Hemolytic Anemias

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Anemia

- Reduced red cell mass below the normal limit for age and sex of patient
  - In practice, a hemoglobin level below the normal limit for age and sex of patient

Classifications of Anemias

- Morphological classification- Based on size of RBCs and their hemoglobin content
  - Normocytic vs Microcytic vs Macrocytic
  - Normochromic vs Hypochromic

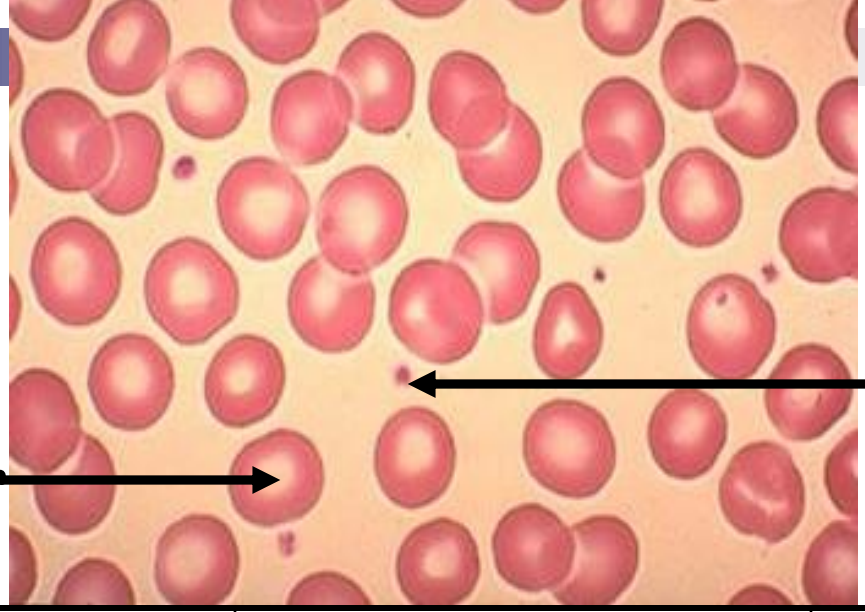
NOTE: The morphological classification suggests an etiologic differential which is confirmed by additional tests

- Etiological Classification
  - Decreased Hgb and/or RBC production
  - Defects of red cell survival **(Hemolytic Anemias)**

Automated Blood Count

AUTO BLOOD CT WITH AUTO DIFF

			Reference
HEMOGLOBIN	15.5	g/dL	[13.7-17.3]
HEMATOCRIT	0.46	L/L	[0.39-0.49]
RED BLOOD CELL COUNT	4.95	X10^12	[4.37-5.74]
MCH **Mean cell Hb**	31.3	pg	[26.5-34.0]
MCHC **Mean cell Hb concentration**	33.4	%	[31.5-36.3]
RDW-CV **Red cell distribution width**	13.4	%	[11.5-14.5]
MCV **Mean cell volume**	94	fL	[80-98]
NUCLEATED RBC %	0.0	/100WC	
NUCLEATED RBC COUNT	0.00	X10^9	[0.00-0.00]
PLATELET COUNT /L	171	X10^9	[150-450]
WHITE BLOOD CELL COUNT	4.5	X10^9	[3.2-9.8]
NEUTROPHIL %	60.2	%	[37.0-80.0]
LYMPHOCYTE %	26.8	%	[10.0-50.0]
MONOCYTE %	10.1	%	[0.0-12.0]
EOSINOPHIL %	2.7	%	[0.0-7.0]
BASOPHIL%	0.2	%	[0.0-2.0]
NEUTROPHIL COUNT	2.7	X10^9	[2.0-8.6]
LYMPHOCYTE COUNT	1.2	X10^9	[0.6-4.2]
MONOCYTE COUNT	0.5	X10^9	[0.0-0.9]
EOSINOPHIL COUNT	0.12	X10^9	[0.00-0.70]
BASOPHIL COUNT	0.01	X10^9	[0.00-0.20]



	Normal Range	Decreased below lower limit =	Increased above upper limit =
Hgb g/dL M F	14 - 18 12 - 16	Anemia	Polycythemia
MCV in fL	80 - 98	Microcytic	Macrocytic
MCH in pg	27 - 34	Hypochromic	Hyperchromic
Reticulocyte: % Abs /c mm	0.5 – 1.5 20k –100k	(usually seen in aplastic anemia or myeoldysplasia)	(usually seen in hemolytic anemia)

Use of reticulocyte count in the evaluation of anemias

HIGH Retic Index >2% or Absolute count >100,000/ul

- HIGH Production Anemias
- RESPONSE TO BLOOD LOSS
- HYPERSPLENISM
- HEMOLYTIC ANEMIAS

Low Retic Index <2% or Absolute count <100,000/ul

- LOW Production Anemias
- HYPOPROLIFERATIVE
- MATURATION DEFECTS

Classification of Hemolytic anemias

- Red cell abnormality
  - Hereditary
    - Hemoglobin Abnormalities
      (thalassemias, sickle cell anemia)
    - Membrane defect (spherocytosis, elliptocytosis etc)
    - Enzyme defect
      (Glucoze-6-Phosphate-Dehydrogenaze (G6PD) deficiency, Pyruvate kinase (PK) deficiency)
  - Acquired
    - Membrane abnormality-paroxysmal nocturnal hemoglobinuria (PNH)

Classification of Hemolytic anemias …2

- Extracorpuscular factors
  - Immune hemolytic anemias
    - Autoimmune hemolytic anemia
    - Transfusion of incompatible blood
  - Nonimmune hemolytic anemias
    - Chemicals
    - Bacterial infections, parasitic infections (malaria), venons
    - Hemolysis due to physical trauma
      - hemolytic - uremic syndrome (HUS)
      - thrombotic thrombocytopenic purpura (TTP)
      - prosthetic heart valves
    - Hypersplenism

Hereditary Anemias

- Affect over 400 million people worldwide

- Basic mechanisms
  - Reduced hemoglobin synthesis
  - Reduced life span of red cells
  - Reduced / abnormal stem cells

Basic Mechanisms in Hereditary Anemias -1

- Reduced hemoglobin synthesis
  - **Quantitative** defect of globin chain synthesis = **Thalassemias**
  - Note: Iron deficiency is an acquired cause of reduced Hgb synthesis

Basic Mechanisms in Hereditary Anemias -2

- Reduced life span of red cells
  - **Qualitative** detects
    - Gobin chains = **Hemoglobinopathies**
    - RBC membrane or cytoskeleton = Abnormal shape
    - RBC enzymes
  - Hemolysis may occur predominantly in the spleen (=extravascular) or in the vessels (=intravascular)
    - With intravascular hemolysis,
      - Why are serum **haptoglobin** levels are reduced?
      - How can iron deficiency develop in chronic cases?

Basic Mechanisms in Hereditary Anemias -3

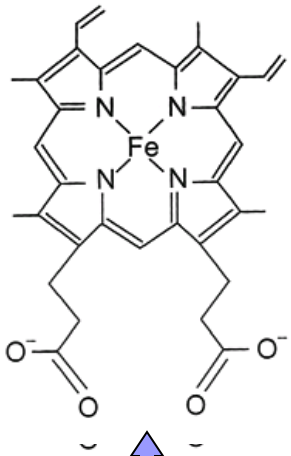
- Reduced / abnormal stem cells
  - Rare disorders like Diamond Blackfan syndrome and congenital dyserythropoietic anemia, etc.

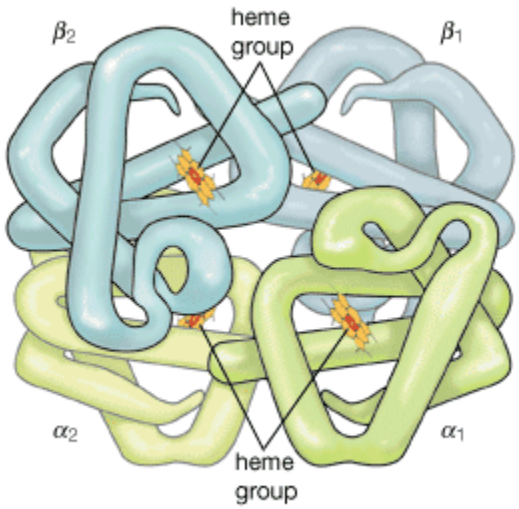
Quantitative defects of Hgb production: Thalassemia

- Thalassemias produce hypochromic and microcytic anemia
- Etiological differential diagnosis of hypochromic/microcytic anemias
  - Iron deficiency anemia
  - Thalassemia
  - Anemia of chronic inflammation
  - Sideroblastic anemia
  - Lead poisoning

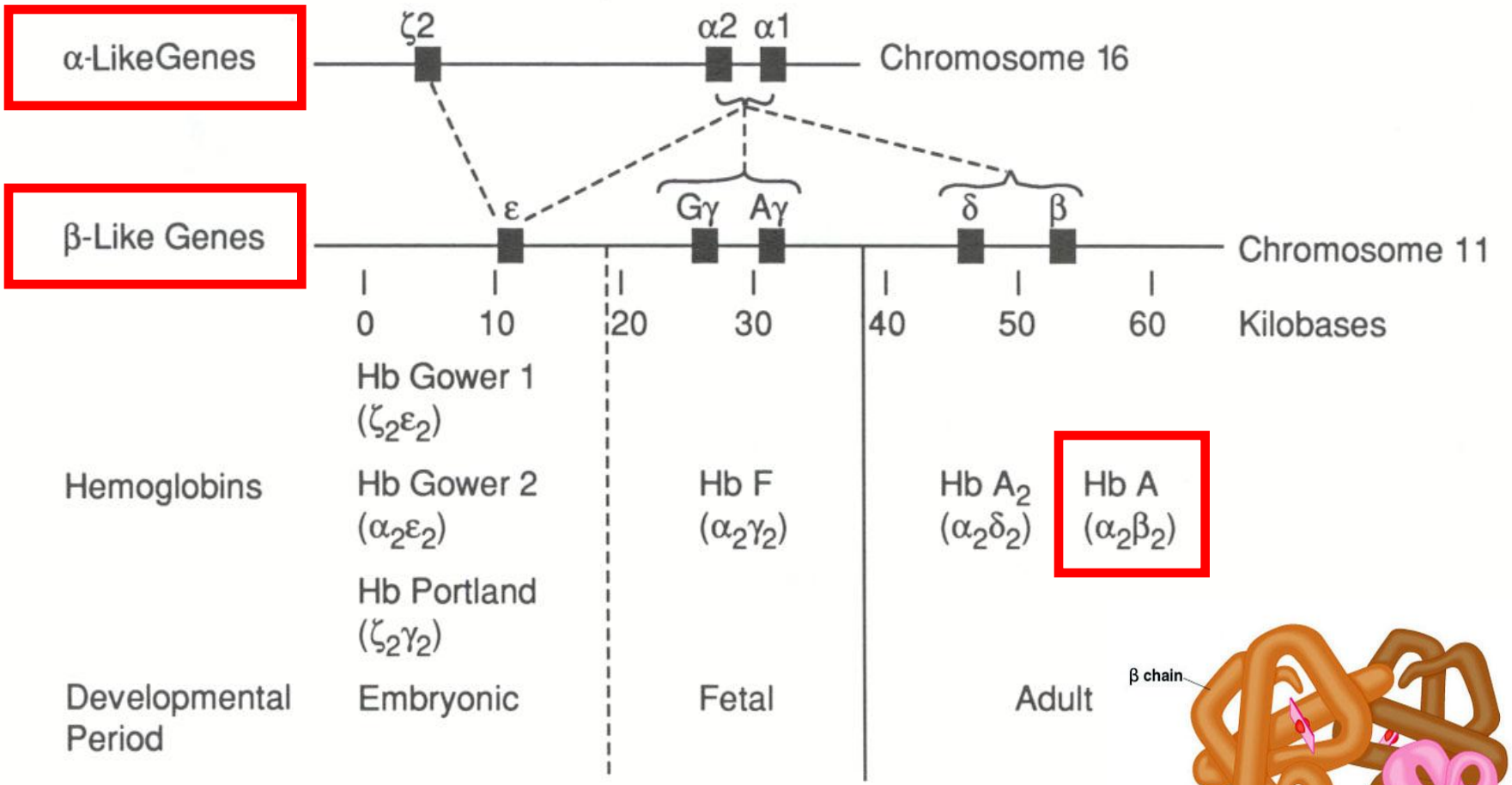
Hemoglobin

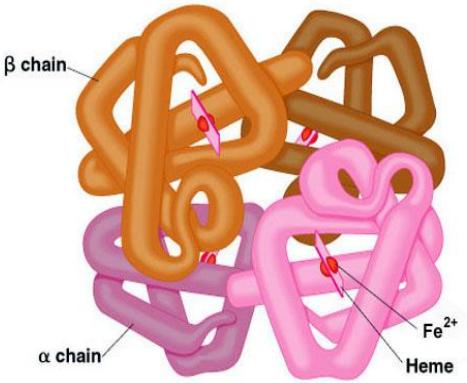
- >90% of an RBC mass is hemoglobin
- Heme – four pyroll rings and a central ferrous iron (Fe^{2+})
- Globin genes – Alpha and similar chains on chromosome 11 and beta and similar chains on chromosome 16
- Hemoglobin: 4 globin chains + 4 heme molecules. Subtypes
  - Adult Hb = α2β2
  - Fetal Hb = α2γ2
  - Hb A2 = α2δ2





Human Globin Genes





Thalassemia - pathogenesis

- Genetic alterations in promoter region of globin genes cause decreased amount of globin chain synthesis
- Decreased globin >> decreased hemoglobin synthesis >> small RBC (microcytic) with less Hgb (hypochromic)
  - The thalassemias are named after the affected globin chain – Alpha thalassemia and Beta thalassemia
  - The unaffected chain is produced in relative excess

Thalassemias – Mechanisms of anemia

Adult Hb = α2β2
Fetal Hb = α2γ2
Hb A2 = α2δ2

- Beta Thalassemias
  - Decreased synthesis of β chains – compensatory increase in γ and δ chains – increased levels of fetal Hb (α2γ2) and Hb A2 (α2δ2)
  - The excess α chain are toxic to RBC -- markedly reduced life span of RBC (= hemolytic anemia)
  - Tetramers of α chains have very high O_2 affinity – poor delivery of oxygen to tissues

Thalassemias – Mechanisms of anemia

Adult Hb = α2β2
Fetal Hb = α2γ2
Hb A2 = α2δ2

- Alpha Thalassemias
  - Alpha chains required for all types of hemoglobins
    - Complete absence of alpha chains is incompatible with normal fetal development
  - Excess β chains are mildly toxic to RBC (= hemolytic anemia)
  - Tetramers of γ chains (Hb Bart) and β chains (Hb H) have very high O_2 affinity – poor delivery of oxygen to tissues

Molecular Basis of Thalassemias

	β-Thalassemia	α-Thalassemia
Number of Globin Genes	2 (1 per chromosome 11)	4 (2 per chromosome 16)
Genetic abnormality	Point mutations in promoter region	Gene deletions
Molecular consequence	Either complete absence (β^0) or reduced transcription (β^+).	No transcription from affected gene(s).
Clinical Severity	Depends on number of β genes affected and type of abnormality	Proportional to number of affected α genes (1 to 4)

Thalassemia: Clinical consequences

- Pathophysiology:
  - Anemia of varying severity (reduced Hb synthesis + Hemolysis)
  - Tissue hypoxia – increased erythropoietin
  - Hyperplasia of bone marrow
  - Consequences of treatment (↑ iron from repeated transfusions)
- Severity of anemia varies greatly, depending on precise genetic defect
  - Normal >> asymptomatic microcytic anemia >> severe anemia >> intra-uterine death

Clinical Syndromes in Thalassemias:

- β-Thalassemia

  (type of genetic abnormality in parenthesis. Remember, β_0 is complete absence of β chain synthesis from that allele, β+ is reduced synthesis of β chain and β is normal level synthesis)

  - Minor (β^0/β or β^+/β)
  - Intermedia (β^0/β or β^+/β^+)
  - Major (β^+/β^+ or β^0/β^0) – Also called Cooley's anemia

- Key to color coding: Normal -- asymptomatic microcytic anemia -- severe anemia -- fetal death

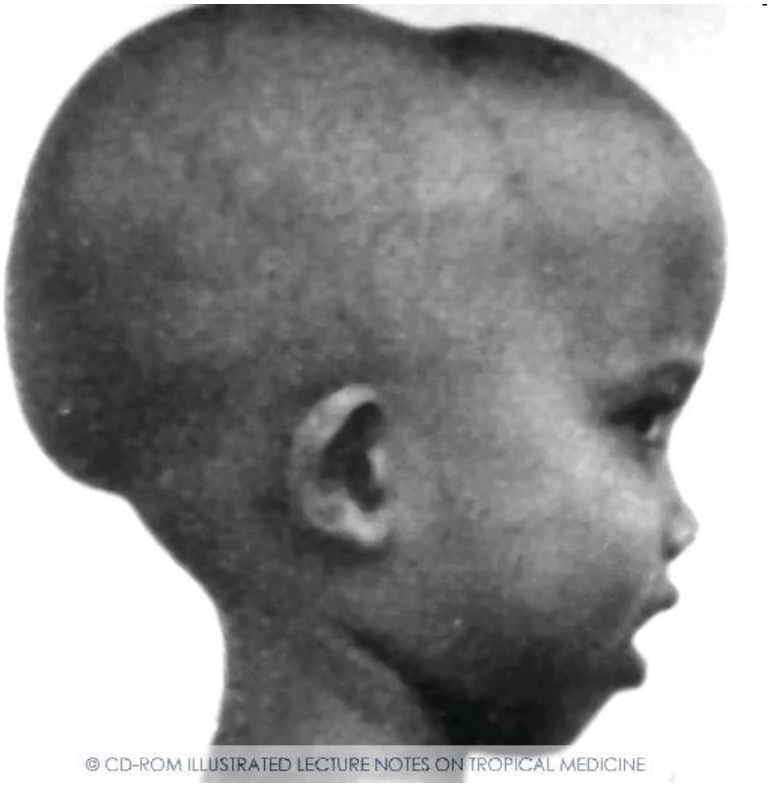
Clinical Syndromes in Thalassemias:

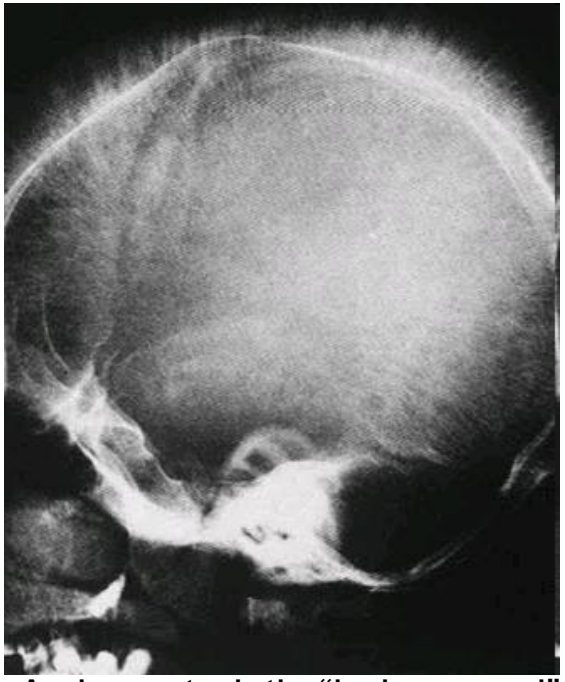
- α-Thalassemia
  (number of α genes with mutations shown in parenthesis)
  - Silent carrier state (1)
  - α-Thalassemia trait (2)
  - HbH (=β4) disese (3)
  - Hydrops fetalis (4)

- Key to color coding: Normal -- asymptomatic microcytic anemia -- severe anemia -- fetal death

Thalassemia Major

1. Severe hypochromic anemia – tissue hypoxia – stunted growth etc
2. Damage to RBC by excess α chains
   a. Destruction in spleen – enlarged spleen
   b. Compensatory increase in red cell production - marrow hyperplasia
   c. Dependence on blood transfusions – iron overload - cirrhosis





A characteristic “hair on end” abnormality produced by bone marrow hyperplasia and diploetic expansion

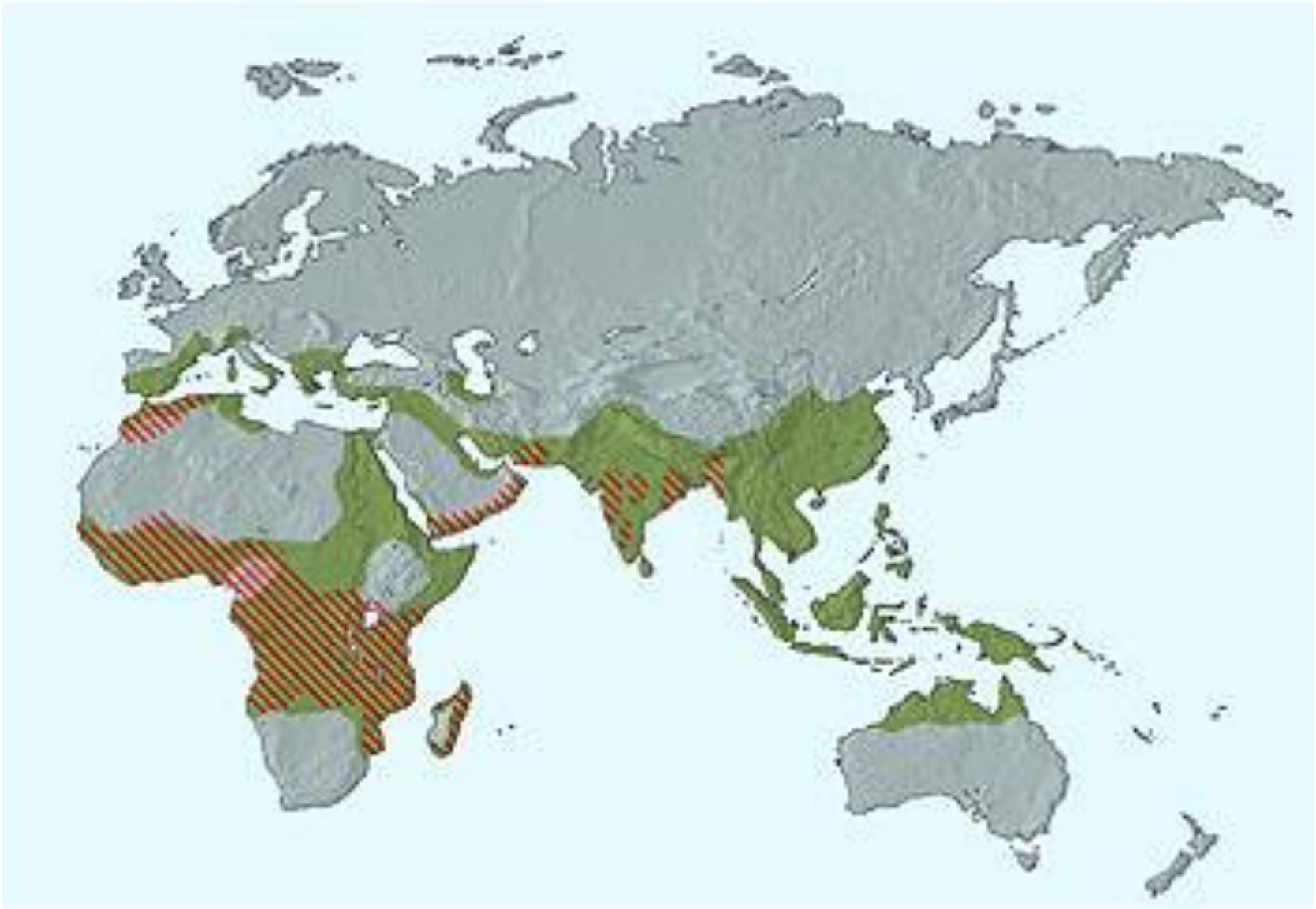
Qualitative Hgb Change : Hemoglobinopathies

- Sickle Cell Disease:
  - Chronic hemolytic anemia characterized by sickle-shaped red cells caused by homozygous inheritance of Hemoglobin S
  - Commonest type of hereditary anemia in US
    - The sickle-cell gene occurs widely throughout Africa and in countries with African immigrant populations, some Mediterranean countries, the Middle East, and parts of India

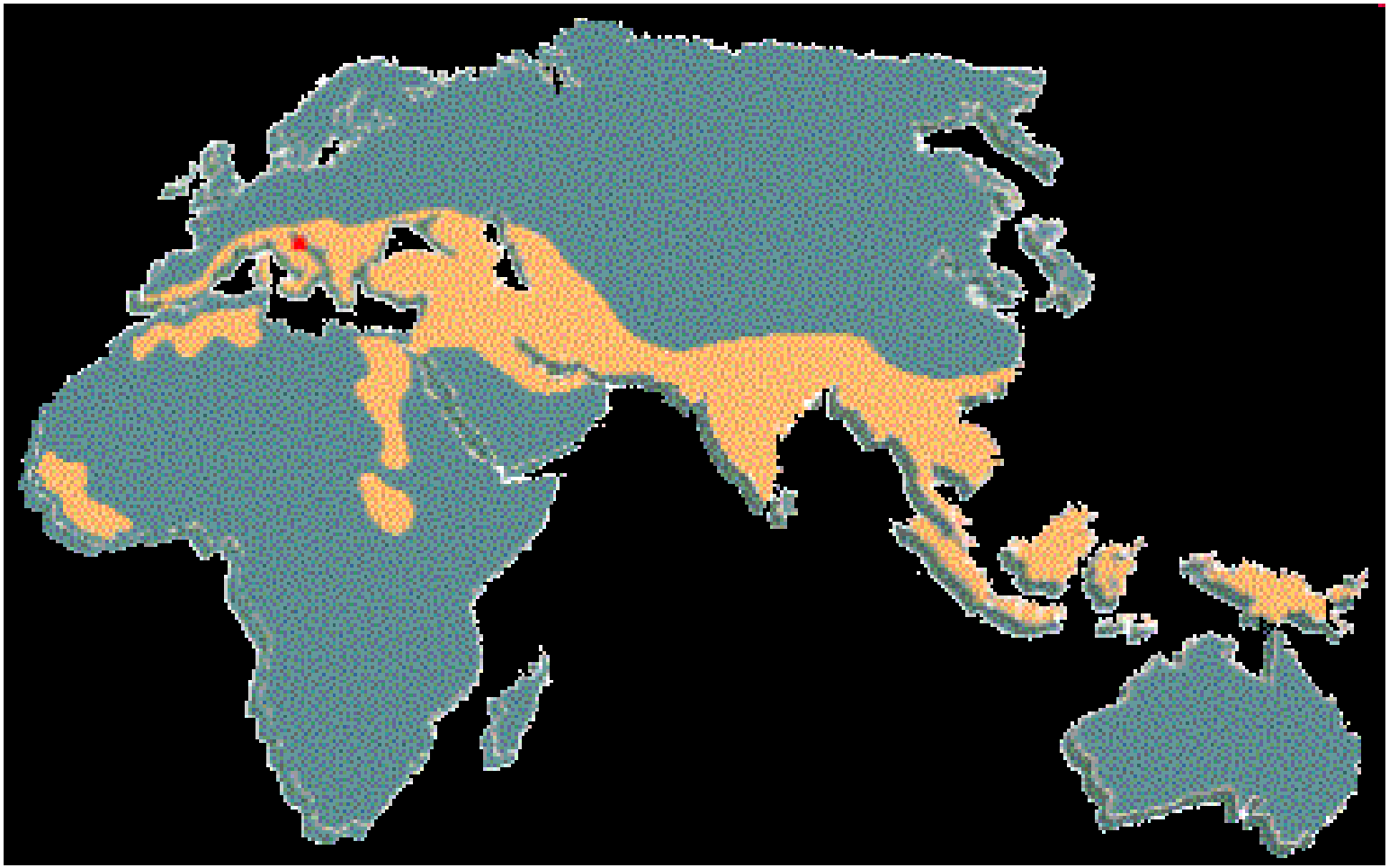
Prevalence of Thalassemia and Hemoglobinopathies

		US	World	Mximum incidence area
Thalassemia	Carriers	Very low	40 million Beta thal carriers	Mediterranean countries, Indian subcontinent, far East
	Disease	1,000	??	
HbS	Trait	2.5 million	<1% to >15%	Equatorial Africa
	Disease	90,000	“millions”	
HbC	Trait	>500,000		Equatorial Africa
	Disease	~6,000		

Sickle Cell trait = shown in orange stripes
High prevalence of malaria = shown in green



Prevalence of Alpha Thalassemia



Qualitative Hgb Changes: Hemoblobinopathies

- Usually single nucleotide difference in the coding region of the globin gene leads to single amino acid change -
  - In sickle Hgb, valine replaces glutamic acid in the 6th position of β chain
  - In Hgb C, lysine replaces the same glutamic acid
  - In Hgb E, lysine replaces glutamic acid at 26th position
- Over 1100 genetic variants of Hgb described
  - http://globin.cse.psu.edu/ for a comprehensive database

Hemoglobinopathies: Basic facts

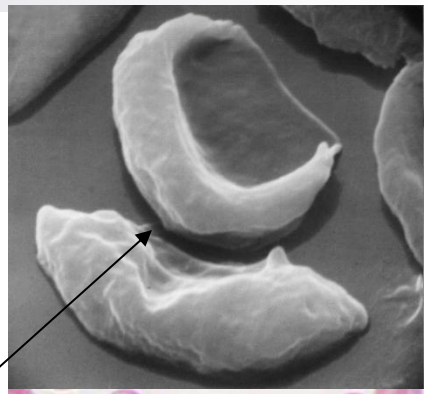
- Hgb S, C, E and D are the most prevalent
  - Heterozygous state = Trait
    - Denoted – AS, AE, AD etc. Or double heterozygous state such as SC, Sickle-Thal, etc
    - Offers some protection against falciparum malaria (Sickle cell trait)
      - 8% of African Americans are heterozygous for Hgb S
    - Can increase the severity of thalassemia (E, D)
  - Homozygous state = Disease
    - Hemolytic anemia (severe in Hb SS, mild in Hb CC)
    - Microcytosis due to reduced Hgb synthesis (E)

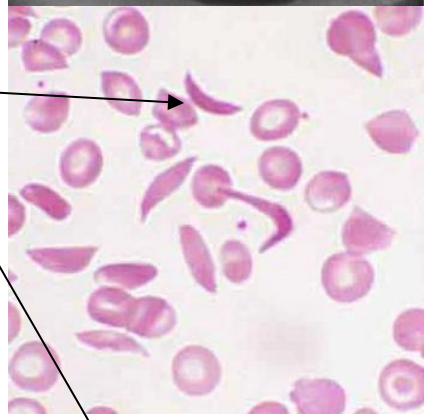
Hemoglobinopathies: More facts

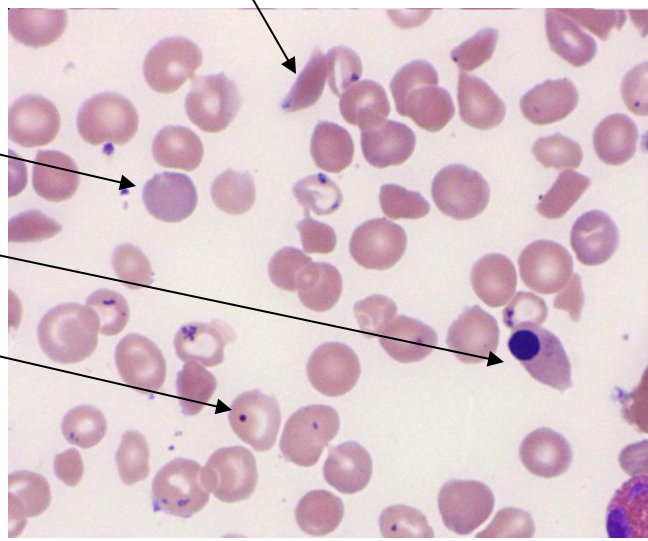
- Other effects of abnormal hemoglobins
  - Altered Oxygen affinity
  - Hemoglobin with oxydized iron (methemoglobin)
  - Unstable hemoglobins.
  - Abnormal chain termination due to “new” stop codon >> abnormal length of globin chain and reduced amount of globin chain (= like thalassemia).

Sickle Cell Anemia – Laboratory Findinges

- Anemia-normocytic or slightly macrocytic
- Leukocytosis (chronic neutrophilia)
- Thrombocytosis-usually mild<1000k/cmm
- Reticulocytosis
- Peripheral smear: few sickle shaped red cells, polychromatophilia, Nucleated RBC, Howell-Jolly bodies
- Hb - electrophoresis

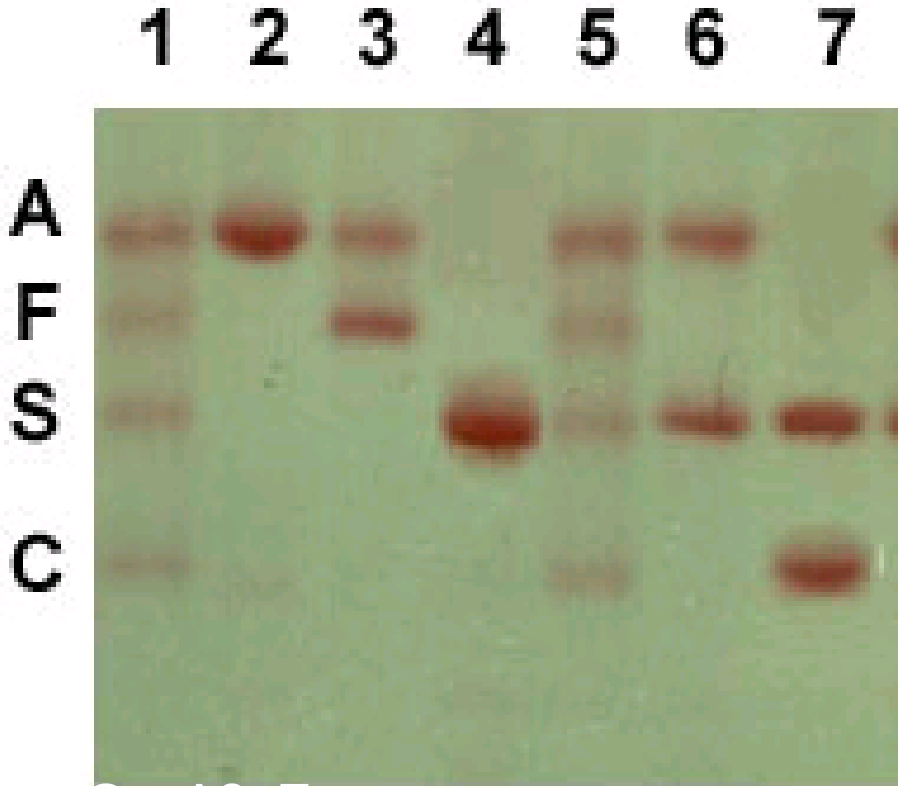




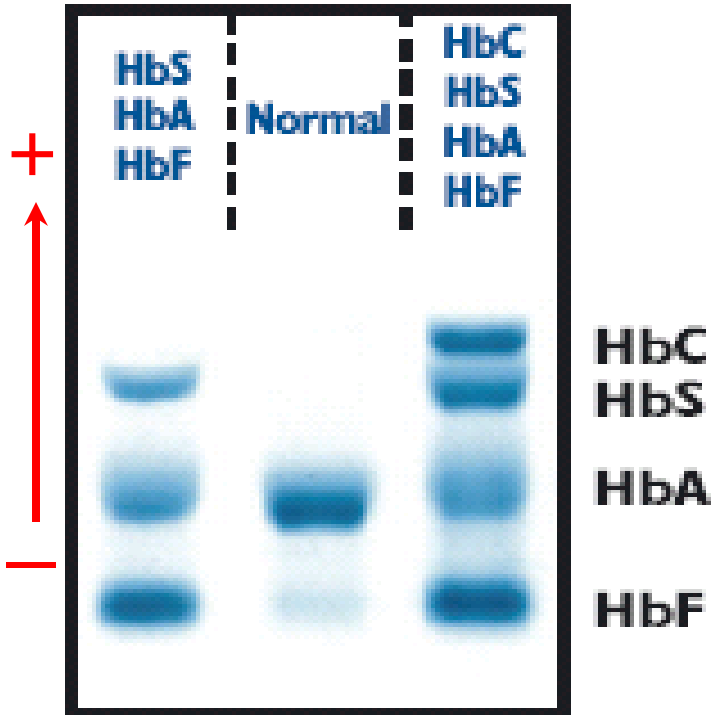


Hemoglobin Electrophoresis

At Alkaline pH



At Acid pH



Sickle Cell Anemia - Clinical Features

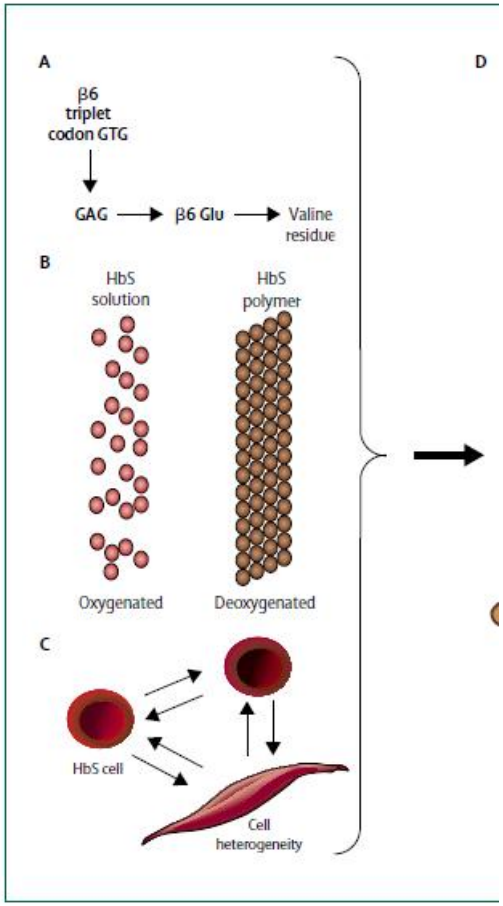
- Due to severe hemolytic anaemia
  - slow growth and development in children
  - bilirubin stones
  - congestive heart failure from chronic anemias and cardiac overload compensation
- Consequences of vaso-occlusion of the microcirculations (tissue ischemia and infarction)
  - infarction of spleen (“auto-spelnectomy”), brain, marrow, kidney, lung, aseptic necrosis of bone, and ophtalmic vascular lesions

Splenic sequestration, Acute lung syndrome, Priapism

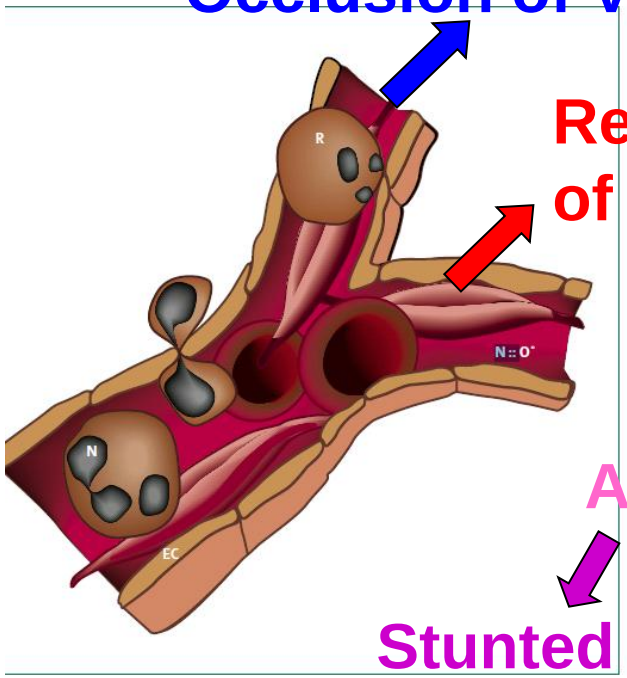
Pain, infarcts, ulcers, papillary necrosis

RBC Sequestration

Ischemia



Occlusion of Vessels



Reduced elasticity of red cells

Hemolysis

Anemia

Jaundice

Stunted Growth

Increased Erythropoiesis

Parvovirus

Aplasia

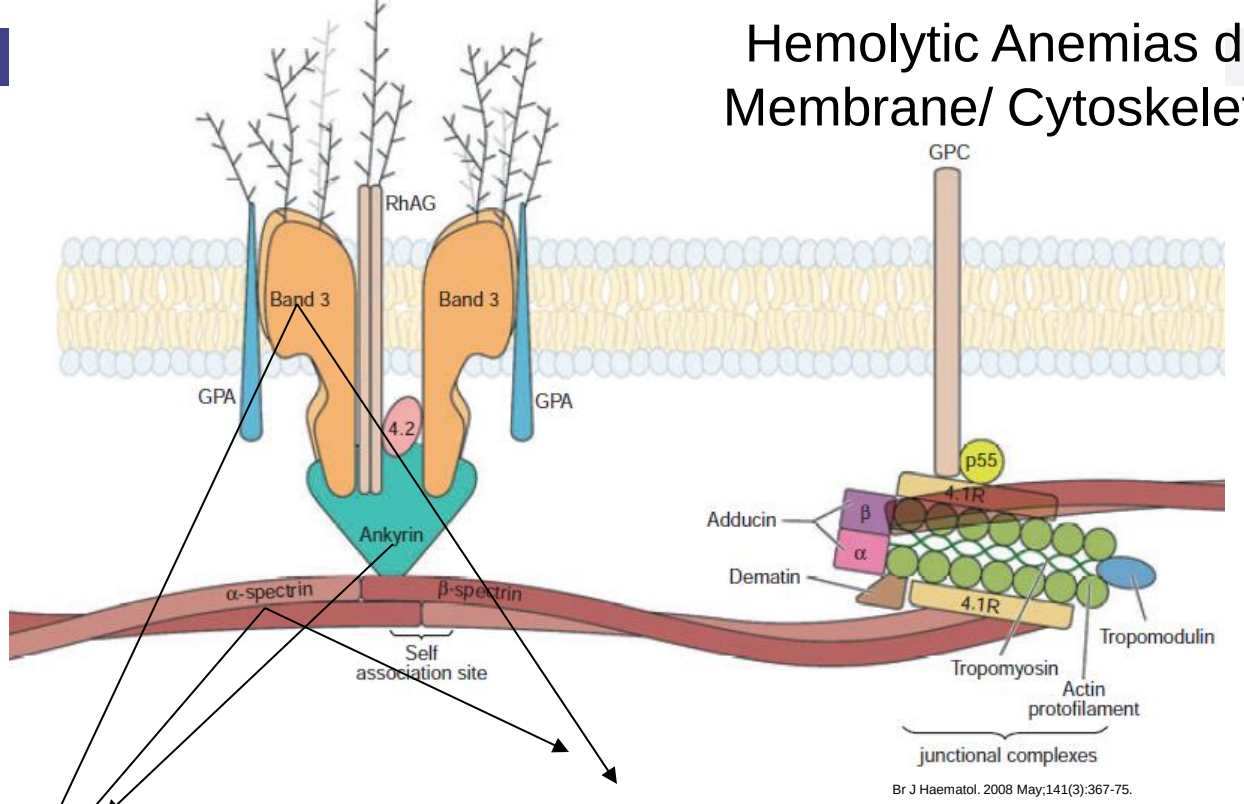
Sickle Cell Anemia - Therapy

- Preventive measures:
  - Infections (penicillin prophylaxis and pneumococcal vaccination)
  - Fever
  - Dehydratation
  - Acidosis
  - Hypoxemia
  - Cold exposure
- Blood transfusions for severe anemia
- New approaches to therapy
  - Activation of Hb F synthesis -5-azacytidine
  - Antisickling agents acting on hemoglobin or membrane
  - Stem cell transplantation, including umbilical blood stem cells

Hereditary Hemolytic Anemias: Other Qualitative Defects

- Red cell membrane/ cytoskeleton
- Enzymes

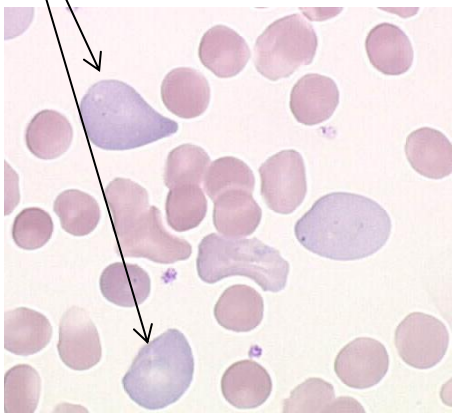
Hemolytic Anemias due to RBC Membrane/ Cytoskeleton Defects



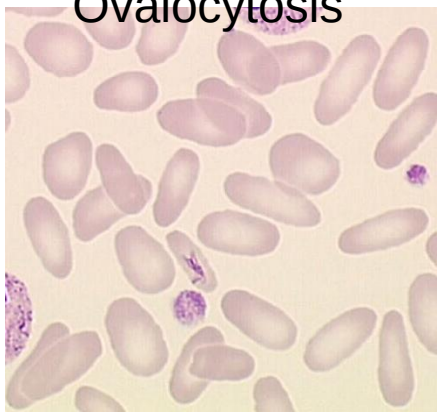
Br J Haematol. 2008 May;141(3):367-75.

Increased Reticulocytes = Increased RBC production in response to hemolysis

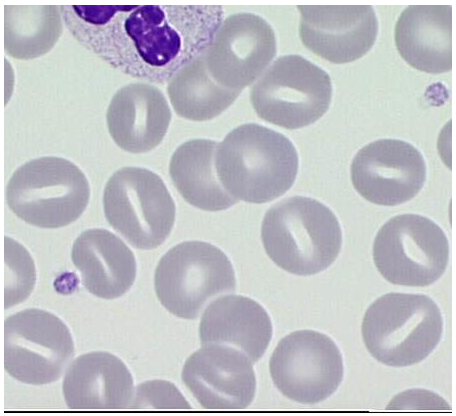
Spherocytosis



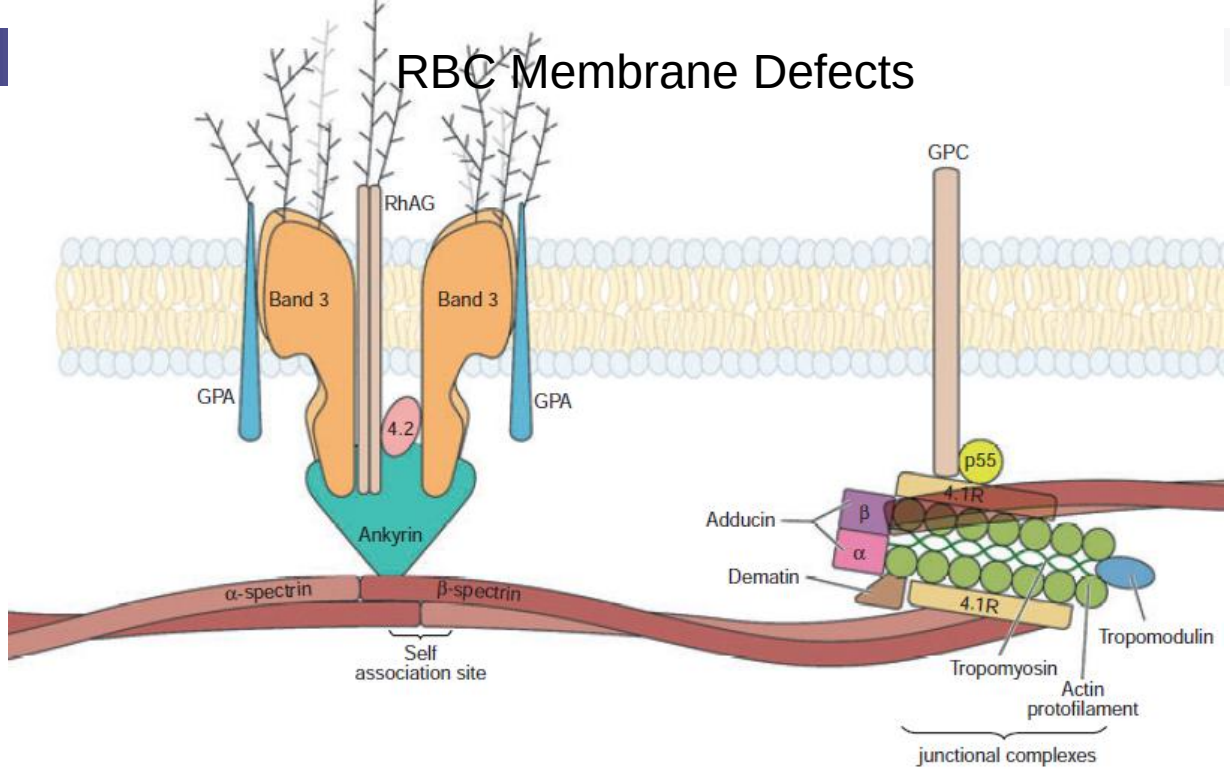
Elliptocytosis/ Ovalocytosis



Stomatocytosis



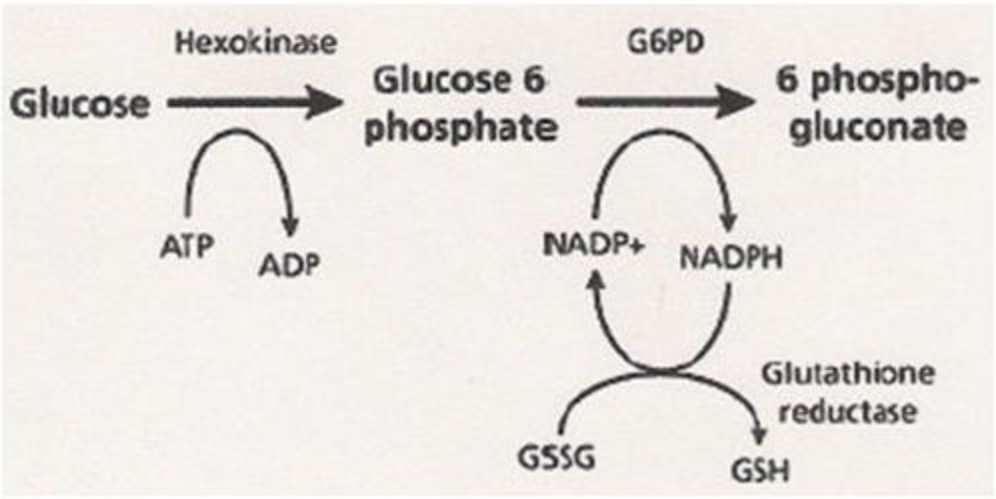
RBC Membrane Defects



	Genes involved	Population Affected	Mutation Frequency	Severity of Anemia	Splenectomy effective?
Spherocytosis	Ankyrin, Spctrin, Band 3	N European	1 in 3000	Mild 20%, Mod 60%, Severe 20%	Yes
Elliptocytosis	Spectrin	W Africa	1 in 50	No/mild 90%, Severe 10%	Yes
Ovalocytosis	Band 3	S Asia	1 in 20	No/minimal	Some
Stomatocytosis	Ion transporters	Worldwide	Rare	Mild	Contraindicated

RBC Enzyme Defects

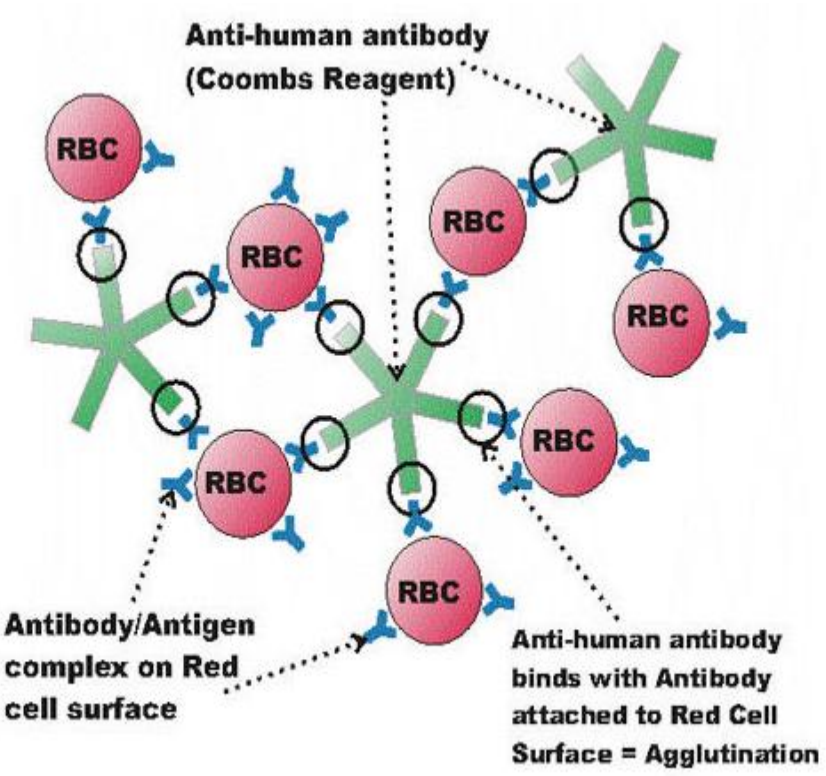
- G-6-PD Deficiency
  - Affects about 10% of US black population. Also many in Africa, middle east, and south Asia – offers protection against falciparum malaria
  - Enzyme required to regenerate NADPH and glutathione, which are critical to avoid oxidative injury



  - Mutation leads to shorter half life of enzyme in RBC
    - No protein synthesis in mature RBCs ∴ Older RBC become deficient in G-6-PD
  - Exposure to oxidative drugs or certain beans precipitates attack of hemolysis
    - Hemoglobin is oxidized and precipitates as Heinz bodies.
    - Self-limited because only older RBCs are eliminated but young ones are unaffected

Acquired Hemolytic Anemias

- Immune:
  - Autoimmune
  - Allo-immune
    - Transfusion
    - Feto-maternal
- Non-immune
  - Infections
  - Mechanical
  - Others



Autoimmune Hemolytic Anemia

- Warm AIHA:
  - Abs bind at 37 °C
  - Usually IgG and not C' binding,
  - extravascular hemolysis.
- Cold AIHA:
  - Abs bind 4-30 °C.
  - Usually IgM and fix C'.
  - Usually Intravascular hemolysis.

Autoimmune Hemolytic Anemia

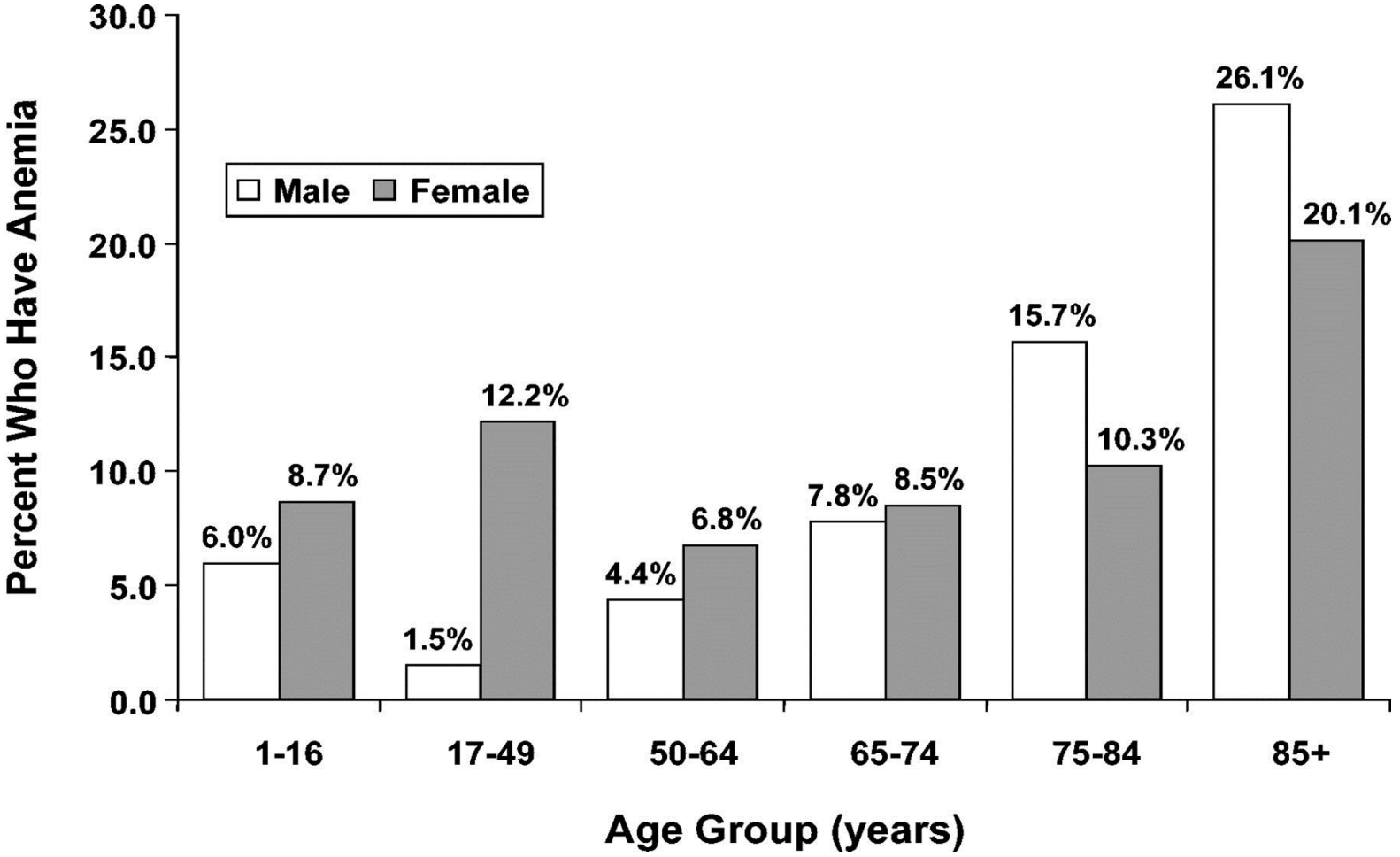
Warm Antibody Type	
Primary	Idiopathic
Secondary	Lymphoproliferative disease (e.g. CLL, lymphoma) Autoimmune disorders (e.g. SLE) Drugs (e.g. penicillin, quinidine or α-methyldopa)
Cold Antibody Type (less dangerous if cold exposure can be avoided)	
Acute	Mycoplasma infection, infectious mononucleosis
Chronic	Lymphoproliferative diseases.


TEST EARLY FOR
SICKLE CELL
USA
37
2004

Brief History of HbS

- 1910: Chicago physician, James B. Herrick, described patient of anemia with "sickle shaped.“ red cells.
- 1927, Hahn and Gillespie showed that sickling was related to low oxygen.
- 1940, Sherman noted alteration of Hgb due to low O2
- 1948, Janet Watson noted that fetal Hgb does not cause sickling
- 1948, Linus Pauling and Harvey Itano showed HbS to be different by protein electrophoresis
- 1956, Vernon Ingram and J.A. Hunt sequenced sickle hemoglobin. This made sickle cell disease the first genetic disorder whose molecular basis was known.

Percentage of persons considered anemic according to age and sex



Guralnik, J. M. et al. Blood 2004;104:2263-2268