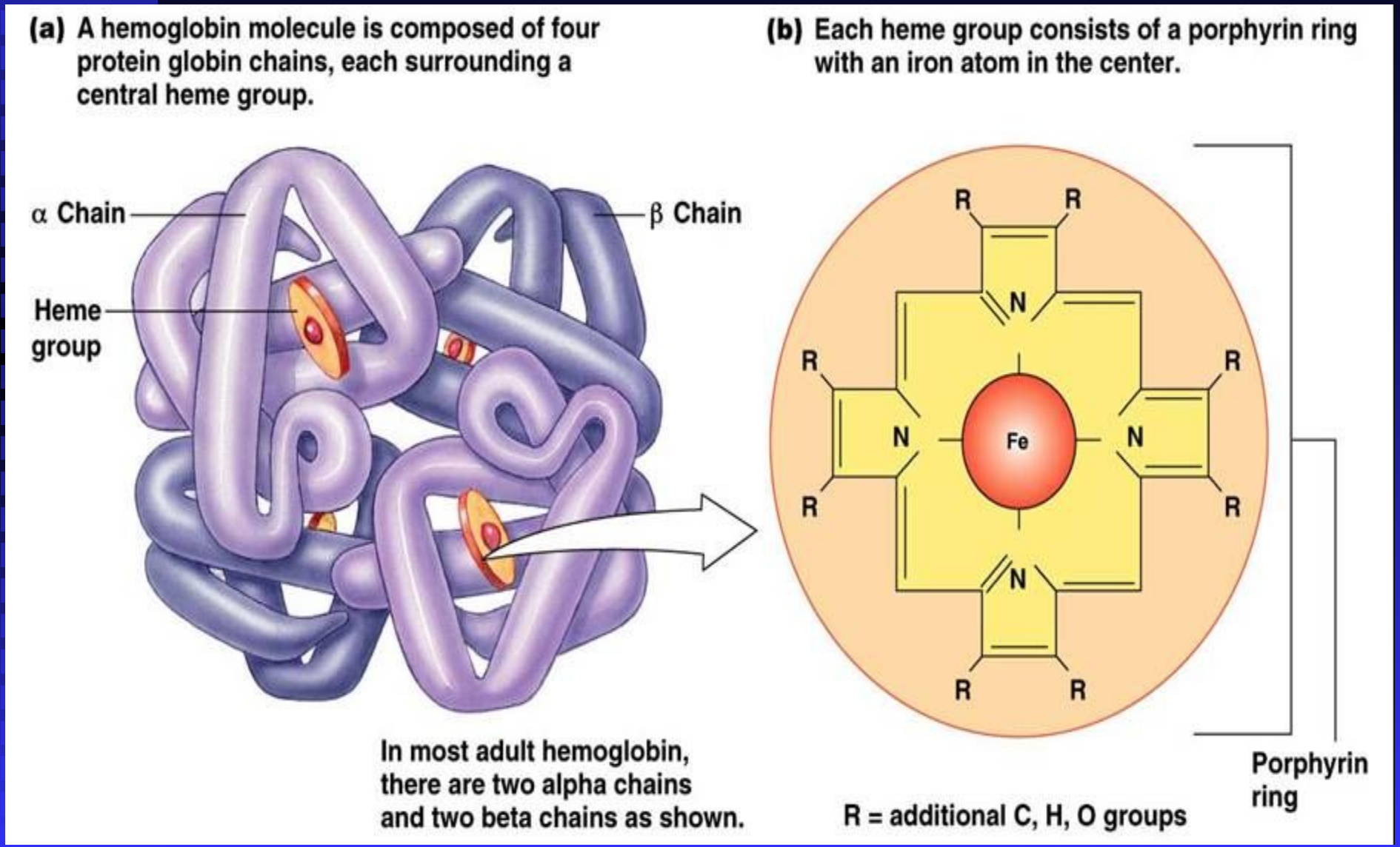
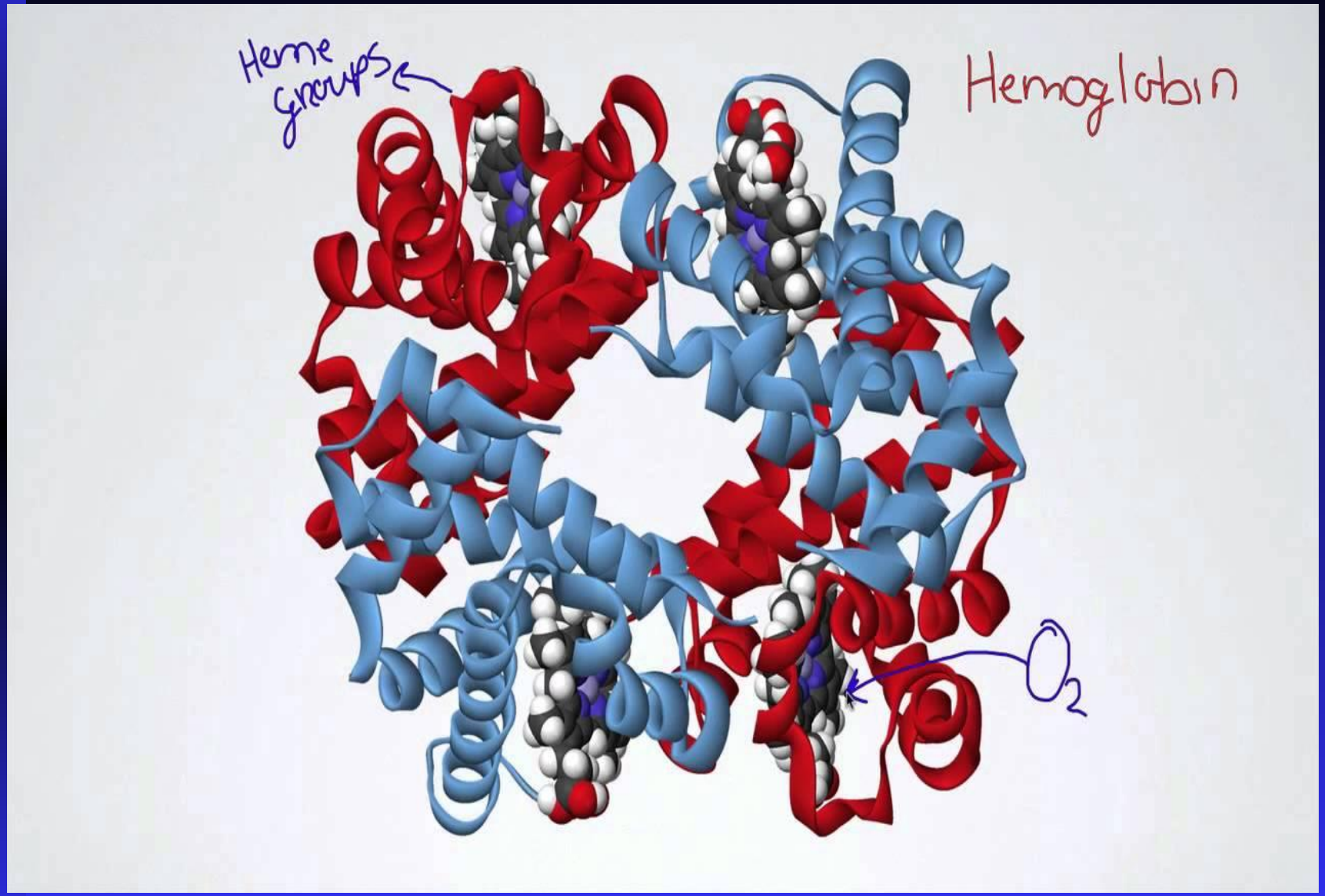
Hemoglobinopathies

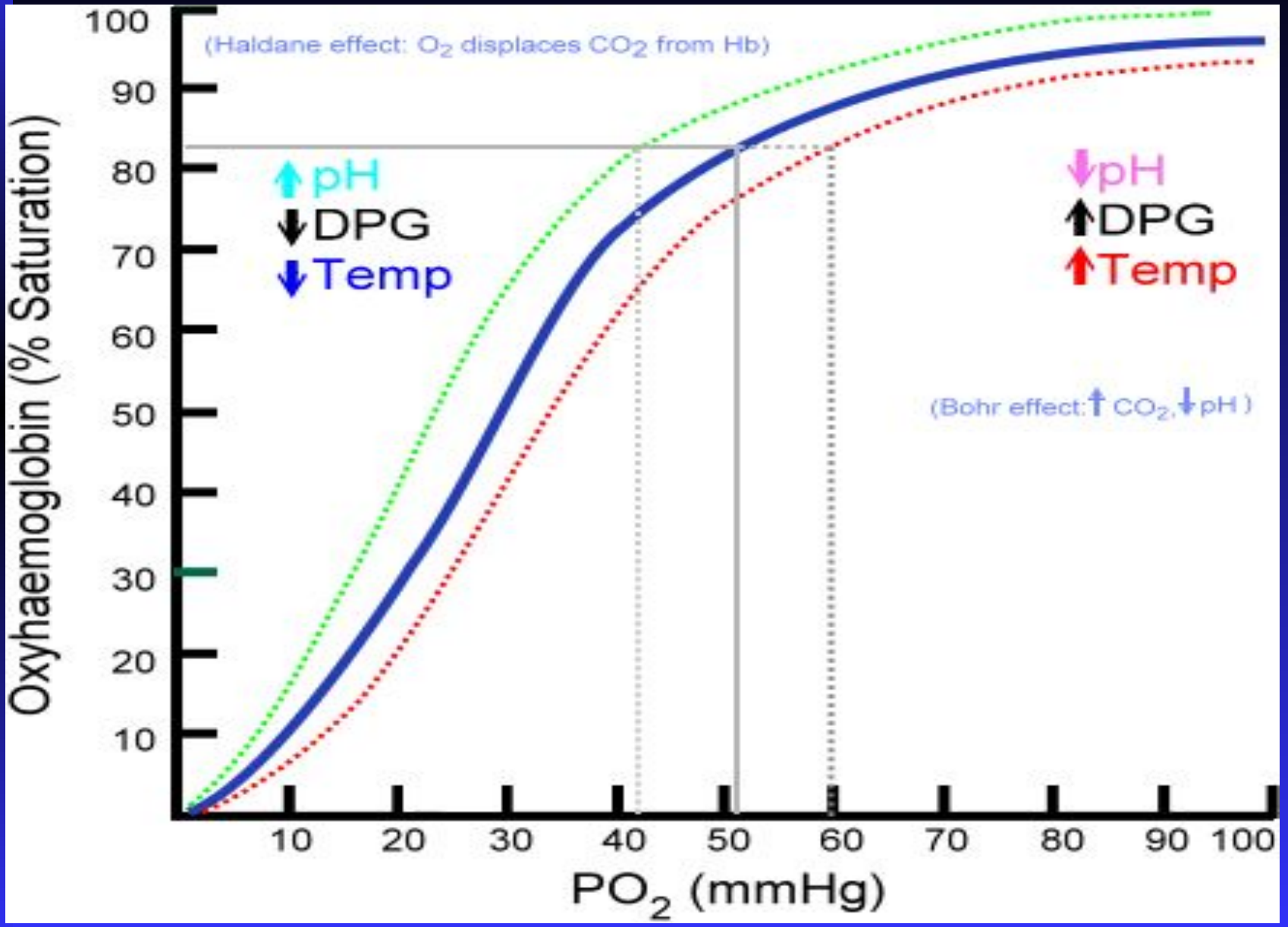
- Hemoglobinopathies
- Thalassemia genetics
- Hb synthesis
- Hb A, A2, F
- Hb ELP
- Hb Constant-Spring
- Hb Bart's
- Hb H
- Hb Lepore
- Hb E
- Hb S
- Hb C
- Hb SC disease
- HPFH

Hb structure

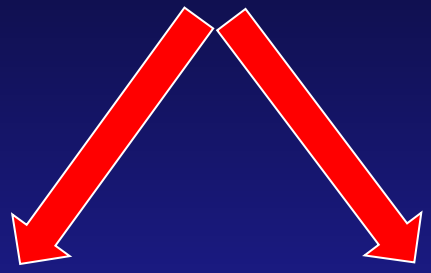



Heme groups
Hemoglobin
O_2

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/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/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)
- Erythropoetin ⇩
- 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

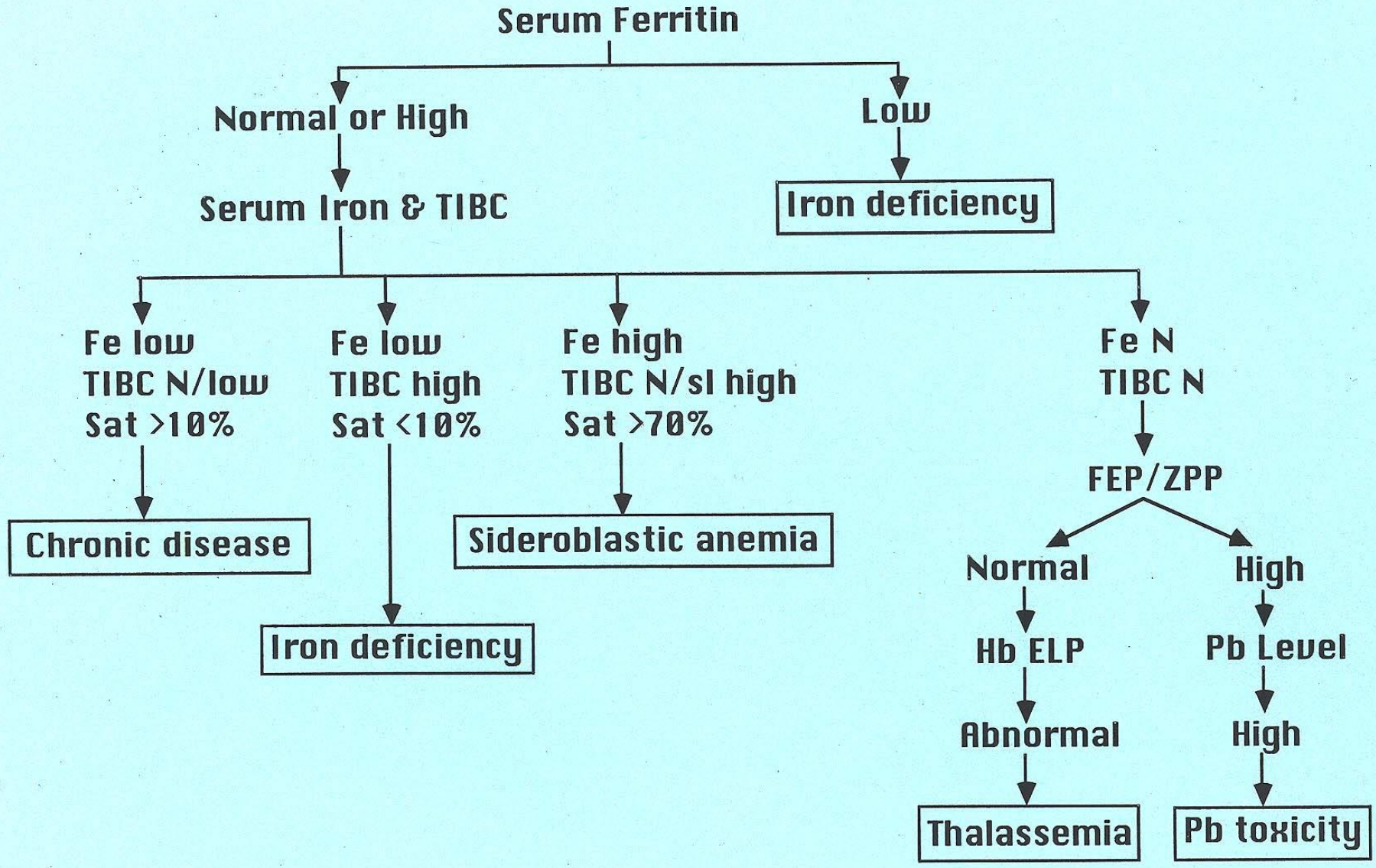
- Elevated reticulocyte count
- Mechanical
- Autoimmune
- Drug
- Congenital

Hb Problems

- Heme production problem: porphyria
- Fe incorporation into Heme: Sideroblastic anemia
- Fe^{++} problems: IDA, hemochromatosis

- Globin problem: sickle cell disease, thalassemia

MICROCYTIC HYPOCHROMIC ANEMIA



From: SB McKenzie, Texbook of Hematology, 2nd ed,1996, p.118 (modified)

Hemoglobinopathies

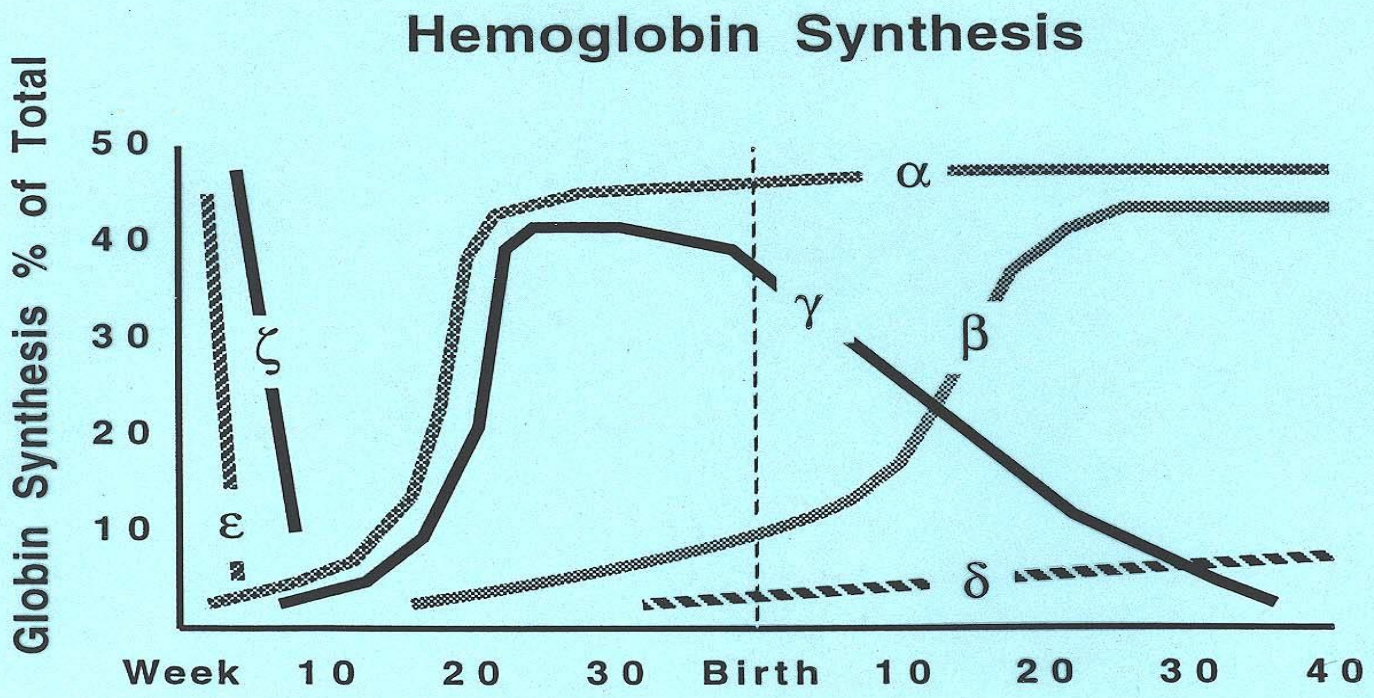
- Decrease, lack of, or abnormal globin
- May be severe hemolytic anemia
- Abnormal Hb with low functionality
- Mutation may be deletion, substitution, elongation
- Hb electrophoresis may be helpful

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]−−



		Adult	Newborn
α2β2	Hb A	97 %	20 %
α2δ2	Hb A2	2.5	<0.5
α2γ2	Hb F	<1	80

Embryonic:

ζ2ε2	Gower-1
α2ε2	Gower-2
ζ2γ2	Portland

Thalassemia

- Genetic defect in hemoglobin synthesis
  - ↓ synthesis of one of the 2 globin chains (α or β)
  - 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

Thalassemia

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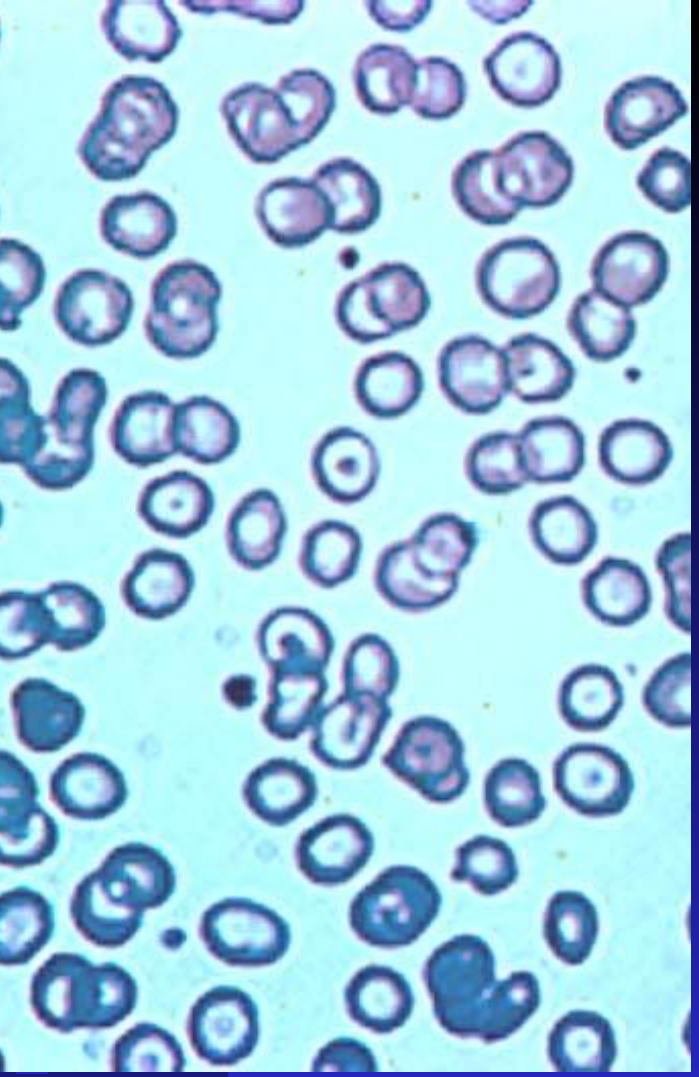
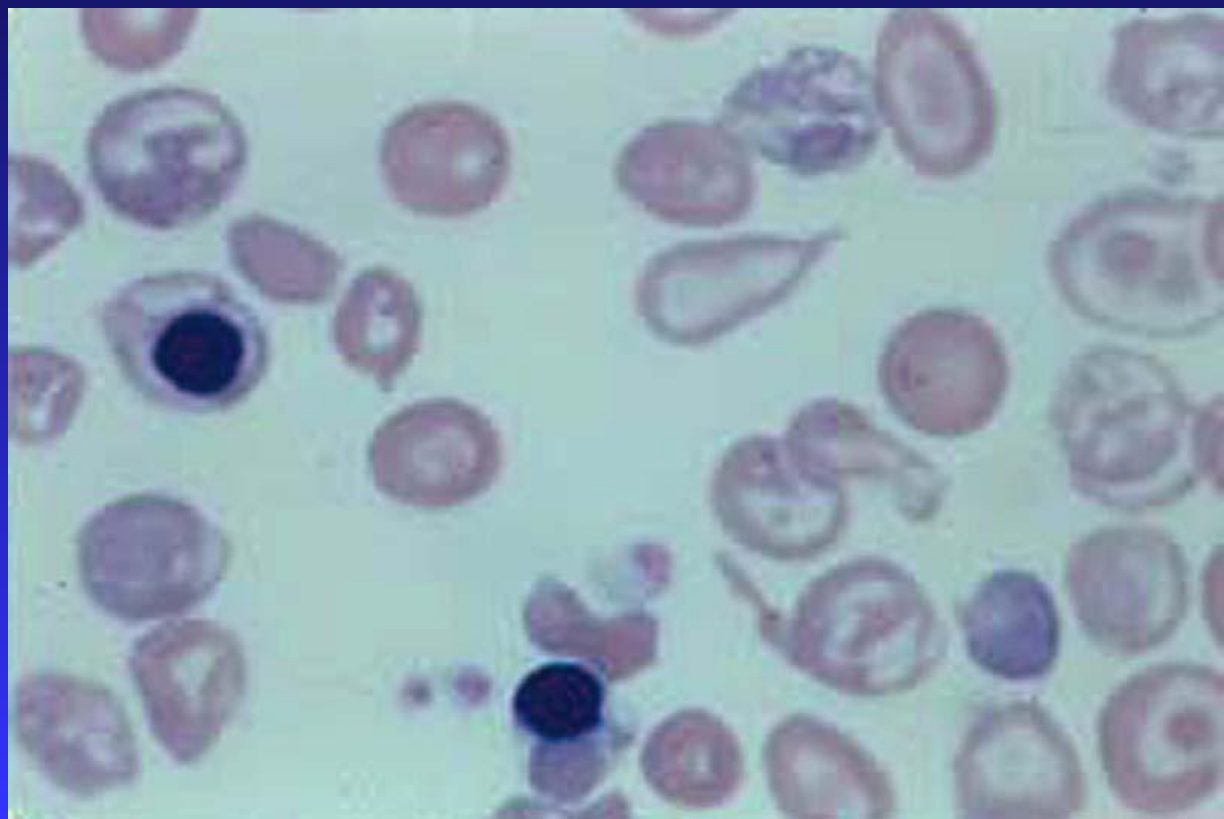
Signs and Symptoms

- 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



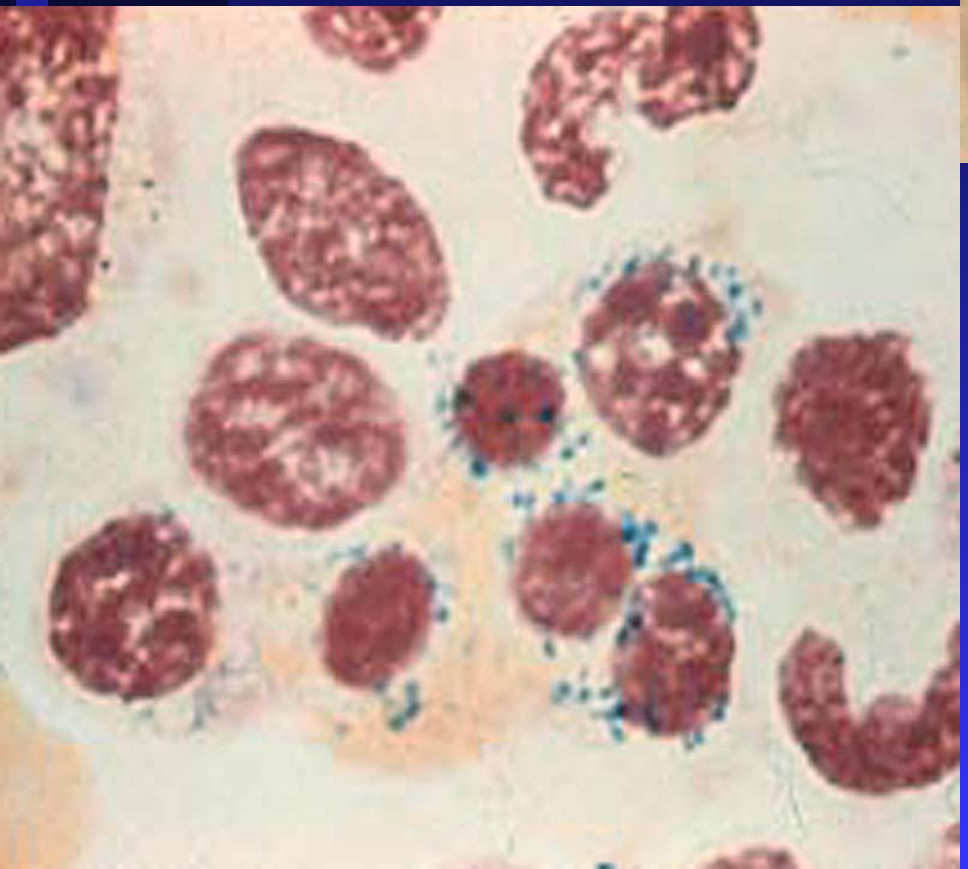


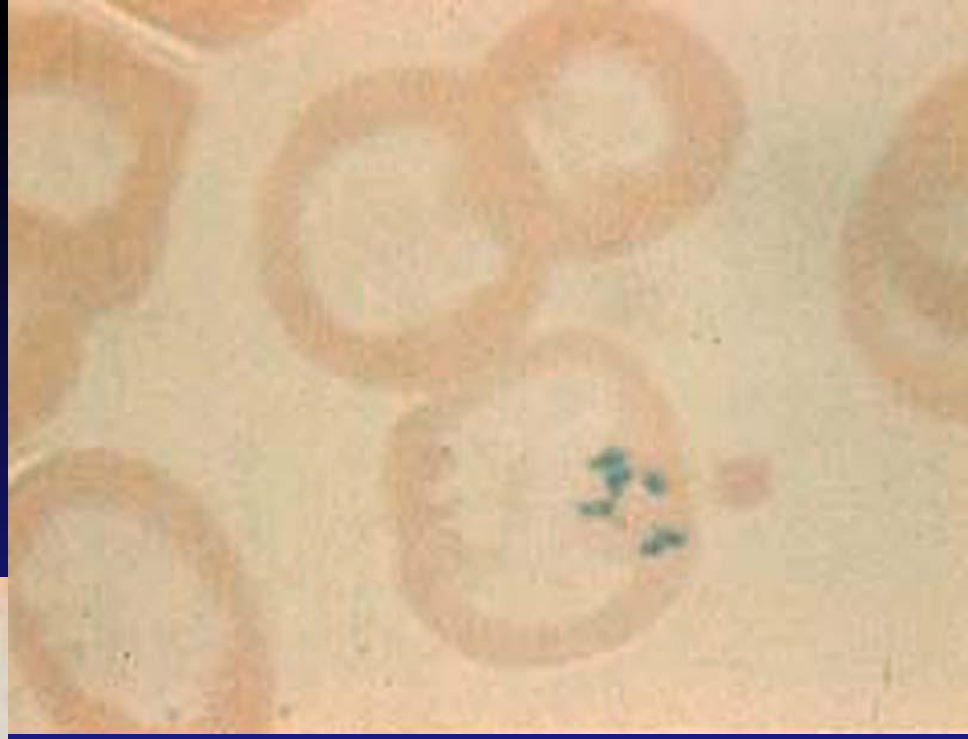
X-ray of scull in Thalassemia:

"Hair-on-end"

Perl's iron stain (Prussian blue)

with potassium ferrocyanide





Siderocyte

Sideroblasts

α Thalassemia

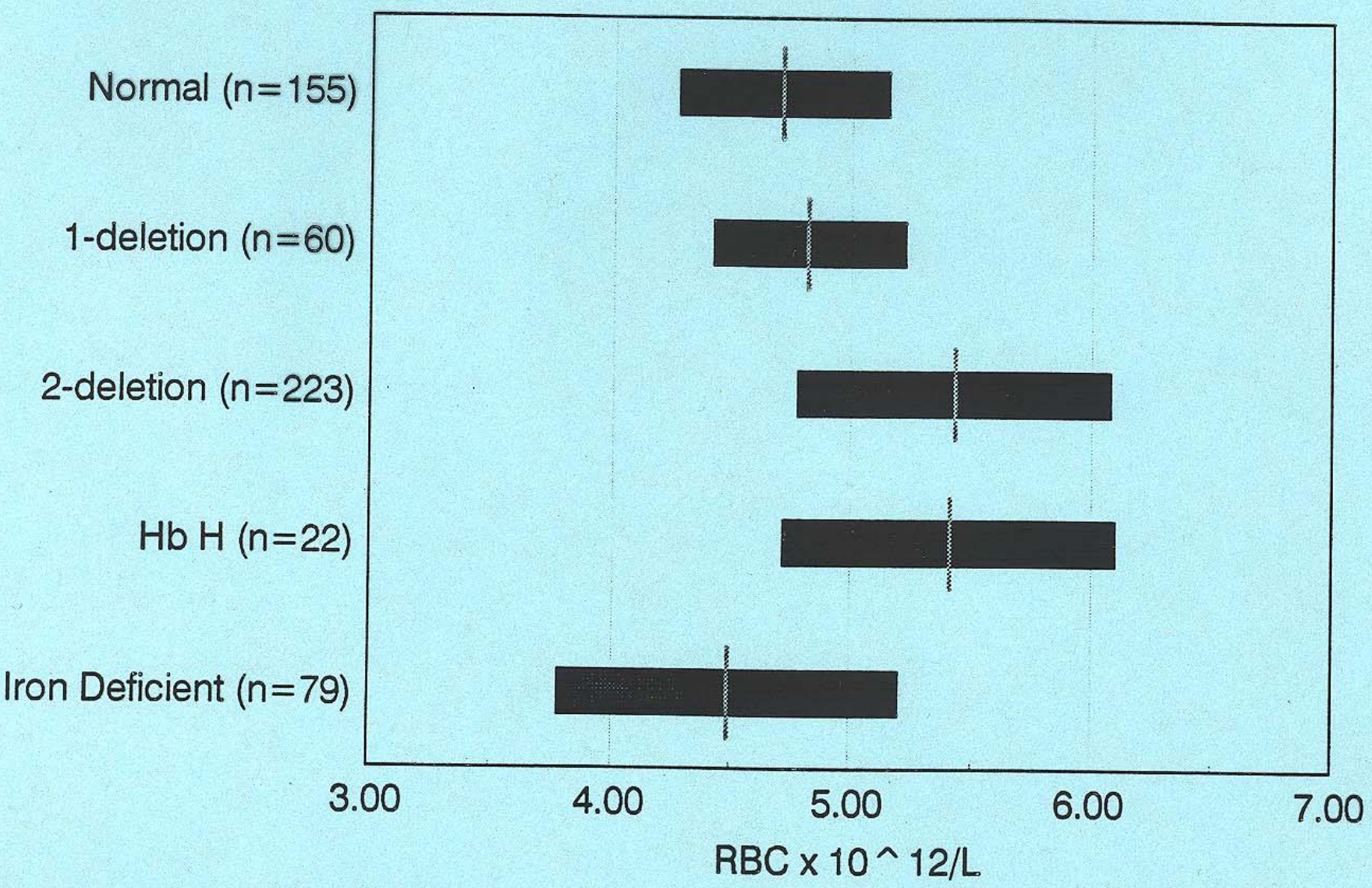
- Deletion of one or more alpha genes from chromosome 16
- -α/αα: silent career with little signs
- --/αα: cis double deletion more common in SEA
- -α/-α: trans double deletion
- --/-α: Hb H disease
- --/--: Hb Bart's hydrops fetalis
- Hb Constant-Spring: elongation (discovered in Kingston, Jamaica;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

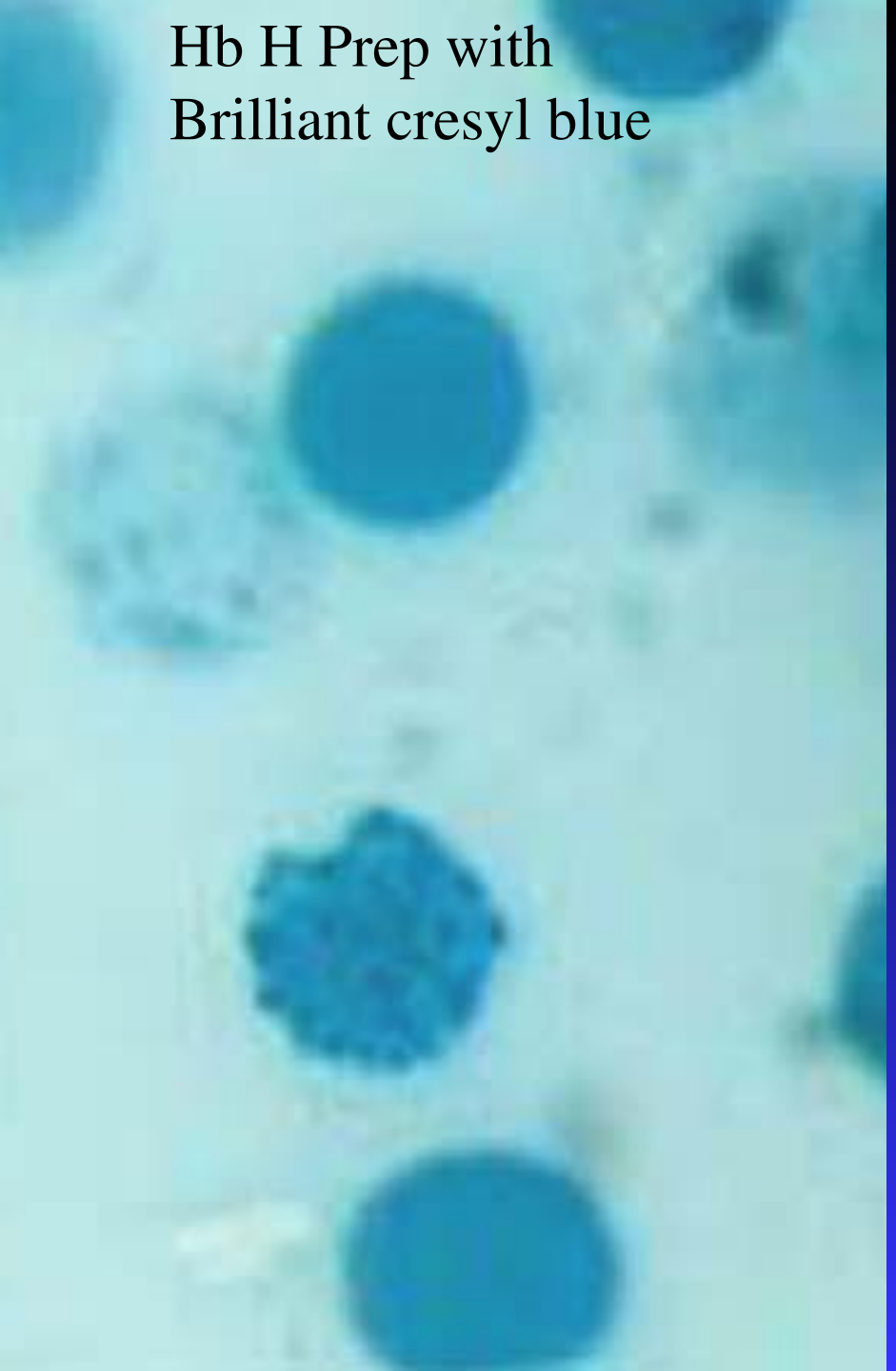
α Thalassemia Lab Changes

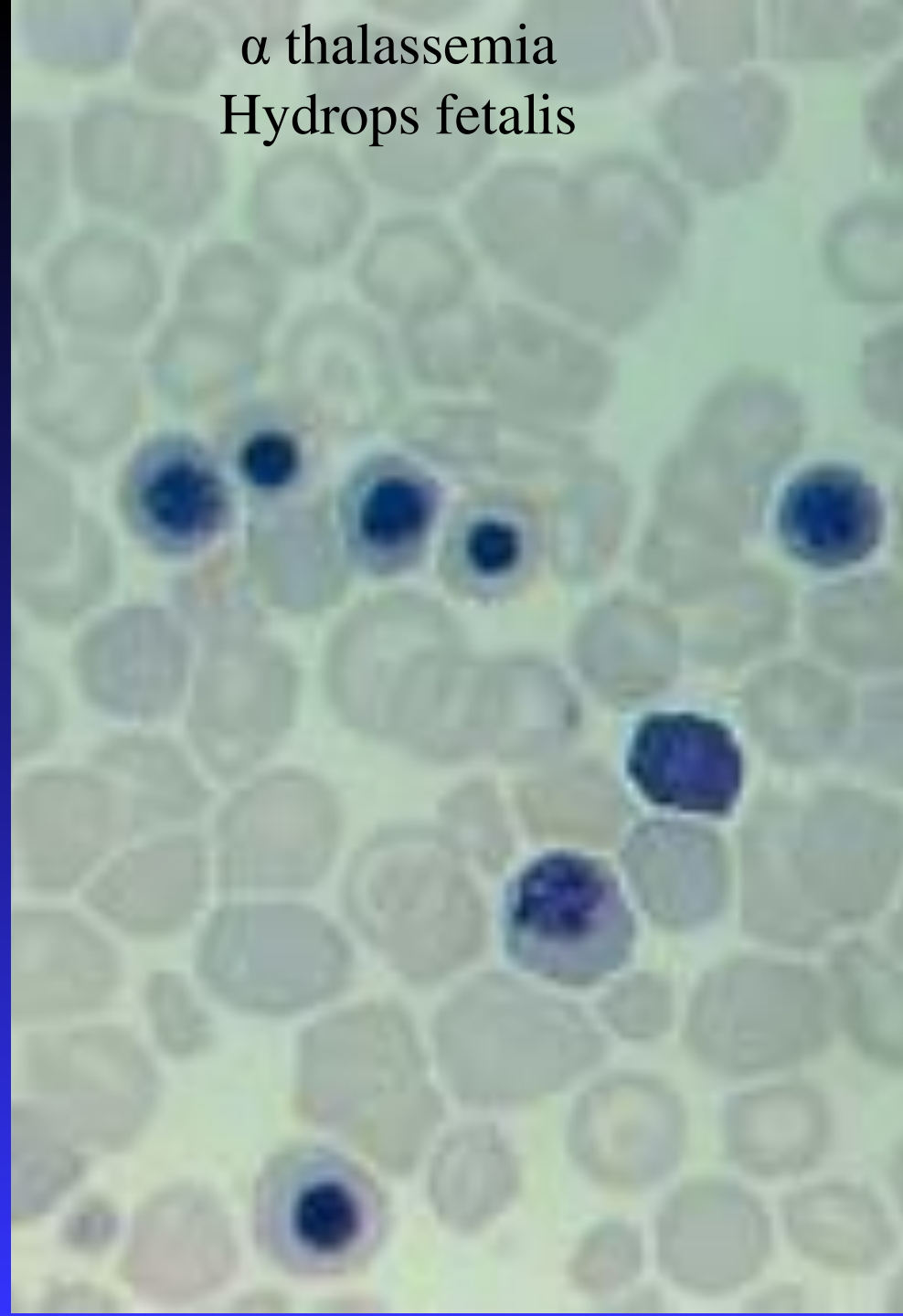
- High RBC
- Low H&H and indices
- High RDW
- May need to rule out IDA
- Hb ELP not useful except in Hb H
- BCB prep for Hb H

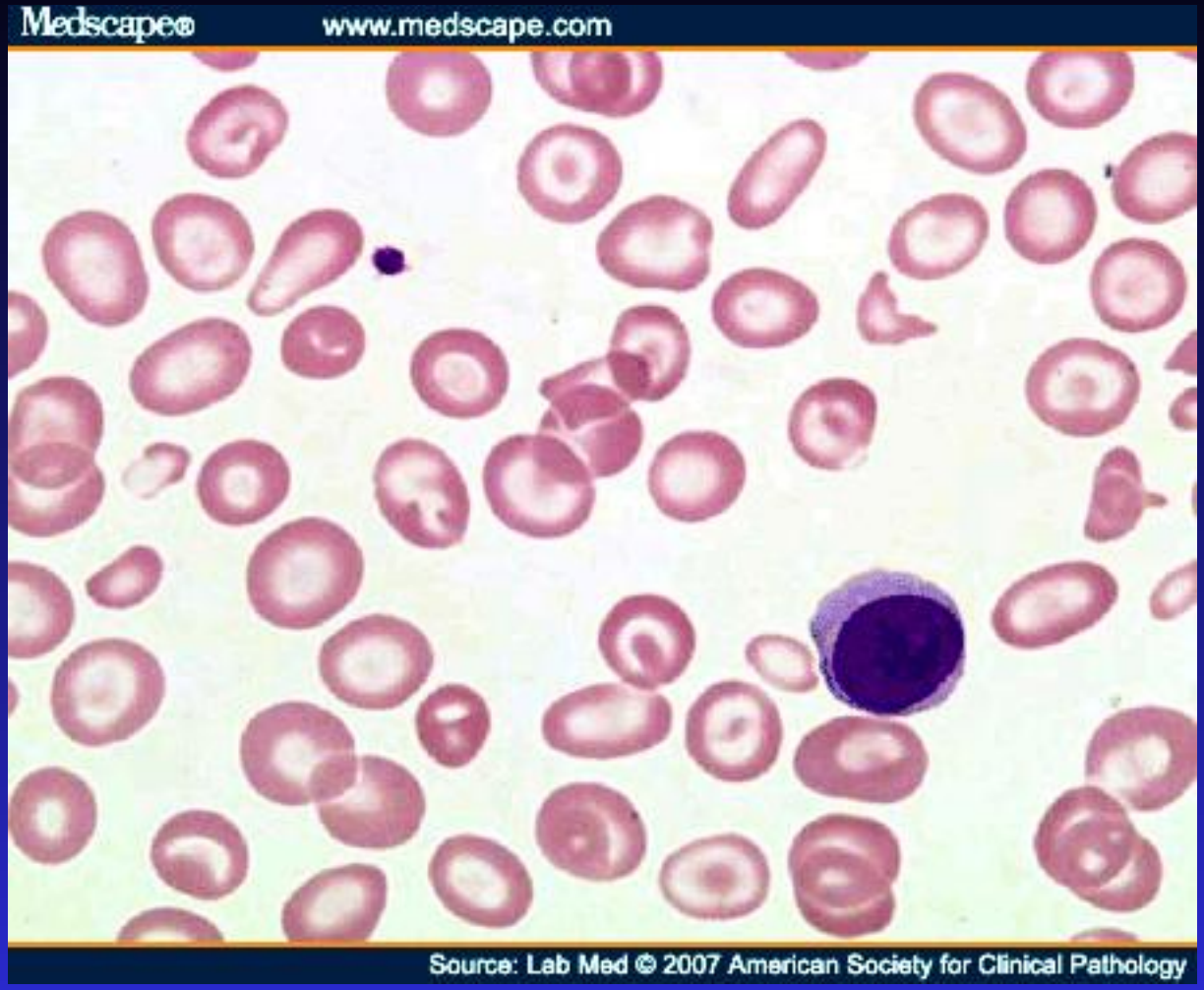
CBC PARAMETERS (mean ± 1sd): Normal, α-Thalassemia, Iron Deficiency

Data from the Hawai`i Hereditary Anemia Project (1992)




Hb H Prep with
Brilliant cresyl blue

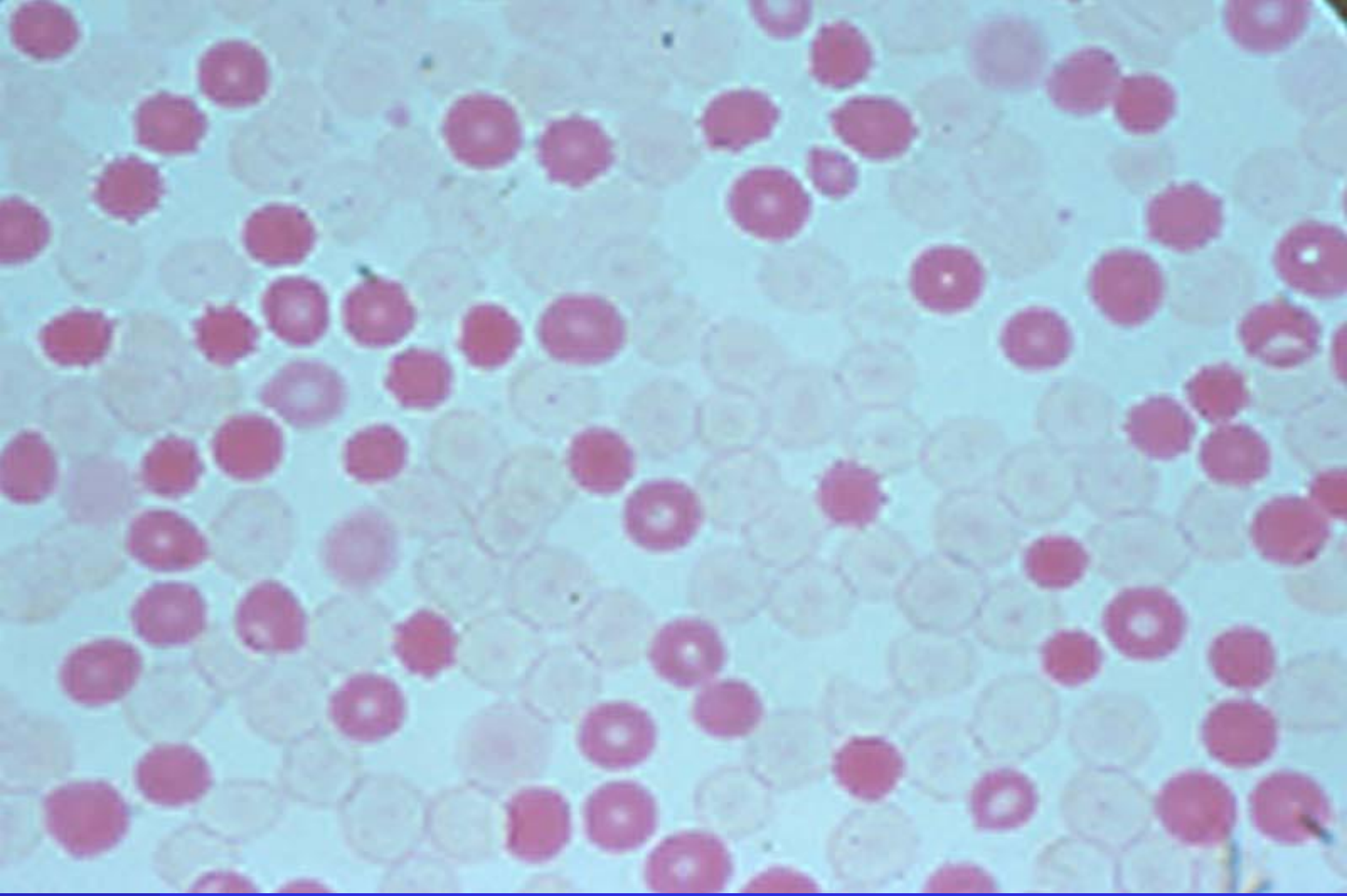

α thalassemia
Hydrops fetalis



Peripheral blood smear: Hb H disease

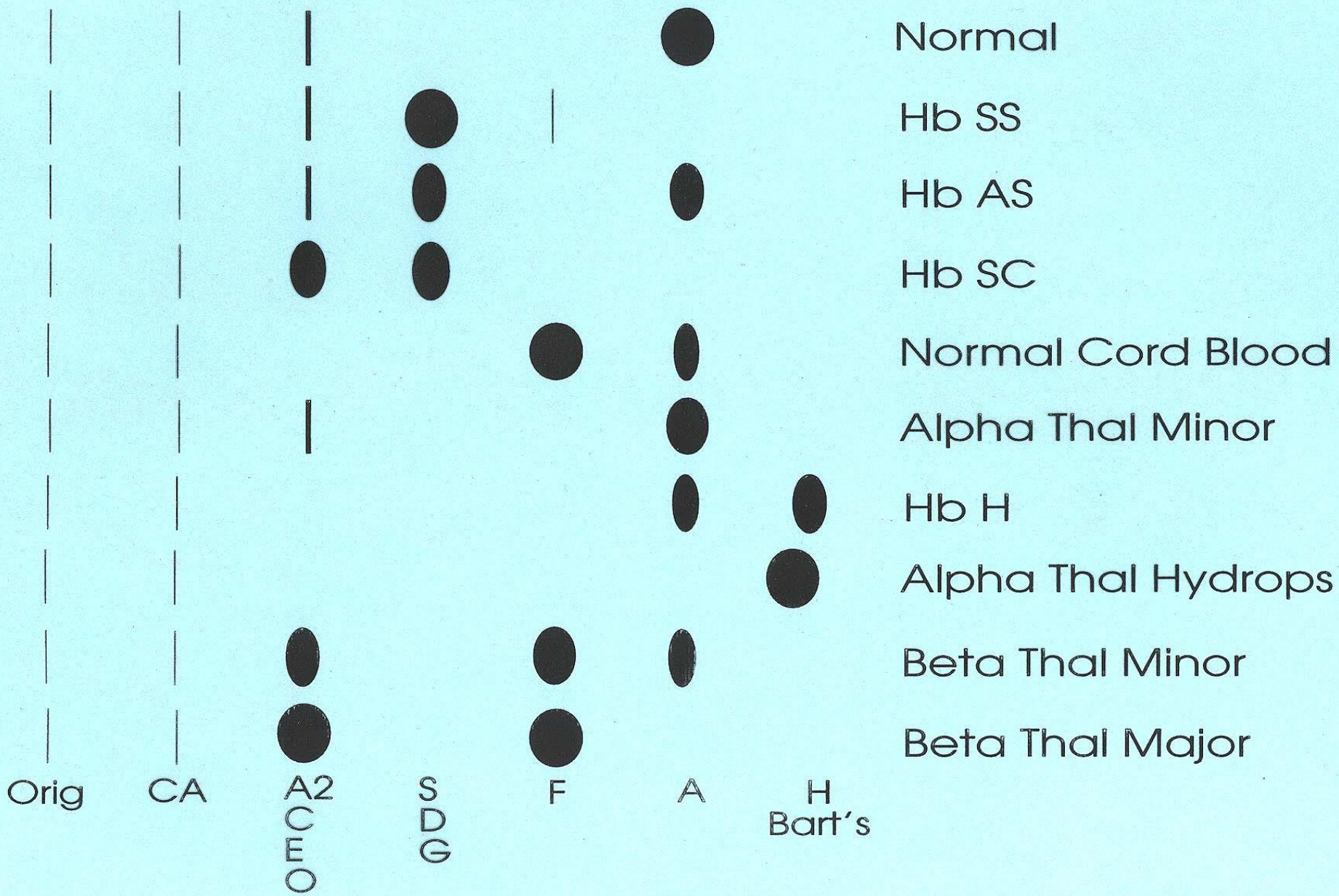
β Thalassemia

- Usually point mutation in the control region chr 11
- β^+ has minimal production
- β^o has no production
- β^+/β^+ or β^o/β^o is β thal major or Cooley's anemia
- Often not apparent at birth until β chain takes over γ chain production
- High Hb A2, Hb F
- Related: Hb Lepore (δ−β 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

	α-Thal Minor	Hb H	β-Thal	Iron Def	Hb E
RBC	↑	↑	↑ ↑	↓	↑
Hb	↓	↓ ↓	↓	↓ ↓	↓
Hct	sl ↓	↓ ↓	↓	↓ ↓	↓
MCV	↓	↓ ↓	↓ ↓	↓	↓
MCH	↓	↓ ↓	↓	↓	↓
MCHC	↓	↓ ↓	↓	↓	N
RDW	↑	↑ ↑	↑	↑ ↑	↑
ZPP/FEP	N	N	sl ↑	↑	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

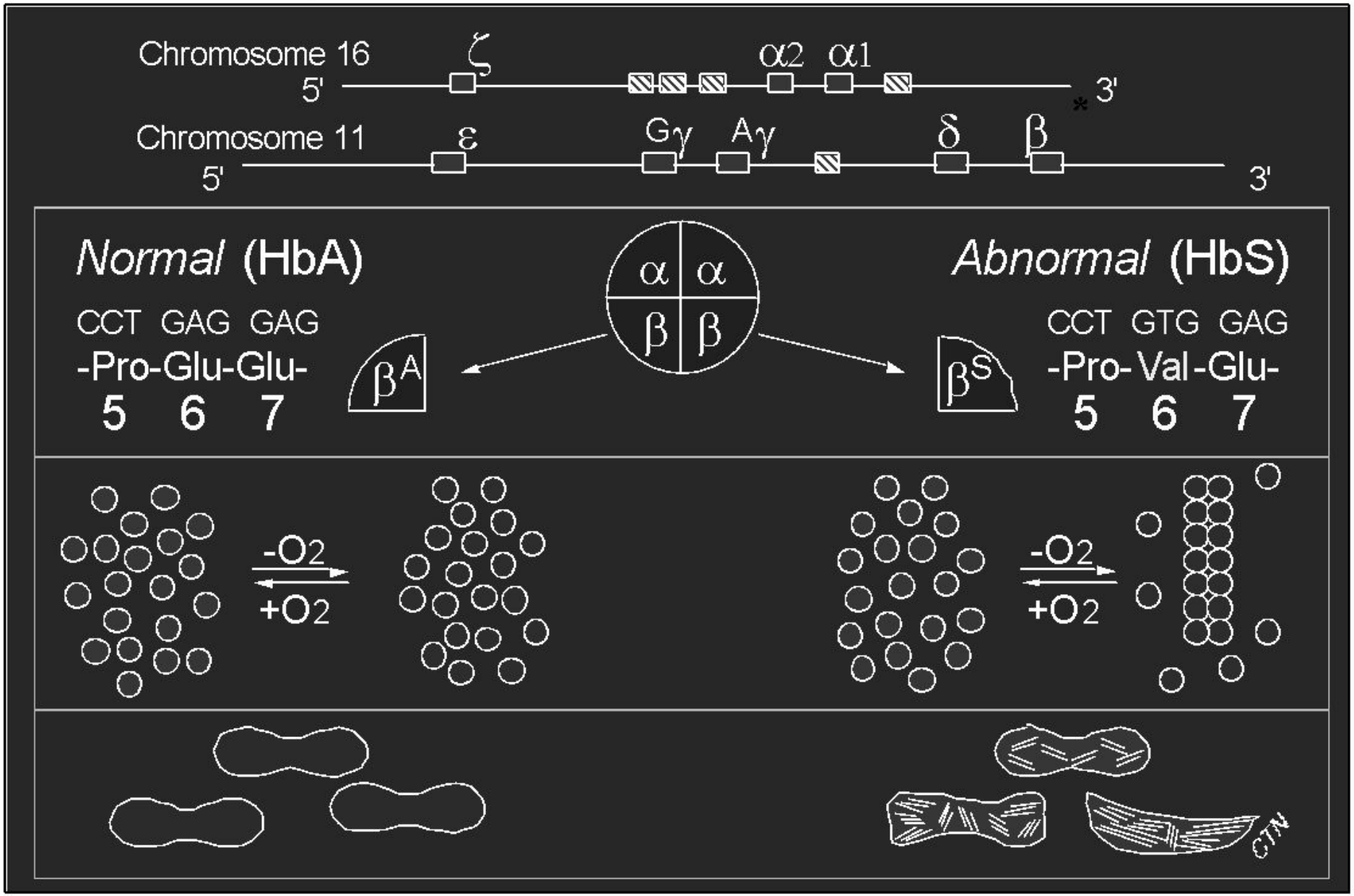
Thalassemia

- 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

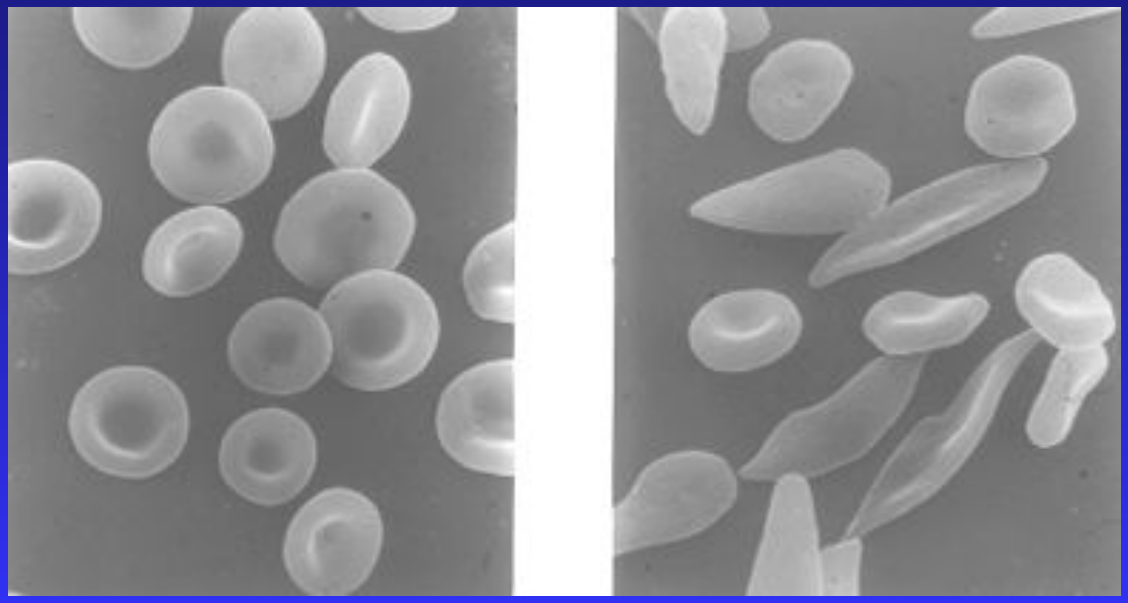
Sickle Cell Mutation



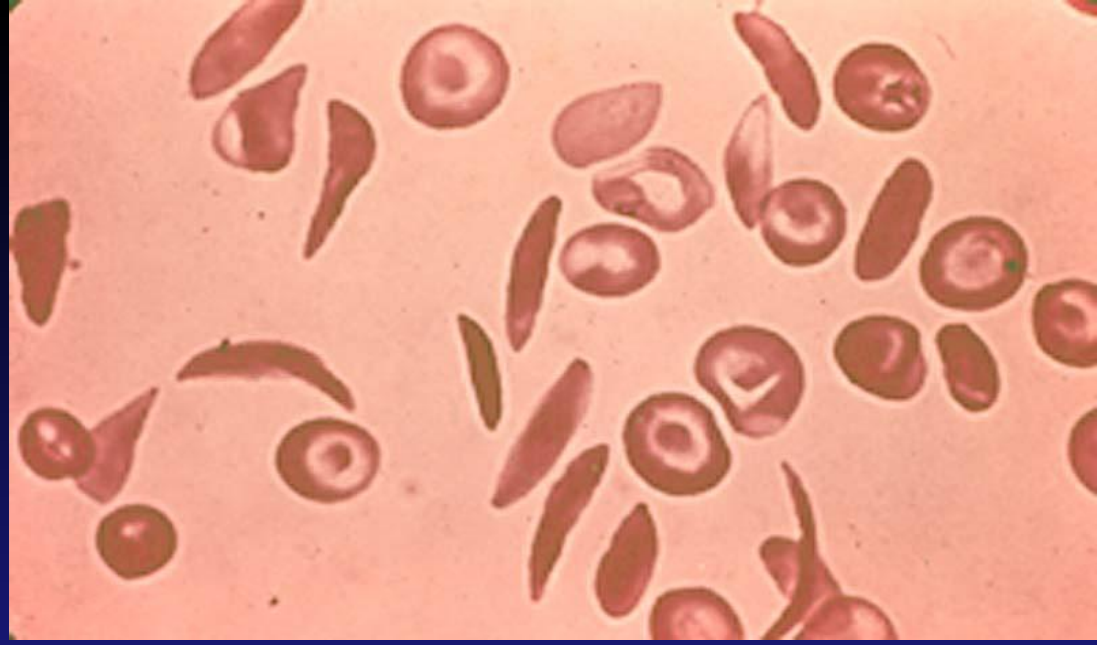
Red Blood Cells from Sickle Cell Anemia

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

OXY-STATE ⟷ **DEOXY-STATE**



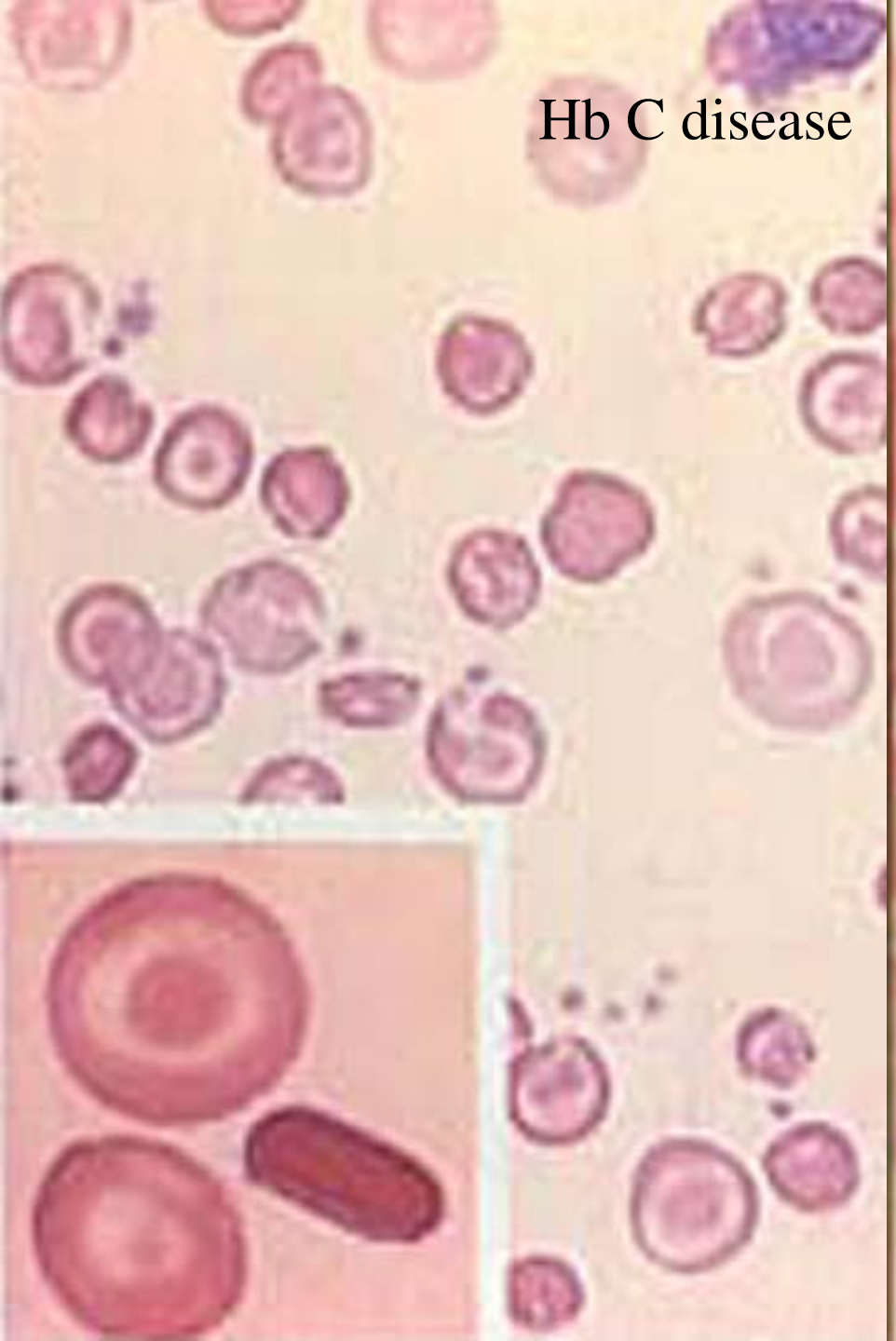
Hb S

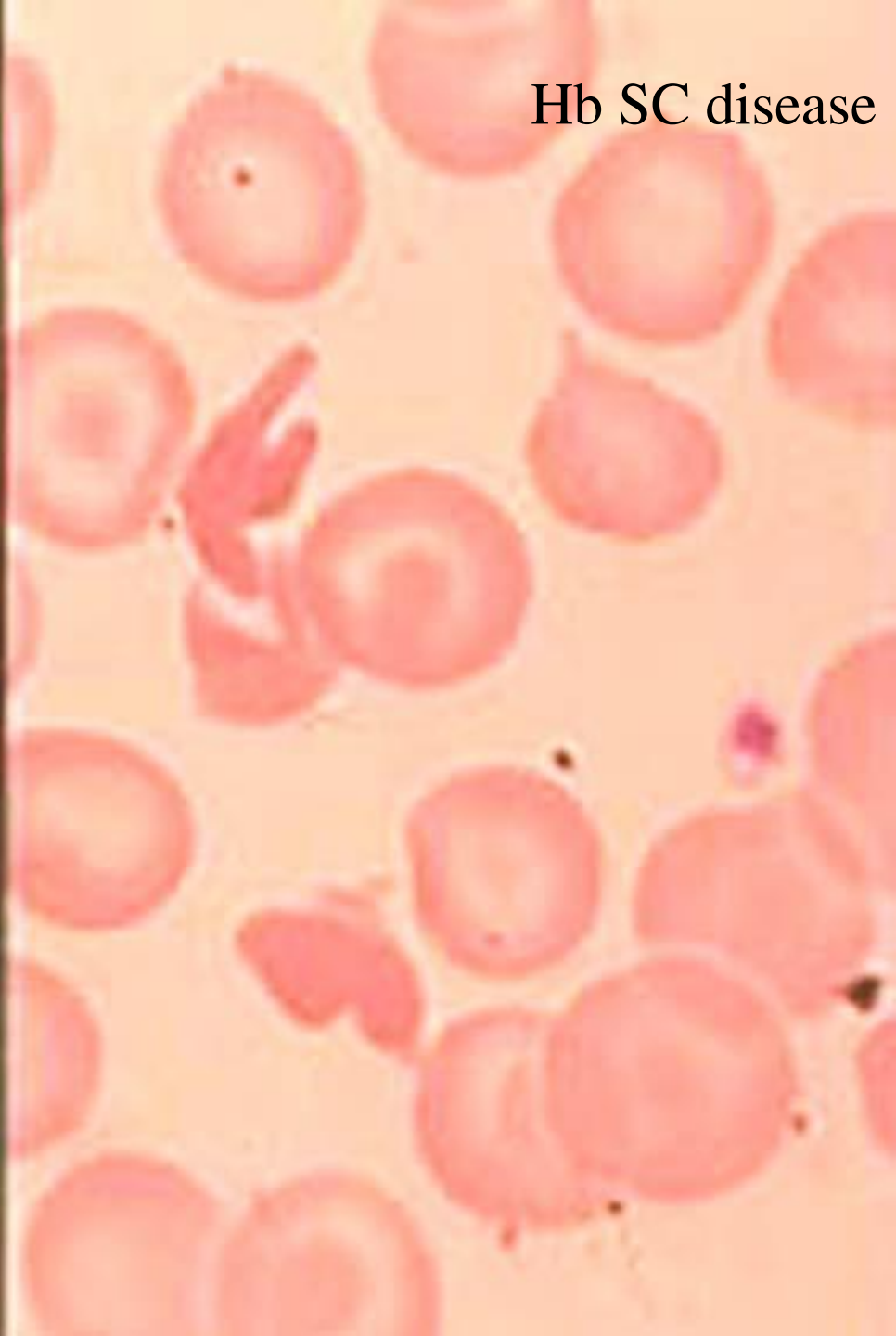


- Sickling Hb
- Autosomal
- Sickle crisis in low oxygen condition
- β6 glutamate to valine substitution
- Prevalent in Eastern Africa
- Solubility test
- Sickling test (meta-bisulfite)

Other Hemoglobinopathies

- Hb C (β6 Glu-Lys) in Western Africa
  - Cigar-like crystals
  - Billiard ball cells
  - Folded cells
- Hb SC disease
  - Washington monument cells
  - Mitten shape
- Hb E (β26 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

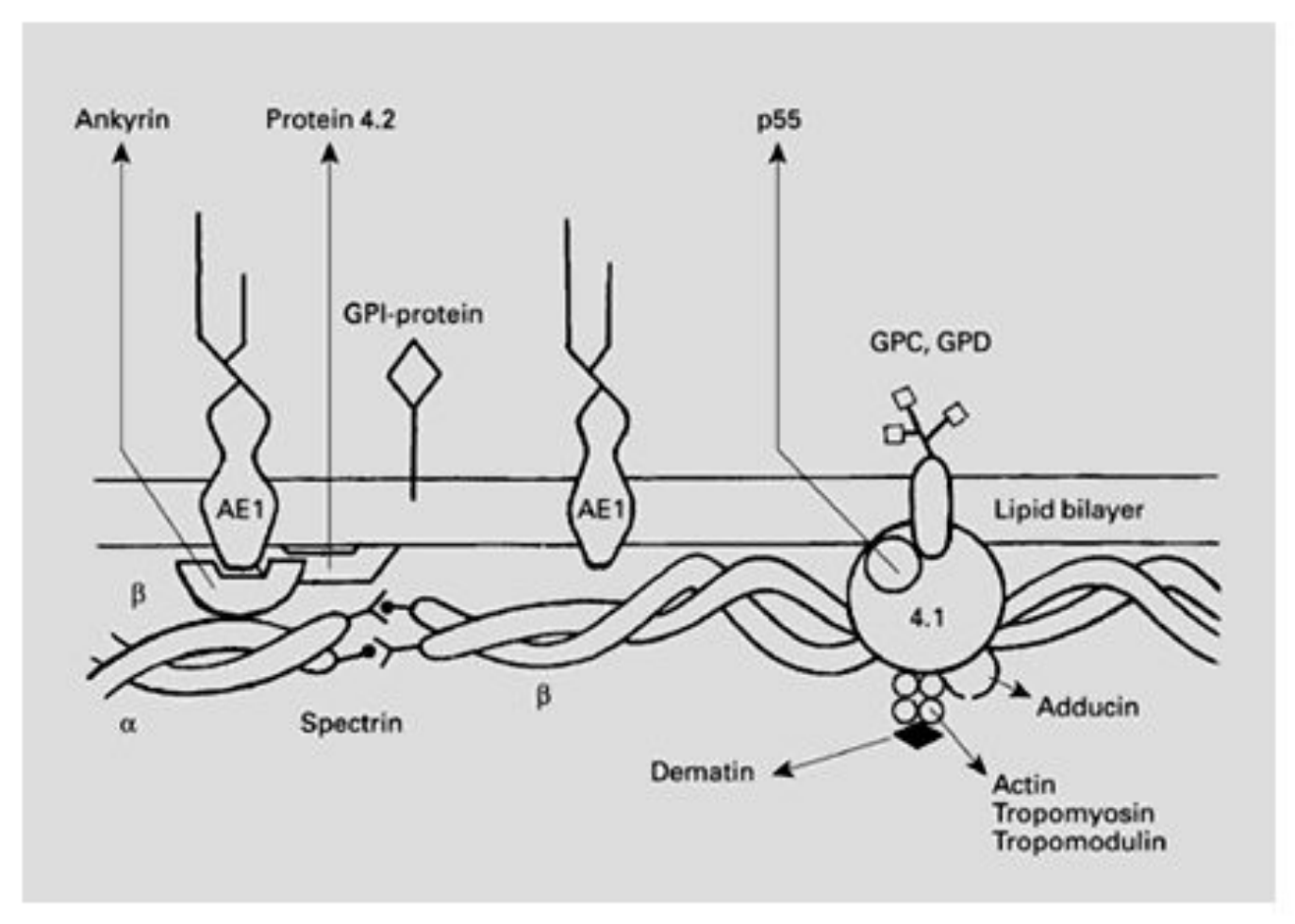
Review red blood cell disorders

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