to intracellular hemoglobin polymerization, loss of deformability and changes in cell morphology.

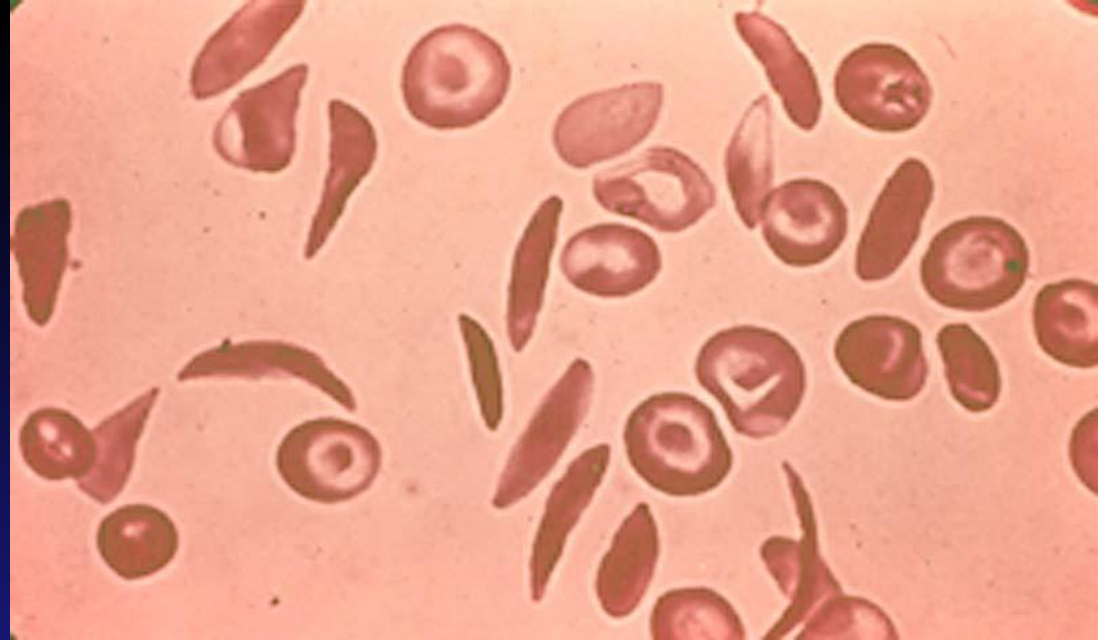
OXY-STATE ↔ DEOXY-STATE





# Hb S

- Sickling Hb
- Autosomal
- Sickle crisis in low oxygen condition
- $\beta 6$  glutamate to valine substitution
- Prevalent in Eastern Africa
- Solubility test
- Sickling test (meta-bisulfite)

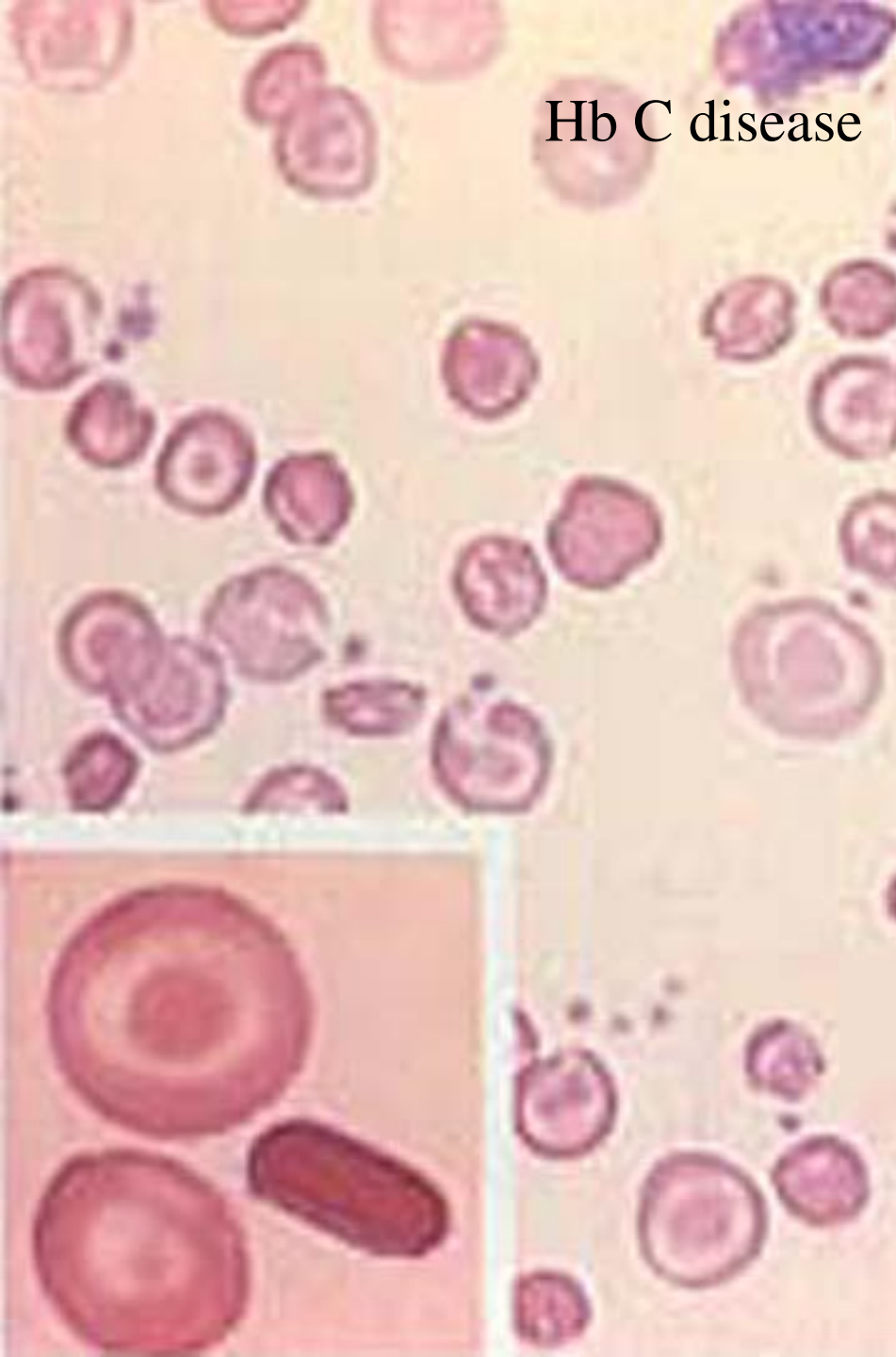


# Other Hemoglobinopathies

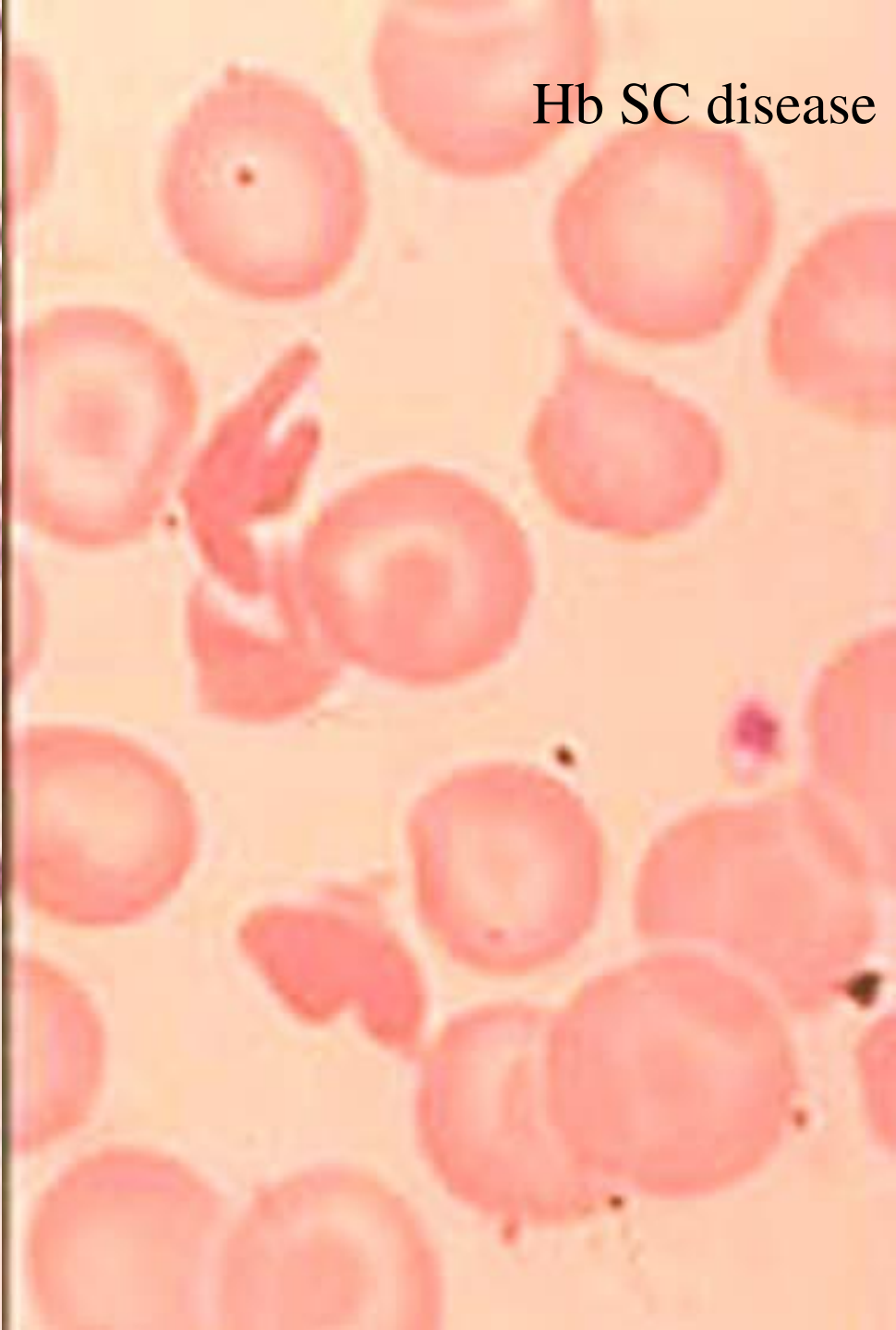
- Hb C ( $\beta 6$  Glu-Lys) in Western Africa
  - ◆ Cigar-like crystals
  - ◆ Billiard ball cells
  - ◆ Folded cells
- Hb SC disease
  - ◆ Washington monument cells
  - ◆ Mitten shape
- Hb E ( $\beta 26$  Glu-Lys) in SEA
  - ◆ Moves with Hb A2 in Hb ELP and A2 column (ie, false elevated Hb A2)



Hb C disease



Hb SC disease





Unusual Hemoglobins in the World

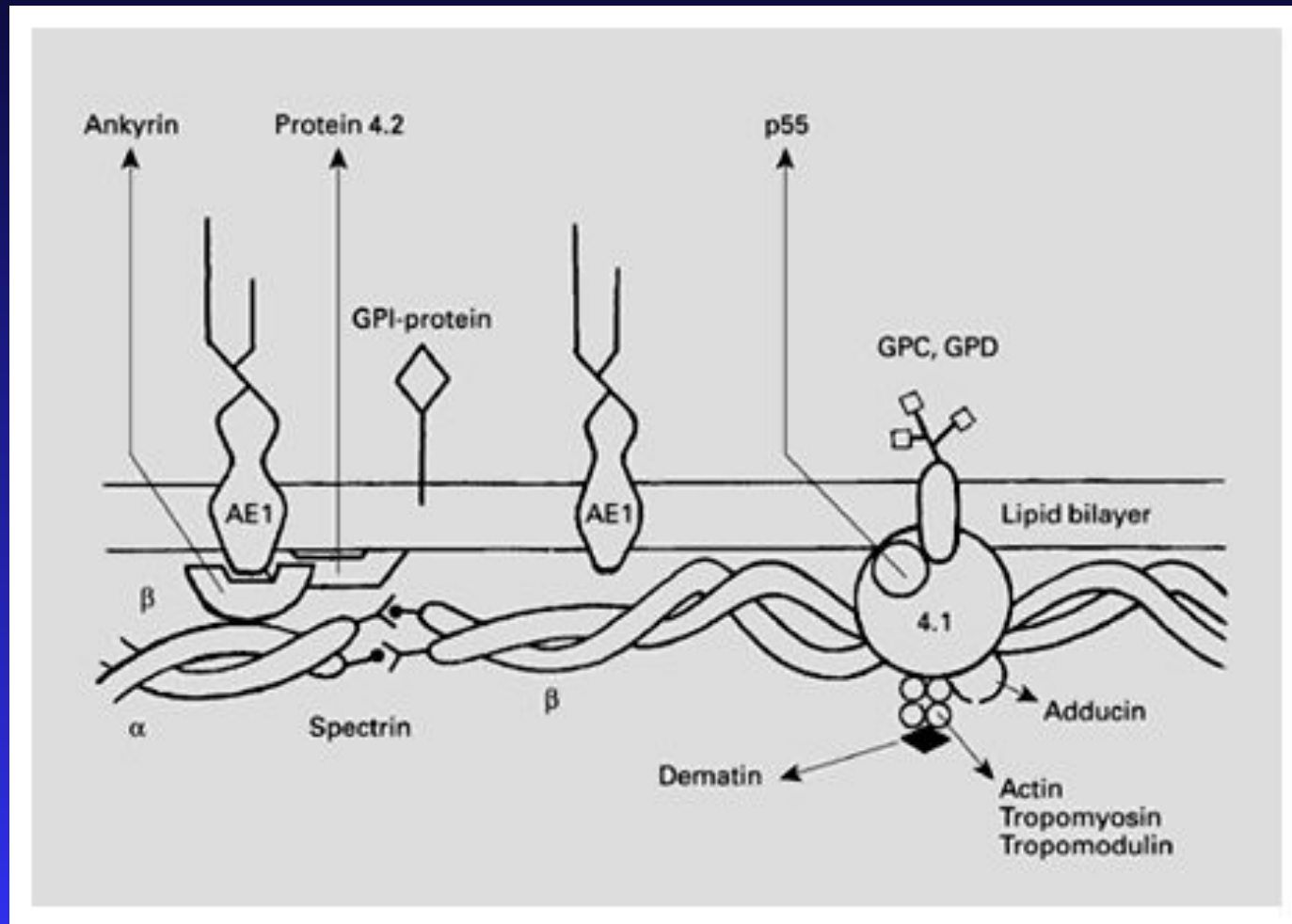
# Review red blood cell disorders

*Red cell destruction – membrane disorders*

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Hereditary pyropoikilocytosis
- Southeast Asian ovalocytosis

# Review red blood cell disorders

## *Red cell destruction – membrane disorders*



# Review red blood cell disorders

## *Red cell destruction – enzymopathies*

- G6PD deficiency
- Pyruvate kinase deficiency
- Other very rare deficiencies

Thank you ★ תודה רבה

