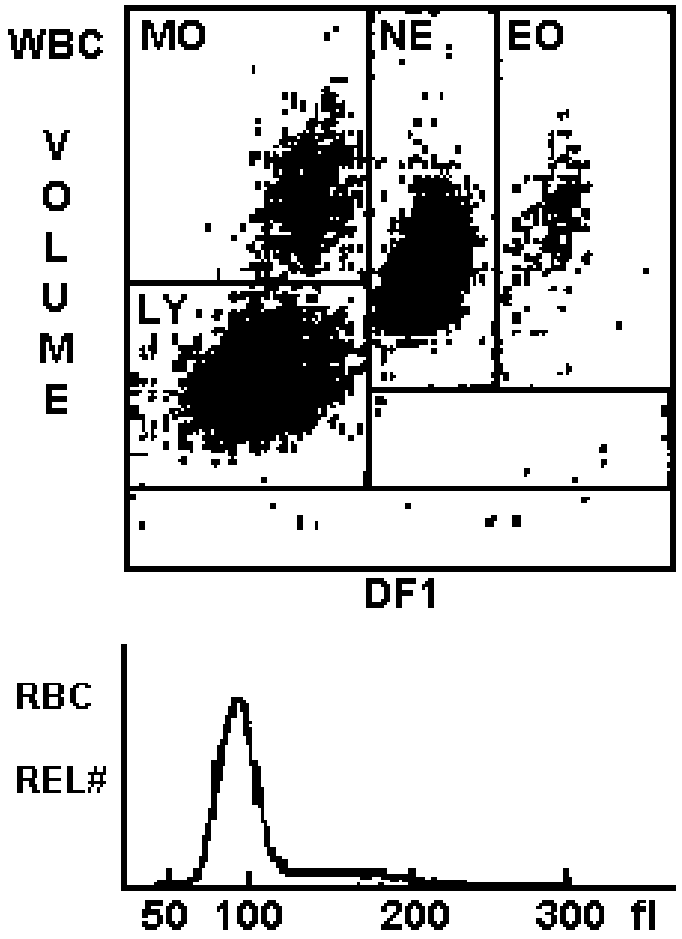
RED BLOOD CELL DISORDERS

Decreased erythrocyte number → ANEMIA
Increased erythrocyte number → POLICYTEMIA

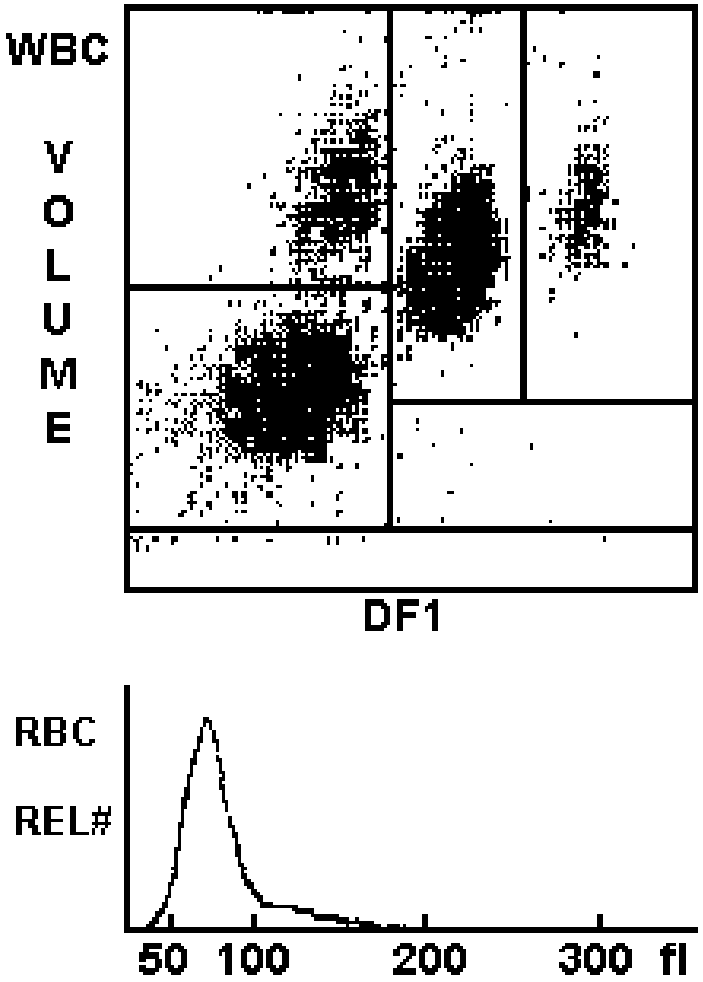
Adult Reference Ranges for Red Blood Cells

	Men	Women
Hemoglobin (g/dL) HGB	13.6-17.2	12.0-15.0
Hematocrit (%) HCT	39-49	33-43
Red cell count (10^6/μL) RBC	4.3-5.9	3.5-5.0
Reticulocyte count (%)	0.5-1.5	
Mean cell volume (MCV; μm^3)	82-96	
Mean corpuscular hemoglobin (MCH; pg)	27-33	
Mean corpuscular hemoglobin concentration (MCHC; g/dL)	33-37	
RBC distribution width (RDW; coefficient of variation of volume)	11.5-14.5	



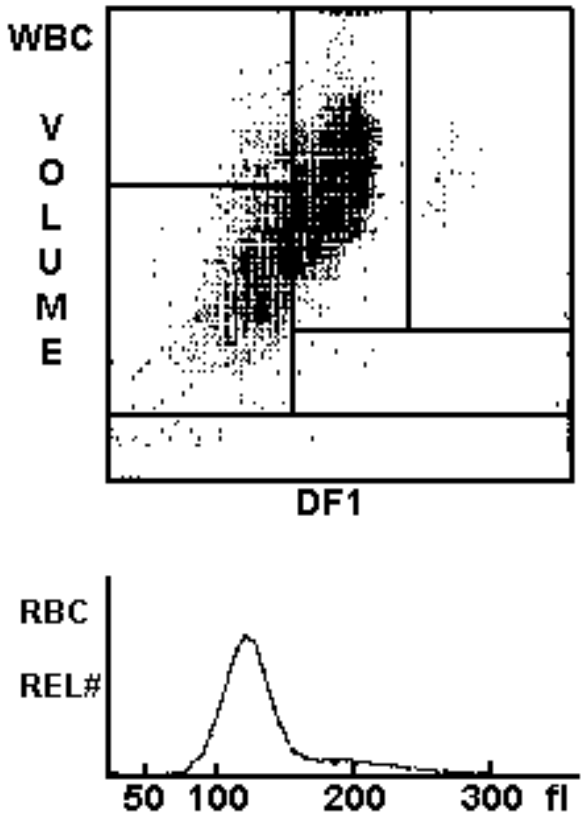
WBC	6.8	
	%	#
NE	52.6	3.6
LY	36.7	2.5
MO	7.8	0.5
EO	2.5	0.2
BA	0.4	0.0
RBC	5.29	
HGB	16.2	
HCT	47.0	
MCV	88.8	
MCH	30.7	
MCHC	34.5	
RDW	12.5	
PLT	179	
MPV	8.4	

This is normal data from a complete blood count as performed on an automated instrument, including an automated WBC differential count.



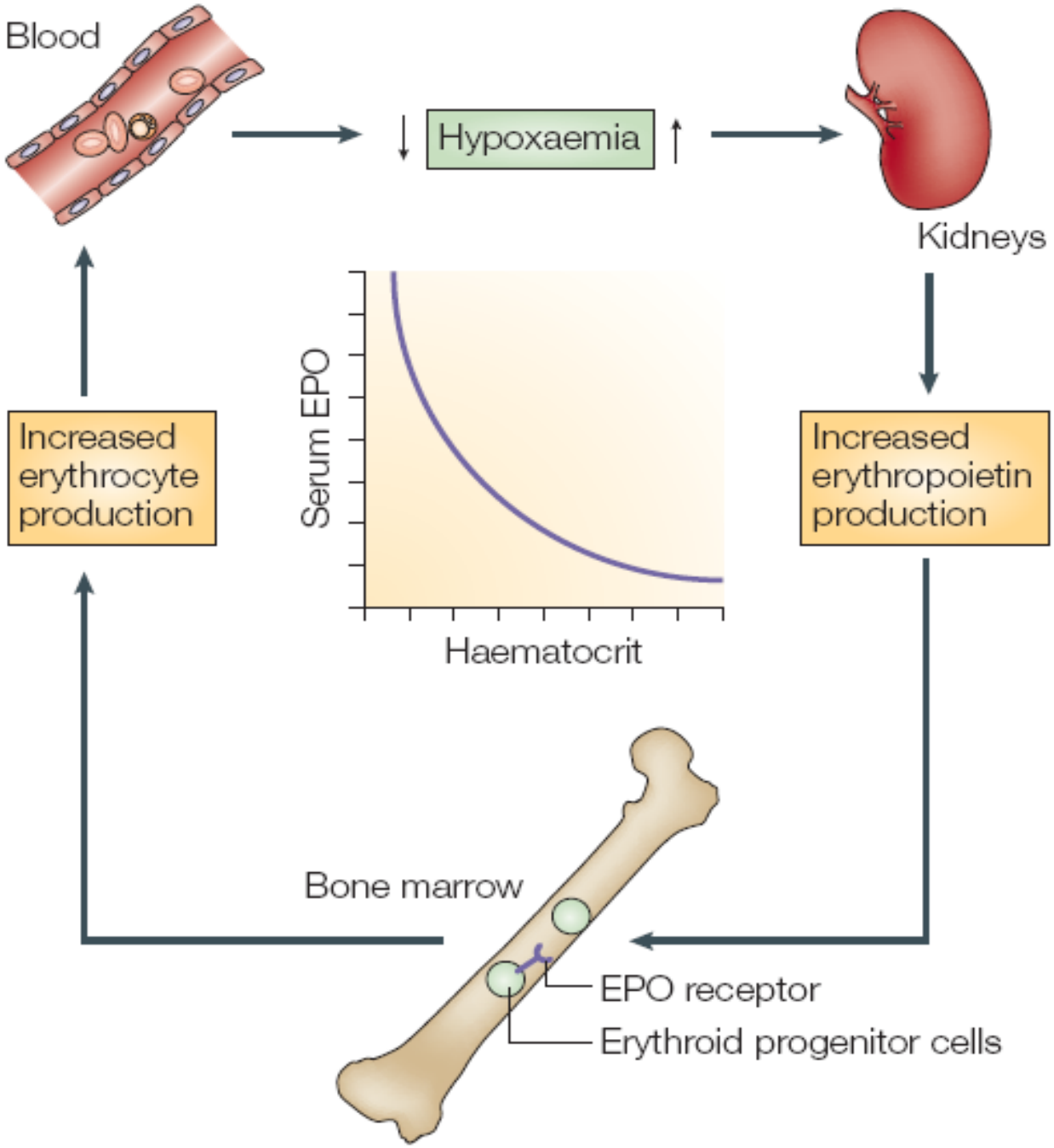
WBC	5.5		
	%		#
NE	54.7		3.0
LY	34.1		1.9
MO	7.5		0.4
EO	3.0		0.2
BA	0.7		0.0
RBC	4.28	L	
HGB	9.7	L	
HCT	29.9	L	
MCV	69.7	L	
MCH	22.6	L	
MCHC	32.4	L	
RDW	18.4	H	
PLT	331		
MPV	8.8		

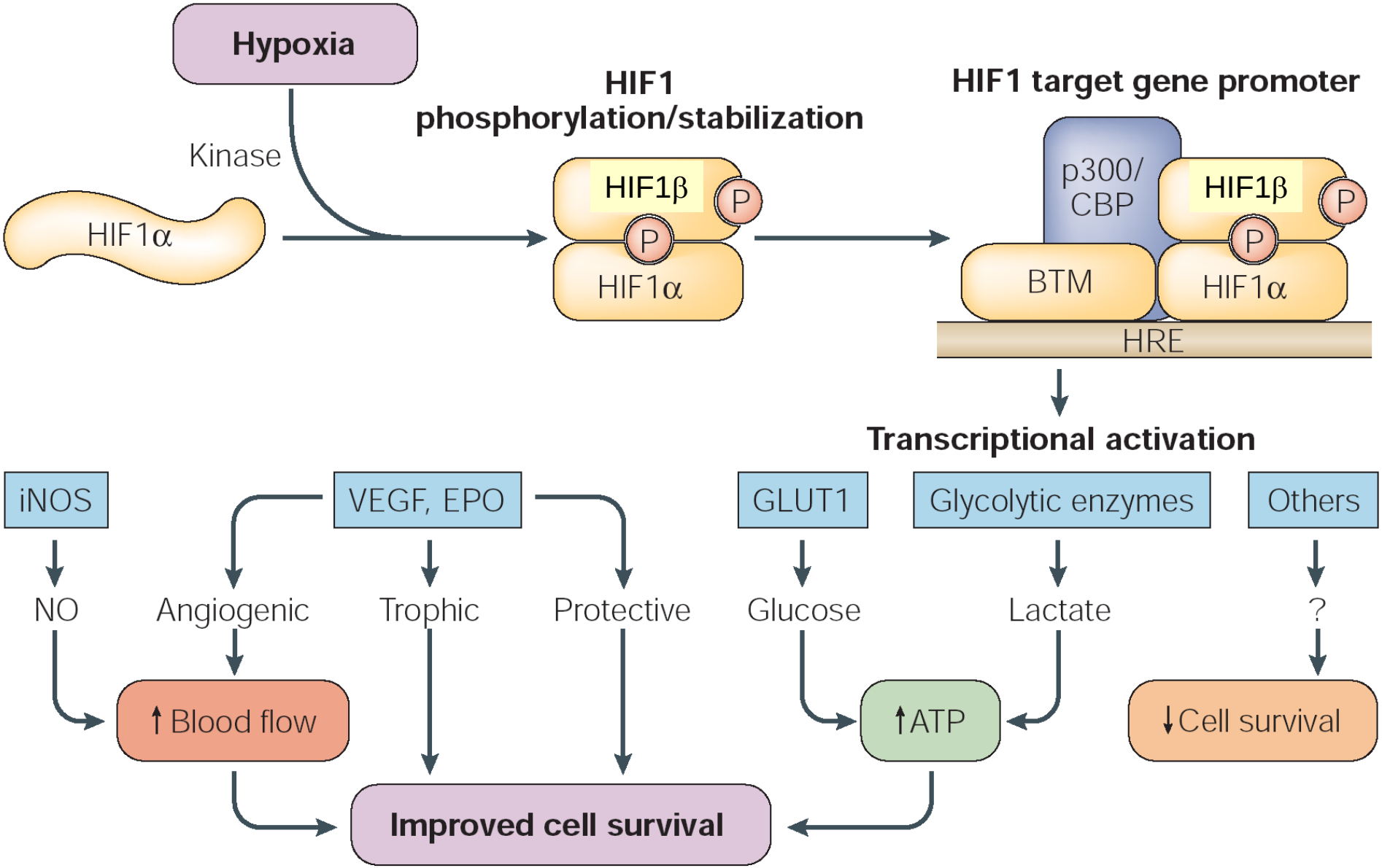
Here is data from a CBC in a person with iron deficiency anemia.
Note the low hemoglobin (HGB). Microcytosis is indicated by the low MCV (mean corpuscular volume). Hypochromia correlates here with the low MCH (mean corpuscular hemoglobin).



WBC	12.1	H			
	%			#	
NE	71.1		H	8.5	H
LY	15.9		L	1.9	
MO	3.3			0.5	
EO	0.5		L	0.1	
BA	8.7		H	1.1	H
RBC	2.69	L			
HGB	10.6	L			
HCT	31.6	L			
MCV	117.6	H			
MCH	39.6	H			
MCHC	33.7				
RDW	14.1				
PLT	578	H			
MPV	7.2	L			

The CBC here shows a markedly increased MCV, typical for megaloblastic anemia. The MCV can be mildly increased in persons recovering from blood loss or hemolytic anemia, because the newly released RBC's, the reticulocytes, are increased in size over normal RBC's, which decrease in size slightly with aging.


Blood
Hypoxaemia
Kidneys
Increased erythrocyte production
Serum EPO
Haematocrit
Increased erythropoietin production
Bone marrow
EPO receptor
Erythroid progenitor cells



Induction of HIF1 target genes by hypoxia

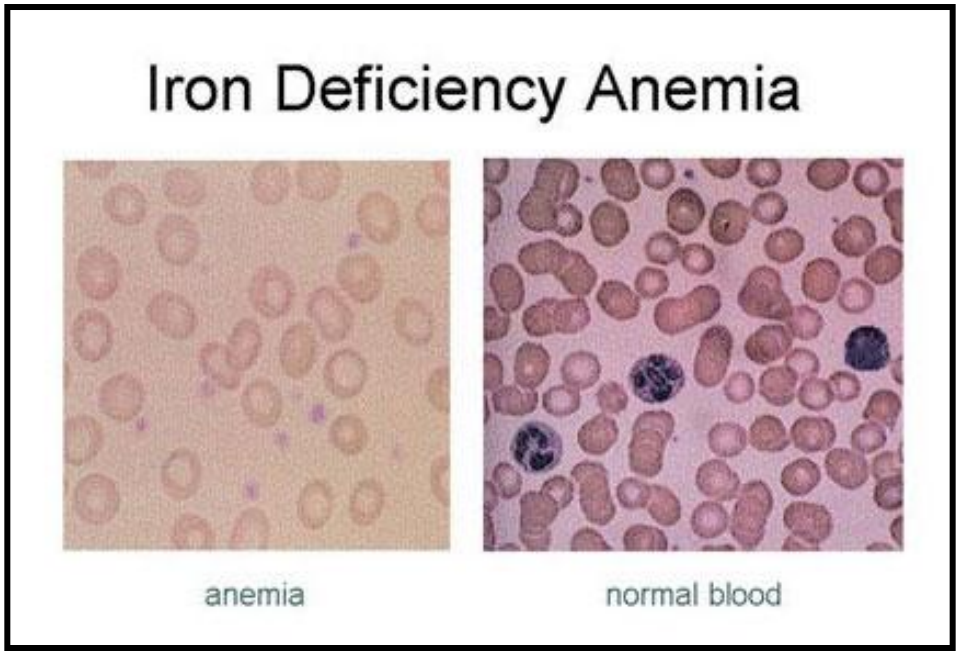
DECREASED RED CELL PRODUCTION

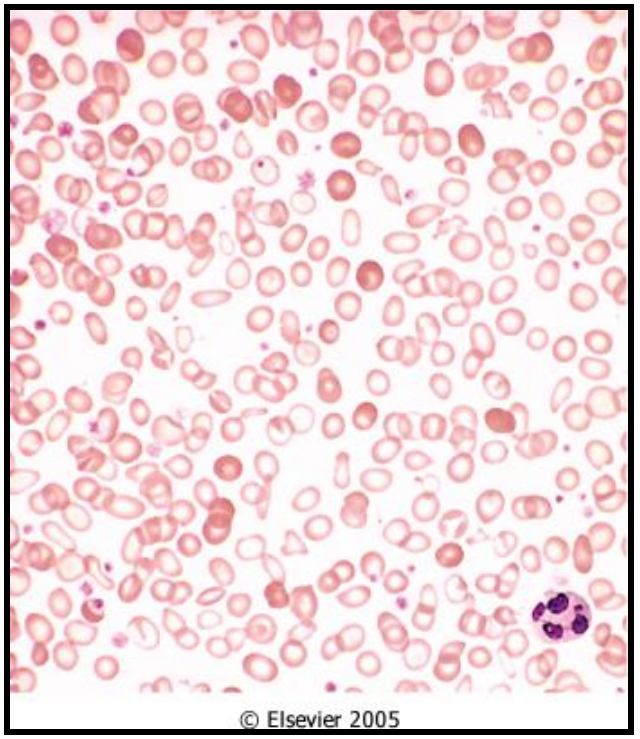
Mechanism	Specific Examples
Inherited genetic defects	
Defects leading to stem cell depletion	Fanconi anemia, telomerase defects
Defects affecting erythroblast maturation	Thalassemia syndromes
Nutritional deficiencies	
Deficiencies affecting DNA synthesis	B_{12} and folate deficiencies
Deficiencies affecting hemoglobin synthesis	Iron deficiency anemia
Erythropoietin deficiency	Renal failure, anemia of chronic disease
Immune-mediated injury of progenitors	Aplastic anemia, pure red cell aplasia
Inflammation-mediated iron sequestration	Anemia of chronic disease
Primary hematopoietic neoplasms	Acute leukemia, myelodysplasia, myeloproliferative disorders
Space-occupying marrow lesions	Metastatic neoplasms, granulomatous disease
Infections of red cell progenitors	Parvovirus B19 infection
Unknown mechanisms	Endocrine disorders, hepatocellular liver disase

SIDEROPENIC ANEMIA

Due to reduced intake or to metabolic problems

Iron need:
•man: < 1.0 mg/day
•fertile woman: 1.5 mg/day, increases during pregnancy





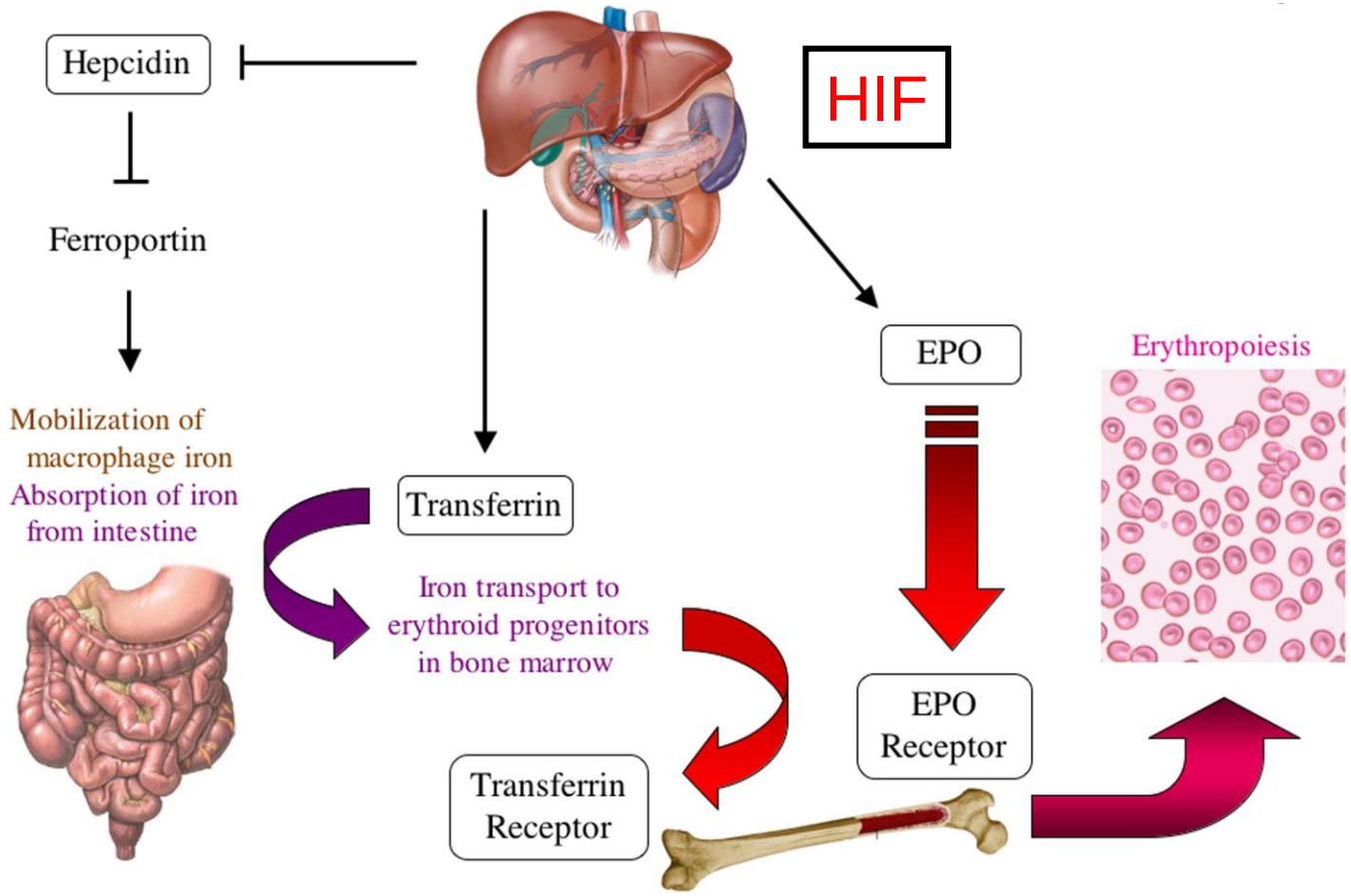
Hypochromic microcytic anemia of iron deficiency (peripheral blood smear). Note the small red cells containing a narrow rim of peripheral hemoglobin. Scattered fully hemoglobinized cells, present due to recent **blood transfusion**, stand in contrast.

Main proteins involved in iron metabolism

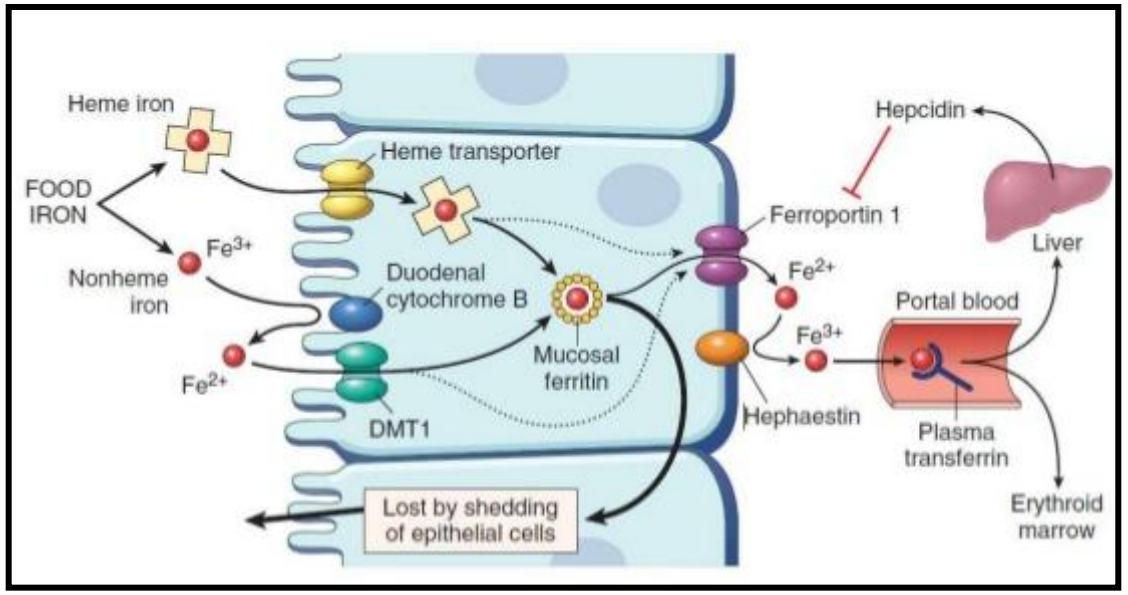
- **transferrin** (Tr; binds 2 iron atoms)
- **Tr receptor** (binds 2 Tr = 4 iron atoms)
- **ferritin** (binds about 4.500 iron atoms)
- **hemosiderin** (insoluble ferritin, in bone marrow, spleen, liver)

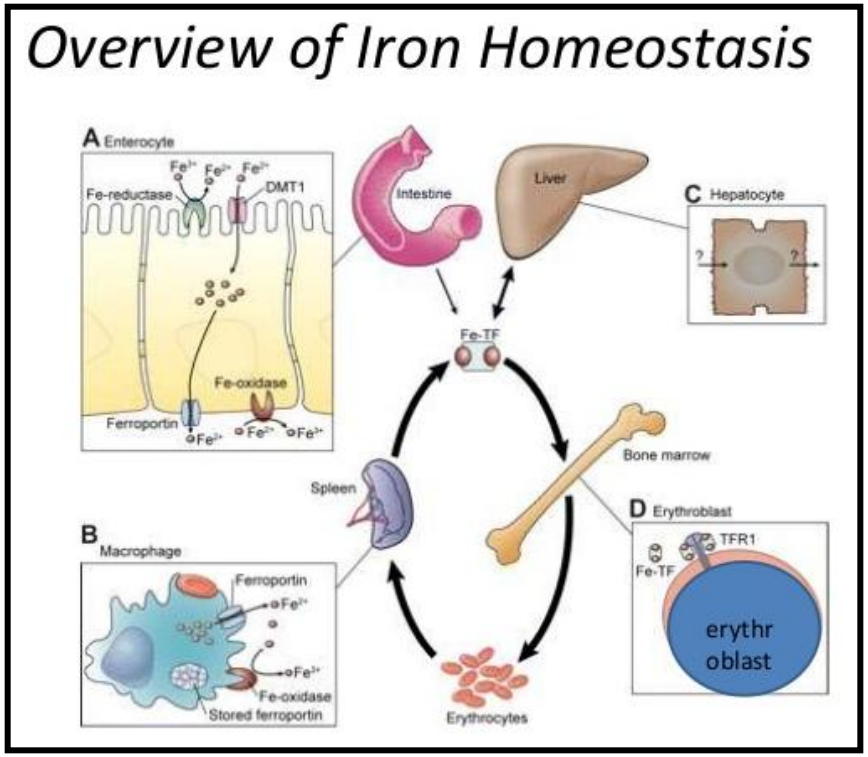
<u>iron distribution in the adult</u> (50-40 mg/kg body weight)

	mg/kg	
	man	woman
functional iron		
• Hb	31	28
• myoglobin	5	4
• enzymes	2	2
transport iron		
• Tr	< 1	< 1
iron stores		
• ferritin	8	4
• hemosiderin	4	2



HIF-1 regulates the expression of multiple genes to stimulate erythropoiesis in response to hypoxia. HIF-1 stimulates production of the EPO in the kidney, which binds to its receptor (EPOR) on erythroid progenitors in the bone marrow (in the adult and yolk sac in the embryo) to stimulate their survival, proliferation, and differentiation. Erythropoiesis involves uptake by the marrow of large amounts of iron, which are used in the synthesis of hemoglobin. In the liver, HIF-1 stimulates iron uptake by repressing the gene encoding hepcidin, which is an inhibitor of ferroportin, the major protein responsible for intestinal iron uptake. HIF-1 also activates hepatic synthesis of transferrin, the major plasma protein responsible for transporting iron from the intestine to the bone marrow via the transferrin receptor. Thus, HIF-1 directly regulates the expression of 5 gene products (EPO, EPOR, hepcidin, transferrin, and transferrin receptor) involving 5 different organs (kidney, liver, intestine, blood, and bone marrow) to control erythropoiesis (Semenza, 2009).





CAUSES OF IRON DEPLETION

A. *Increased need*

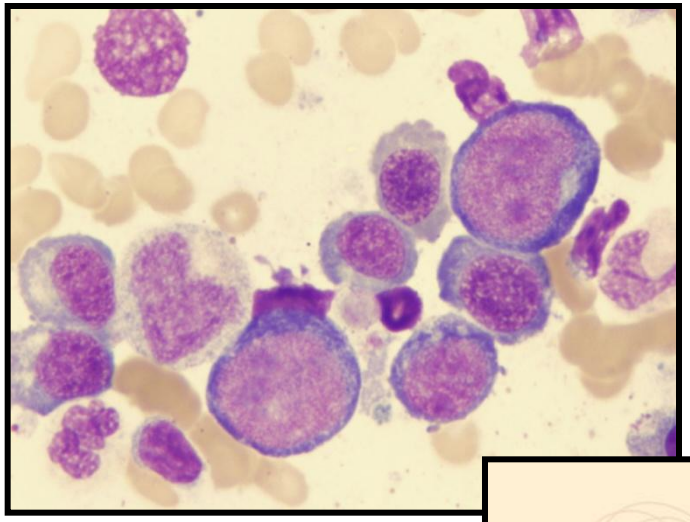
1) bleeding
2) growth
3) pregnancy and milking

B. Insufficient uptake

1) inadequate diet
2) malabsorption

MEGALOBLASTIC ANEMIAS

Most frequent causes: deficiency of vitamin B12 (pernicious anemia) or folic acid (pernicious-like anemias)

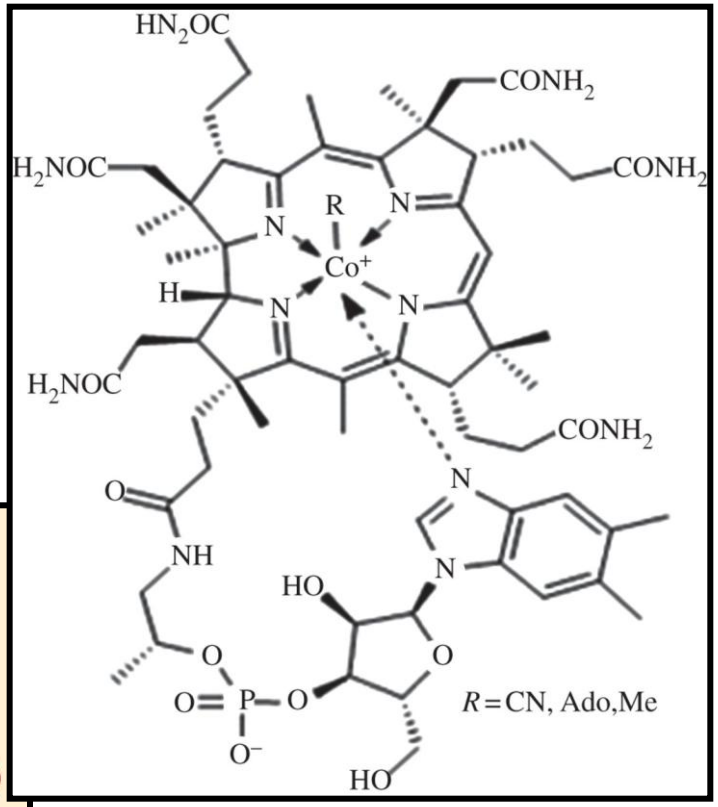


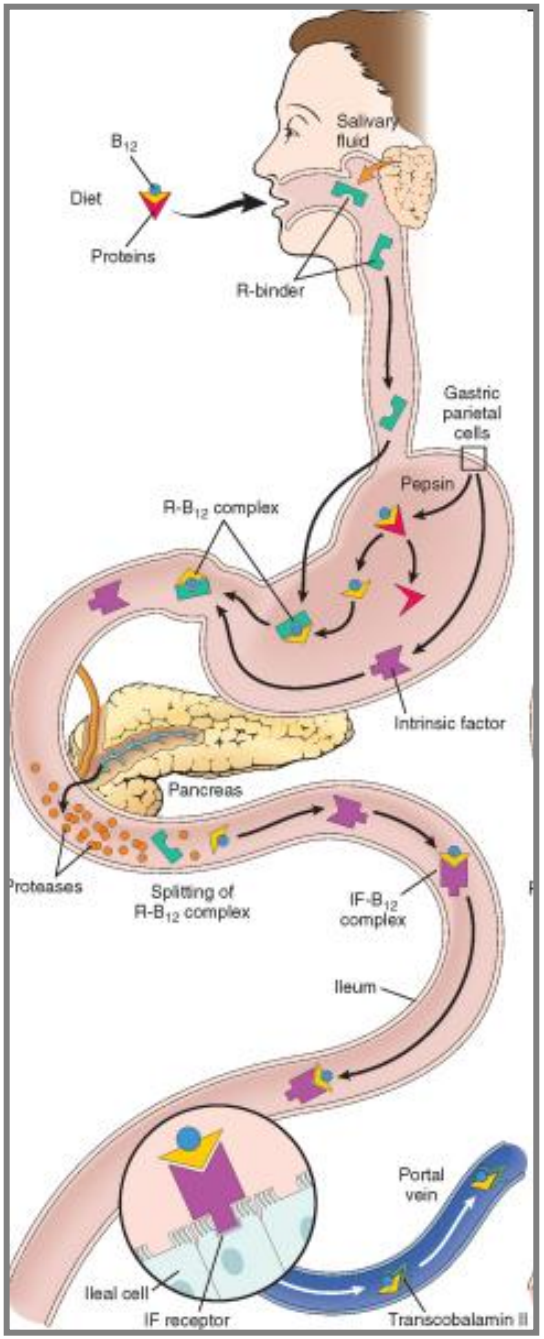
pernicious anemia (www.ourpicture.org)

vitamin B12

daily need: 2-5 mg

conspicuous stores

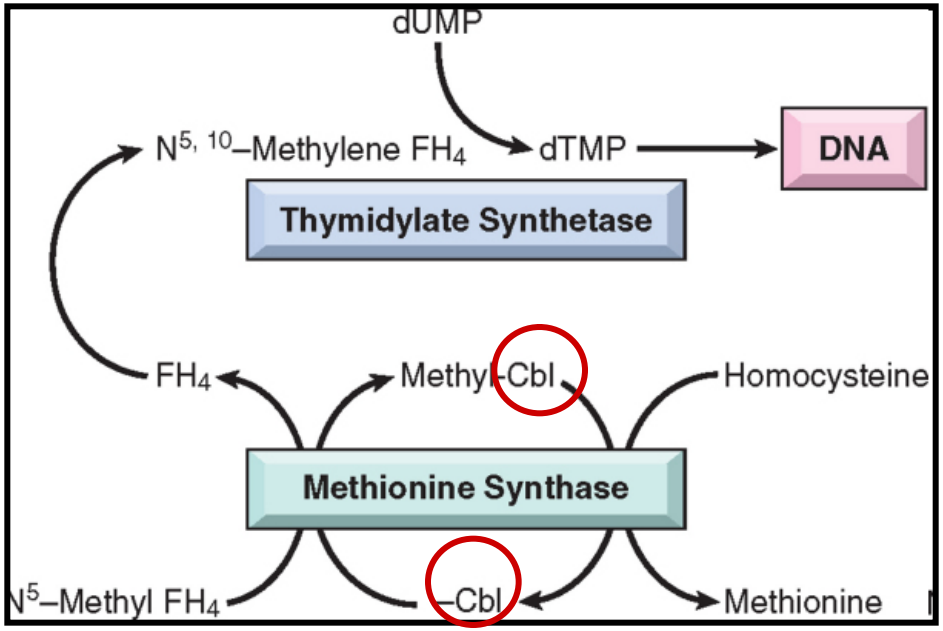




Elsevier, 2010

Causes of vitamin B12 deficiency

- insufficient dietary intake
- gastric factors (insufficient dissociation from food, IF deficiency, autoimmune gastric atrophy, gastrectomy)
- intestinal factors (pancreatic proteolysis deficiency, sequestration by microbiota/parasites, ileal absorption deficiency)
- congenital transport defects
- congenital/acquired metabolic defects



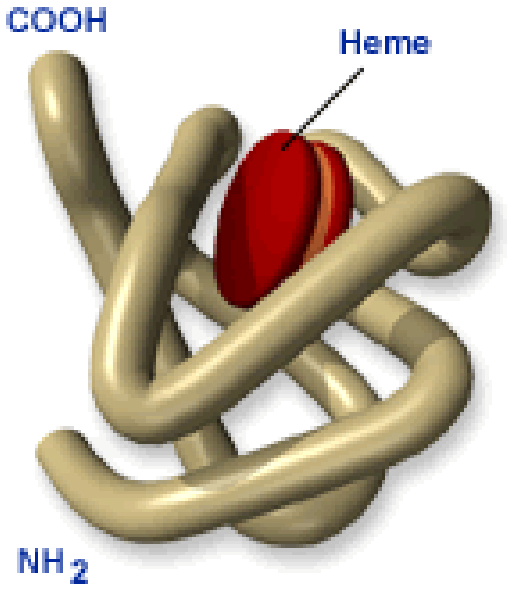
Elsevier, 2010

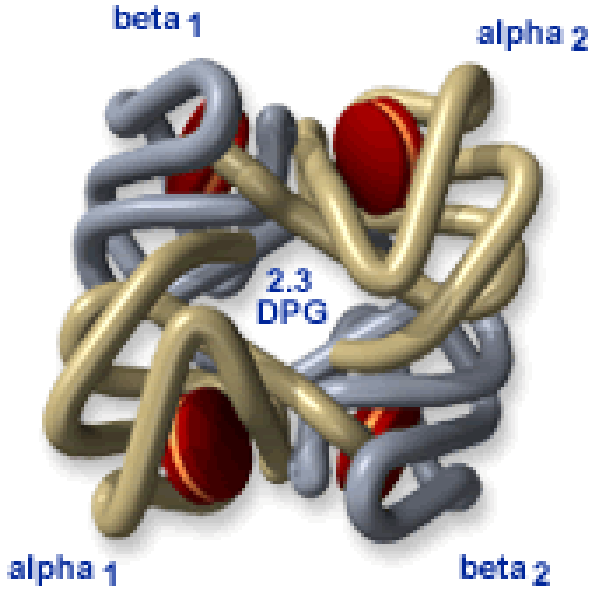
GENETIC DISEASES OF GLOBIN CHAIN (mendelian inheritance)

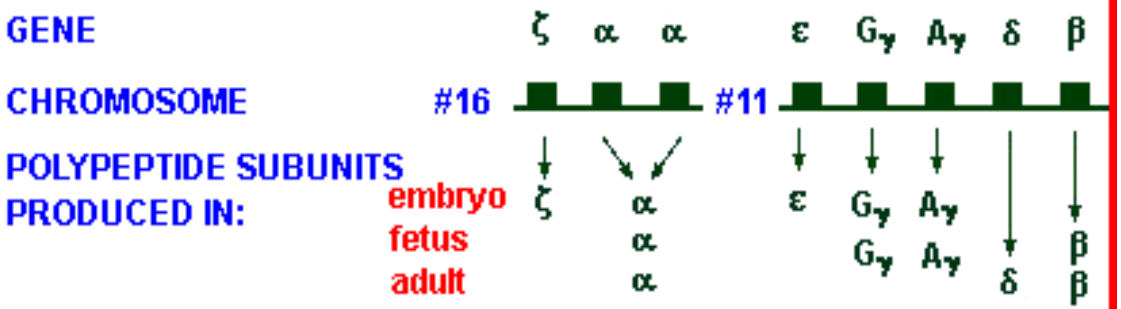
I. Structural defects → **hemoglobinopaties**
II. Synthesis rate defects → **α and β thalassemias**

HEMOGLOBIN (Hb)

$\alpha 2\beta 2$ tetramer (HbA1)
each globin chain bound to one heme (Fe^{2+})
95% of red blood cell content







HEMOGLOBIN	FORMULA	NAME	
embryo	$\zeta_2\varepsilon_2$	Gower I	
	$\alpha_2\varepsilon_2$	Gower II	
	$\zeta_2\gamma_2$	Portland I	
fetus	$\alpha_2\beta_2$	A	
	$\alpha_2\gamma_2$	F	
adult	$\alpha_2\beta_2$	A	97-98%
	$\alpha_2\delta_2$	A_2	2-3,5%
	$\alpha_2\beta_2^{glucose}$	A_{Ic}	< 6%

R. T. Jones. 1997. McGraw Hill Encyclopedia of Science & Technology.

ALTERED Hb SYNTHESIS RATES: α OR β THALASSEMIAS

β-thalassemia

homozygosis → β-thalassemia major (Cooley disease or mediterranean anemia)
heterozygosis → β-thalassemia minor (β-thalassemic tract; compensated by HbA2 and HbF. Need to identify carriers)

- **exon nonsense mutations (recessive phenotype) → β^0-thalassemia (no transcript)**

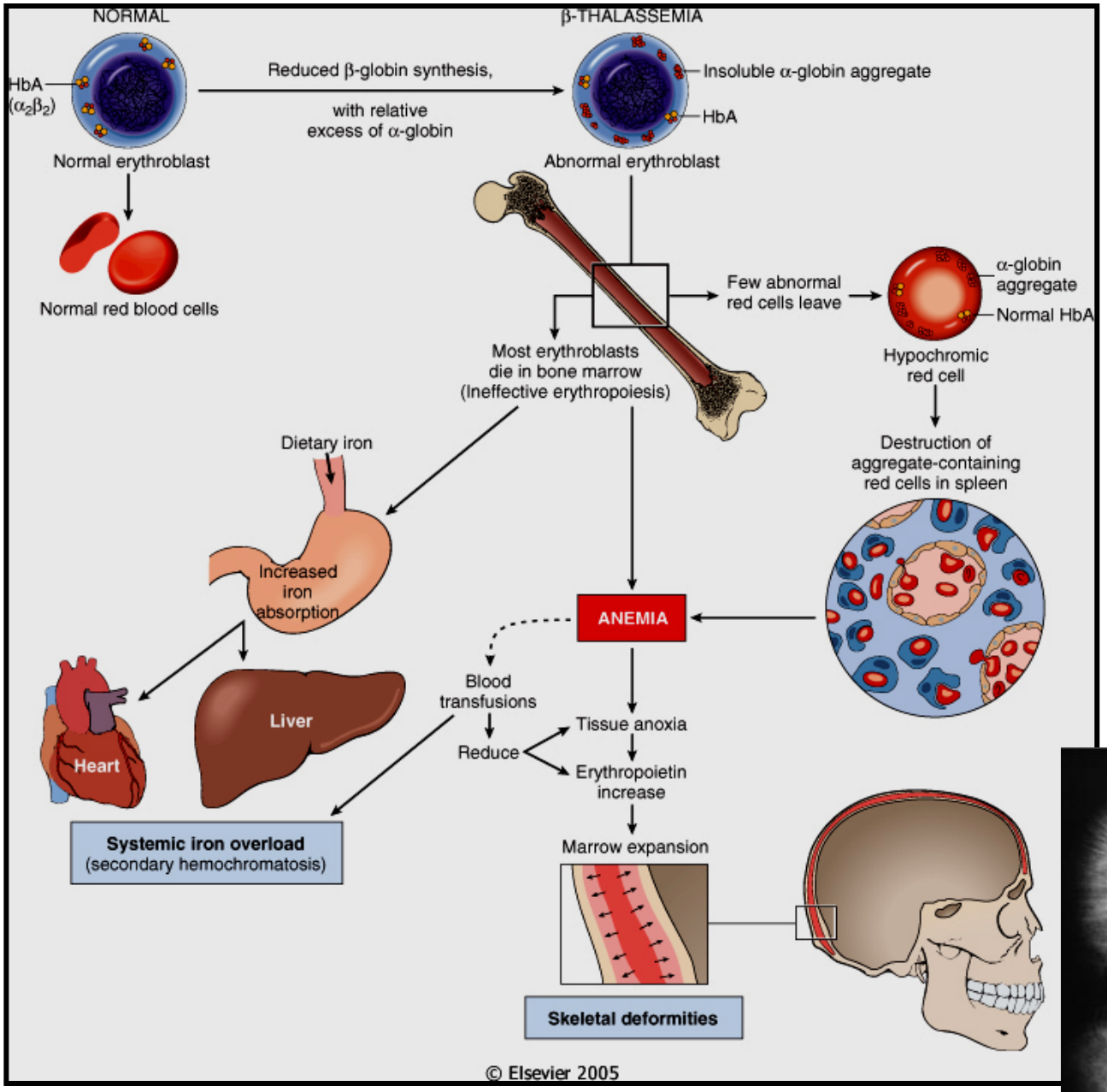
- **intron mutations (recessive phenotype) → β^+-thalassemia (uncorrect mRNA splicing→ reduced β chain synthesis)**

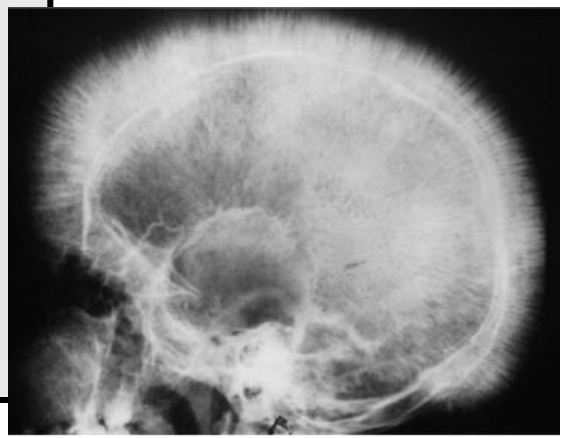
- unbalanced globin chain synthesis → α chains aggregate and precipitate in erythrocytes (reduced half life) and in precursors (ineffective erythropoiesis due to destruction in bone marrow)

- sometimes HbF produced to compensate

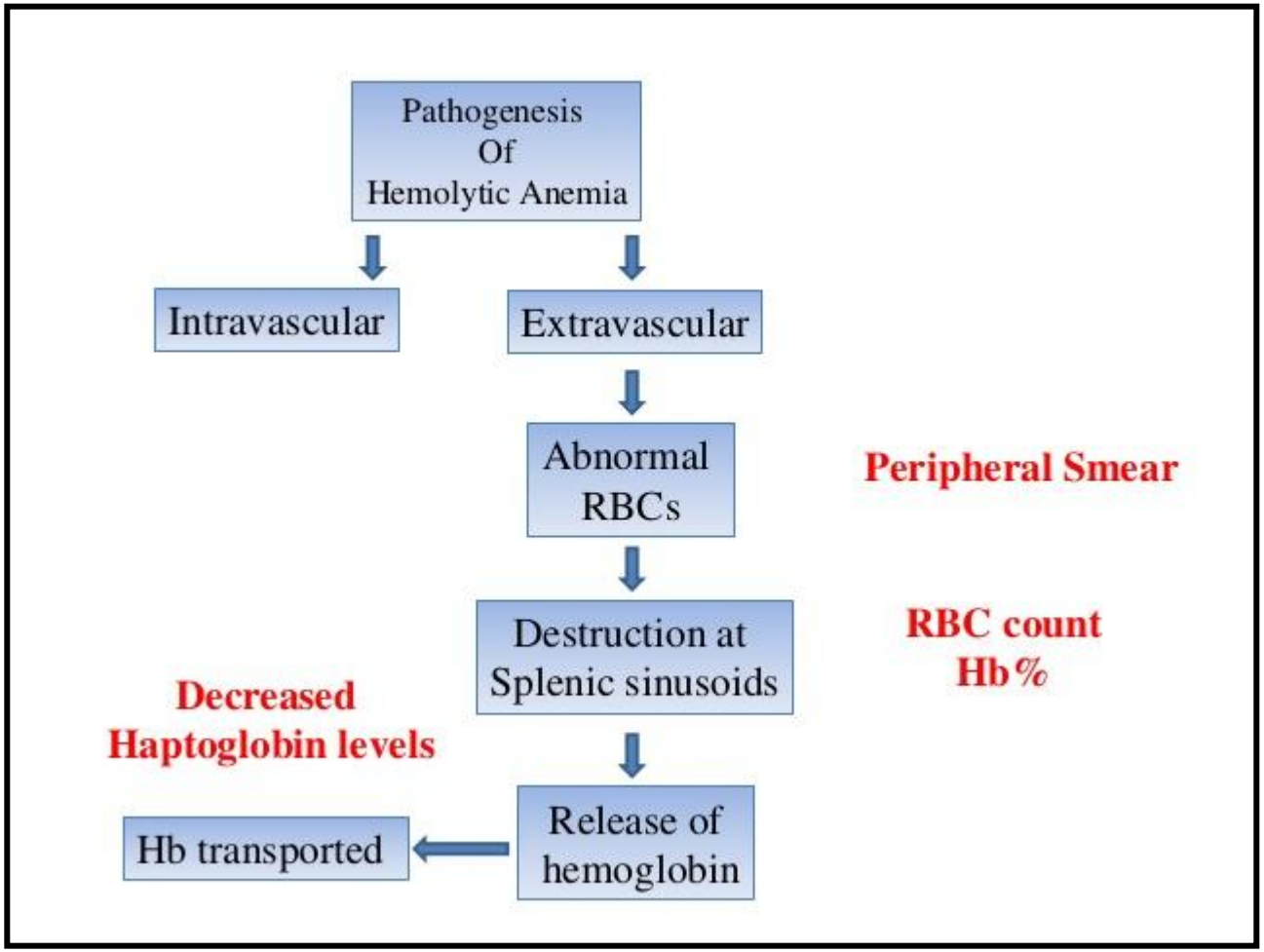
ANEMIA: due to both reduced production and increased destruction of erythrocytes

Thalassemia major: pathogenesis

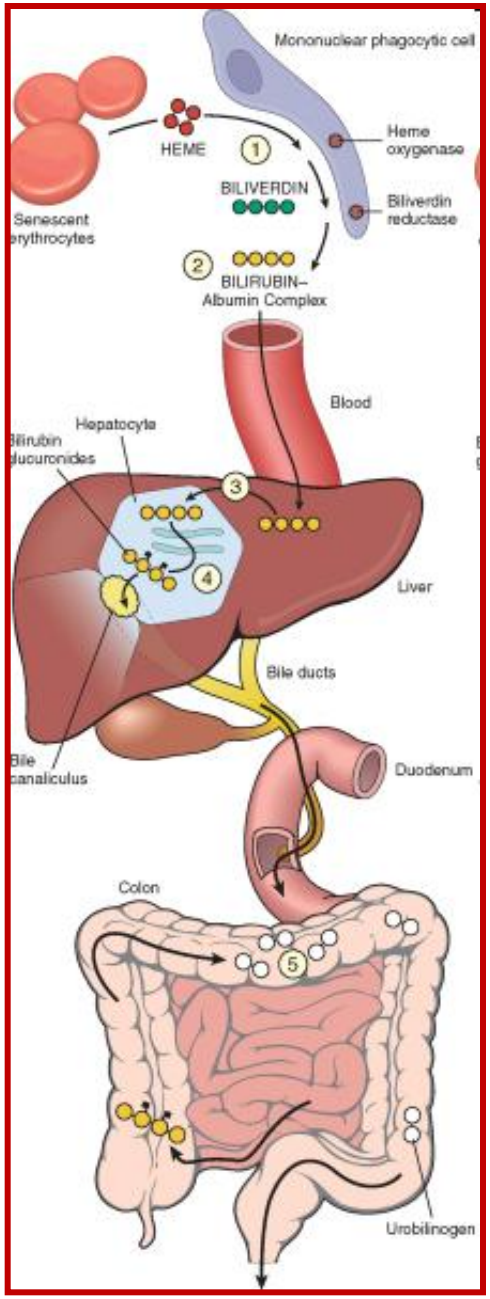


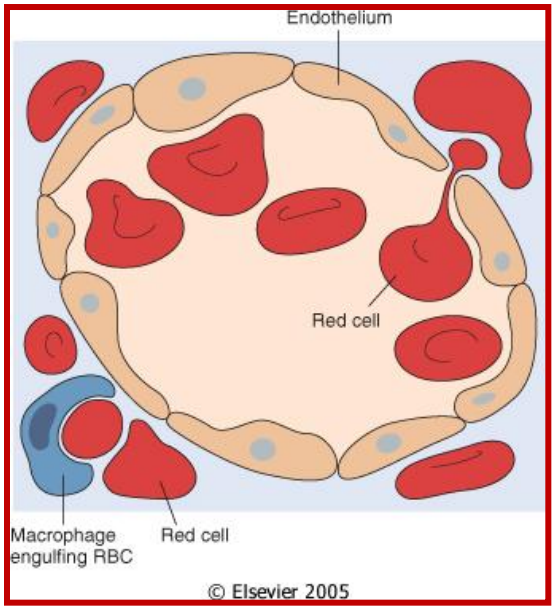


HEMOLYTIC ANEMIAS



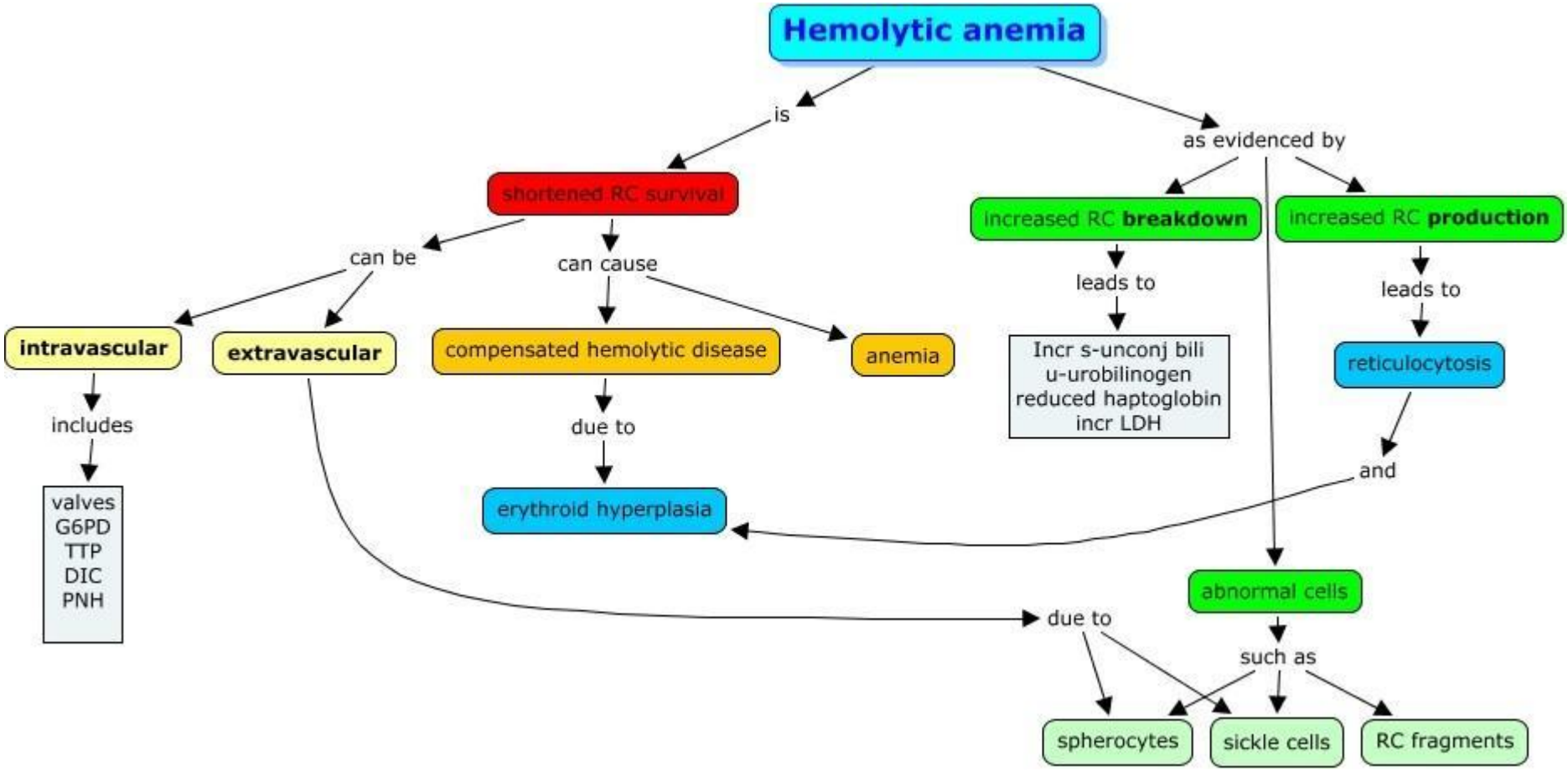
EXTRAVASCULAR HEMOLYSIS





Elsevier, 2010

Mechanism	Specific Examples
A. Inherited genetic defects	
Red cell membrane disorders	Hereditary spherocytosis, hereditary elliptocytosis
1. Enzyme deficiencies	
Hexose monophosphate shunt enzyme deficiencies	G6PD deficiency, glutathione synthetase deficiency
Glycolytic enzyme deficiencies	Pyruvate kinase deficiency, hexokinase deficiency
2. Hemoglobin abnormalities	
Deficient globin synthesis	Thalassemia syndromes
Structurally abnormal globins (hemoglobinopathies)	Sickle cell disease, unstable hemoglobins
B. Acquired genetic defects	
Deficiency of phosphatidylinositol-linked glycoproteins	Paroxysmal nocturnal hemoglobinuria
c. Extrinsic causes	
Antibody-mediated destruction	Hemolytic disease of the newborn (Rh disease), transfusion reactions, drug-induced, autoimmune disorders
Mechanical trauma	
Microangiopathic hemolytic anemias	Hemolytic uremic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenia purpura
Cardiac traumatic hemolysis	Defective cardiac valves
Repetitive physical trauma	Bongo drumming, marathon running, karate chopping
Infections of red cells	Malaria, babesiosis
Toxic or chemical injury	Clostridial sepsis, snake venom, lead poisoning
Membrane lipid abnormalities	Abetalipoproteinemia, severe hepatocellular liver disease
Sequestration	Hypersplenism


Hemolytic anemia
is
as evidenced by
shortened RC survival
increased RC breakdown
increased RC production
can be
can cause
leads to
leads to
intravascular
extravascular
compensated hemolytic disease
anemia
Incr s-unconj bili
u-urobilinogen
reduced haptoglobin
incr LDH
reticulocytosis
includes
due to
and
valves
G6PD
TTP
DIC
PNH
erythroid hyperplasia
abnormal cells
due to
such as
spherocytes
sickle cells
RC fragments

HEMOGLOBINOPATIES

1. Single point mutations: HbS β6glu → val

2. Double point mutations in the same globin chain

3. *Nonsense mutations*: es. β39 CAG(gln) → TAG(stop) frequent in thalassemias

4. Mutations causing elongation

5. Mutationi due to fusion genes Hb Lepore (δβ)

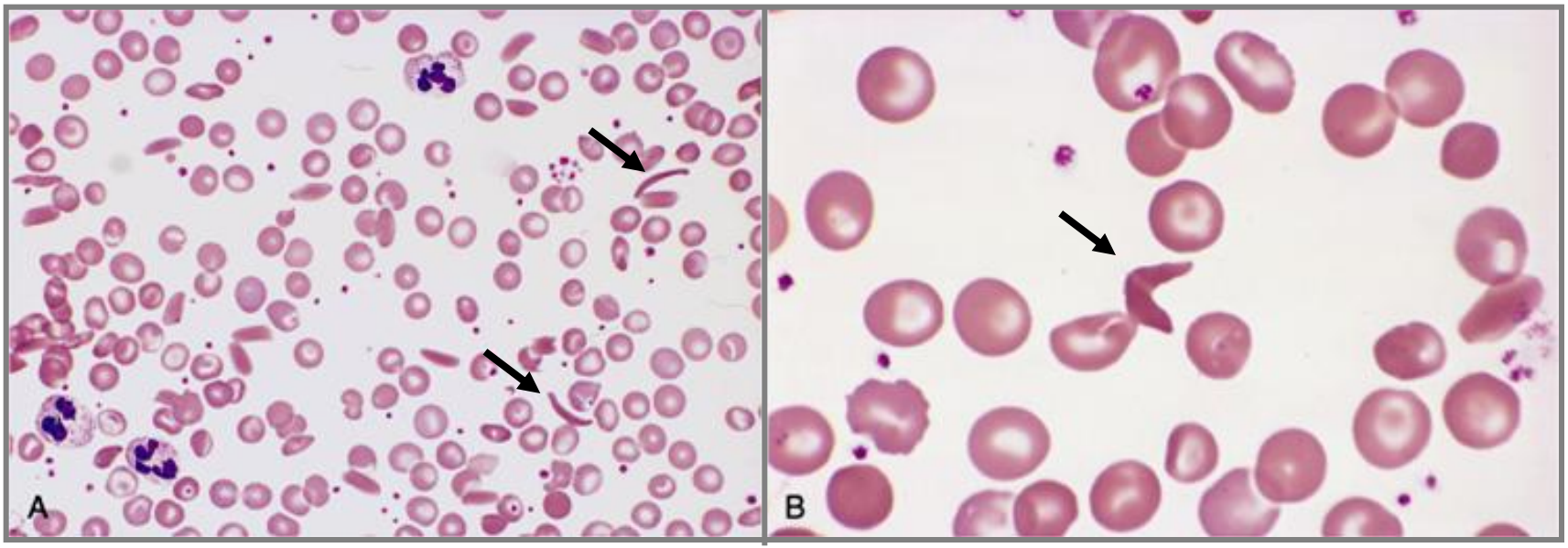
6. Codon deletion/insertion (*in frame* and *frameshift*)

A	**SICKLE CELL DISEASE**
B	**UNSTABLE Hb**
C	**MODIFIED O_2 AFFINITY**
D	**ALTERED O_2 TRANSPORTATION**

SICKLE CELL DISEASE

HbS: β6glu → val; autosomal recessive inheritance

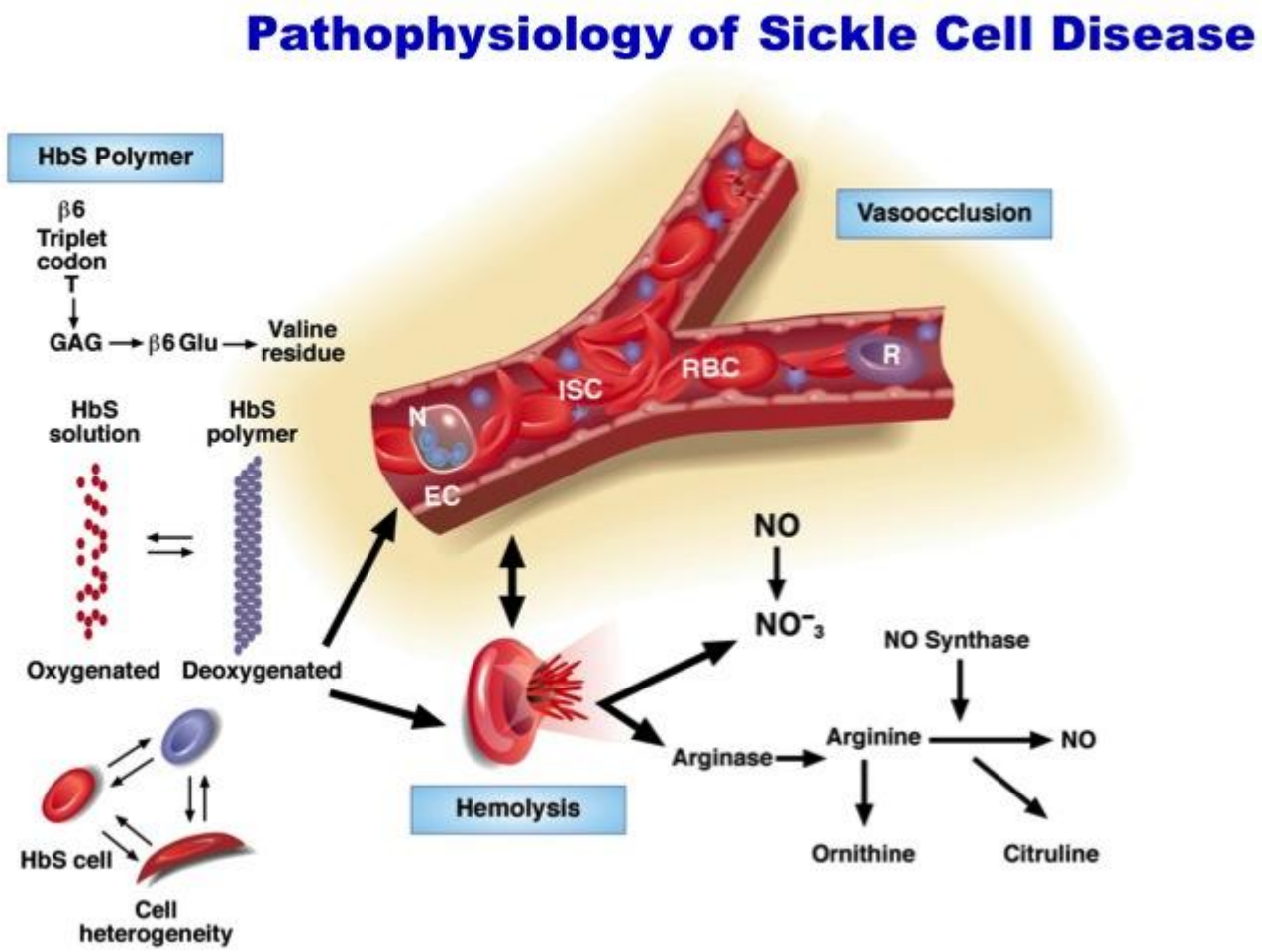
- **heterozygosis** carriers → low frequency clinical signs: HbS 35-40%, HbA1 55-60%
- **homozygosis** SICKLE CELL DISEASE → early death



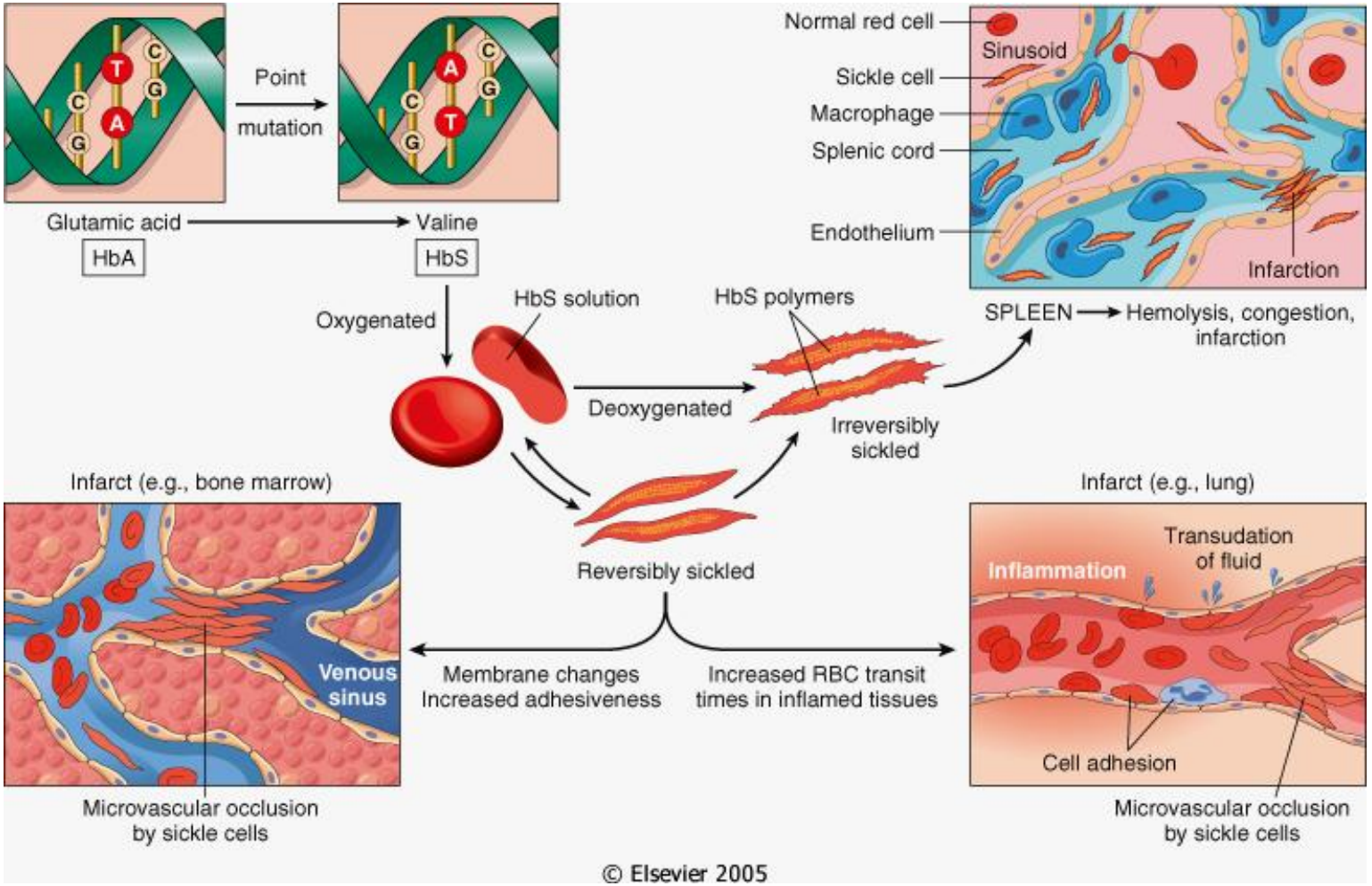
Deoxygenated blood: adjacent mutated β chain polimerize (β6val)

asymptomatic until HbF can compensate (6 months)

SICKLE CELL DISEASE: PATHOGENESIS

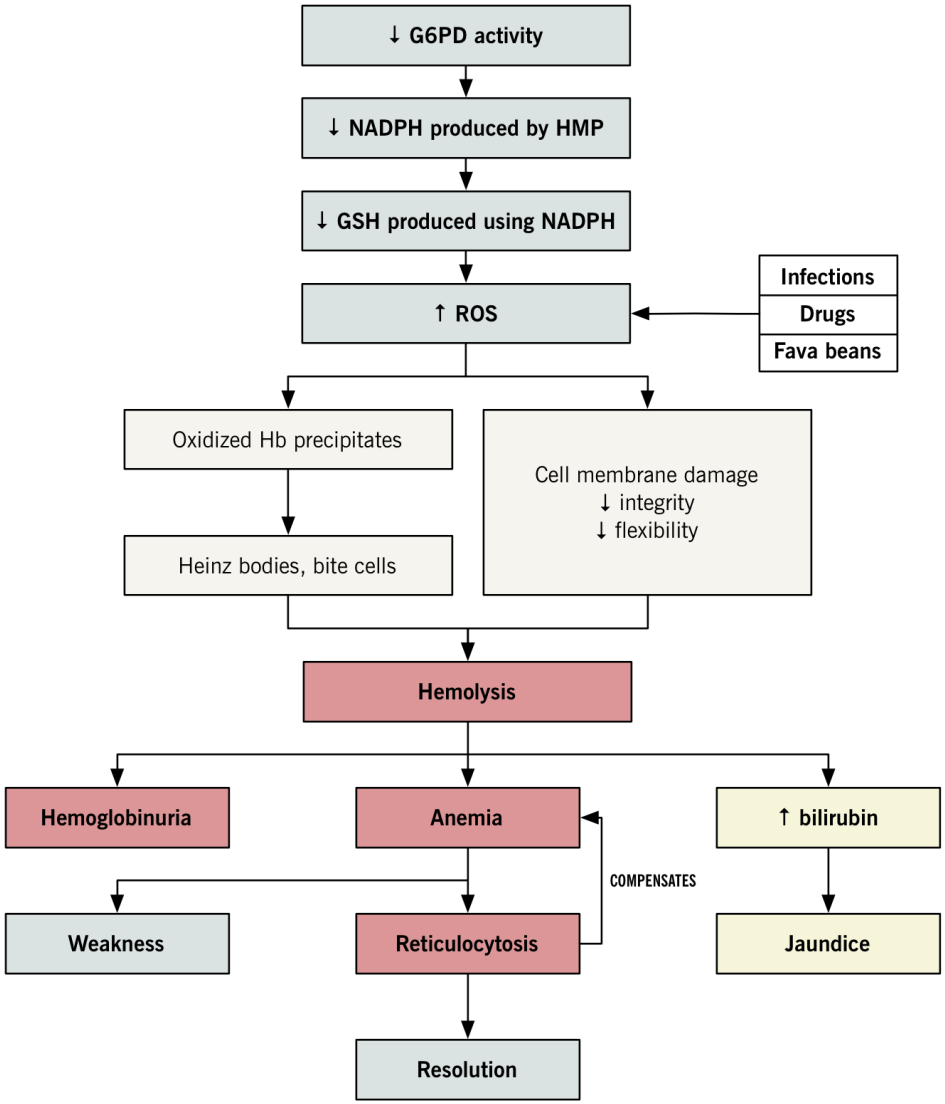


SICKLE CELL DISEASE: PATHOGENESIS

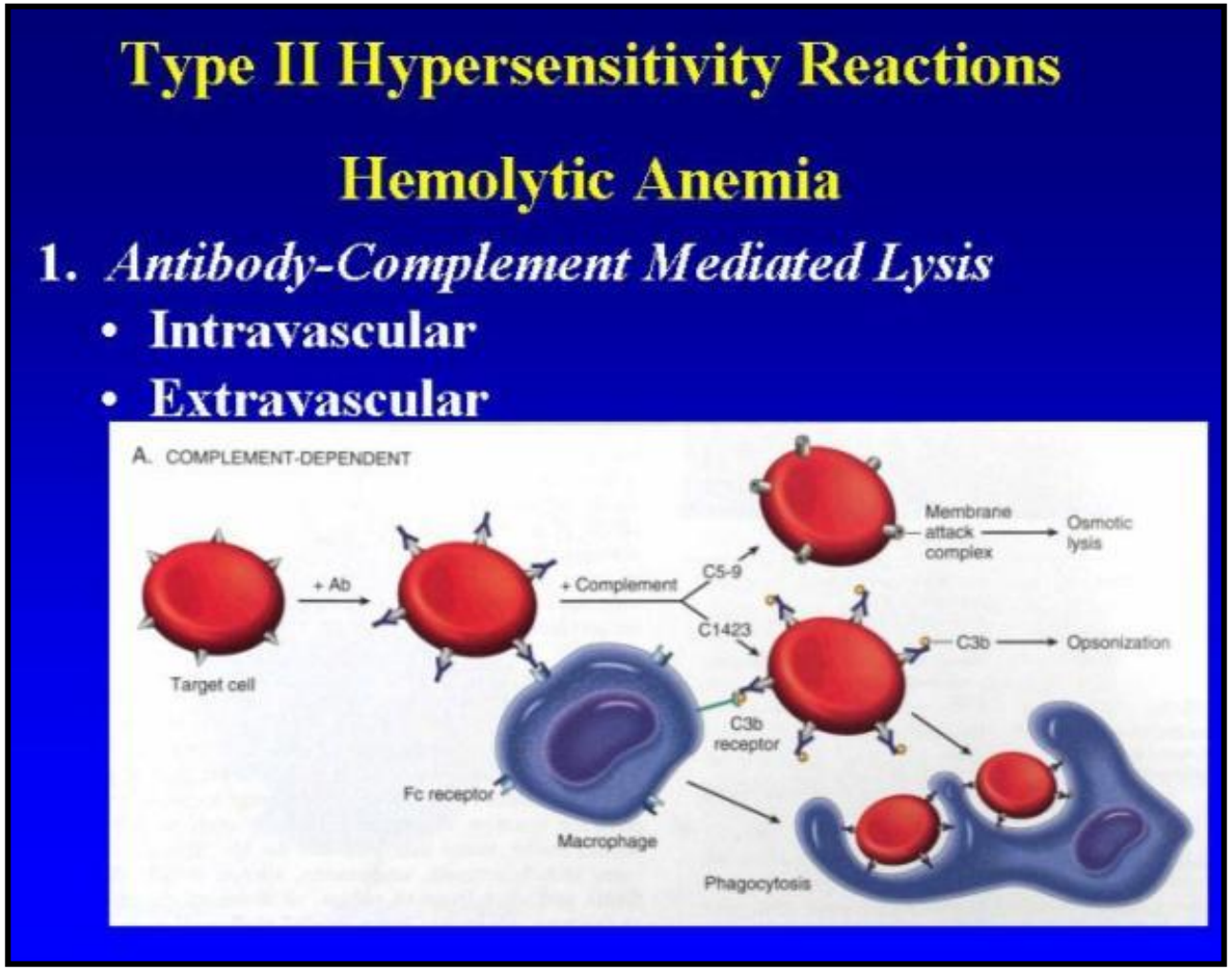


G6PD | Pathophysiology and clinical features

Jennifer Klowak



IMMUNOHEMOLYTIC ANEMIAS



ISOANTIBODIES

non compatible transfusion
newborn hemolytic disease (Rh incompatibility)

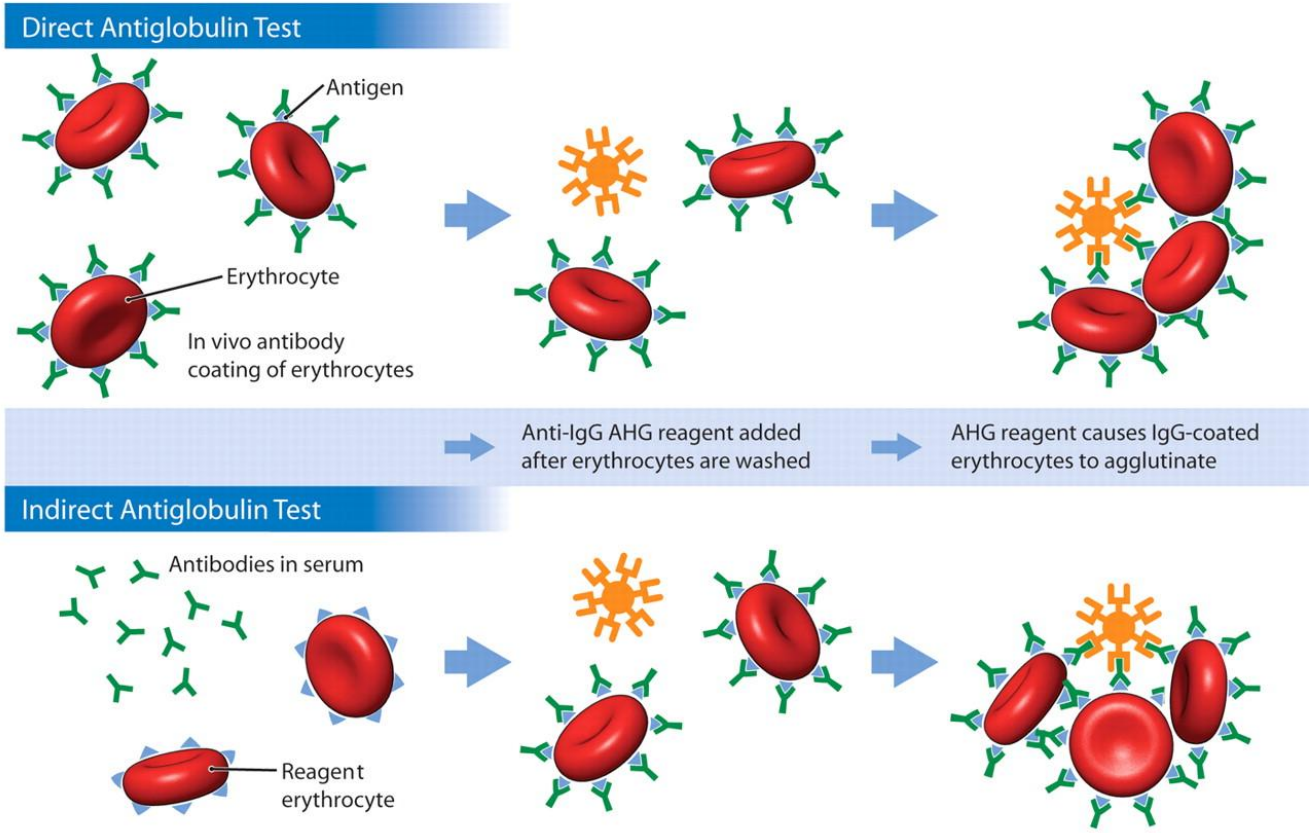
AUTOANTIBODIES

warm Ab caldi (IgG)
cold Ab (agglutinins, hemolysins)
erythrocyte Ag acquiring immunogenicity

Diagnosis of immunohemolytic anemia

- *direct Coombs antiglobulin test*, in which **the patient's red cells** are mixed with sera containing antibodies that are specific for human immunoglobulin or complement.
If either immunoglobulin or complement is present on the surface of the red cells, the multivalent antibodies cause agglutination, which is easily appreciated visually as clumping.

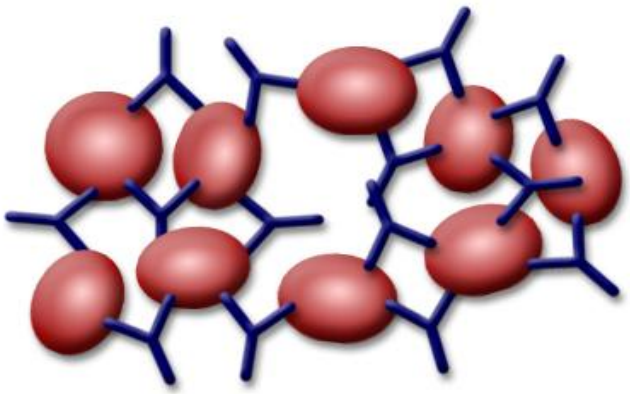
- *indirect Coombs antiglobulin test*, **the patient's serum** is tested for its ability to agglutinate commercially available red cells bearing particular defined antigens.
This test is used to characterize the antigen target and temperature dependence of the responsible antibody. Quantitative immunological tests to measure such antibodies directly are also available.

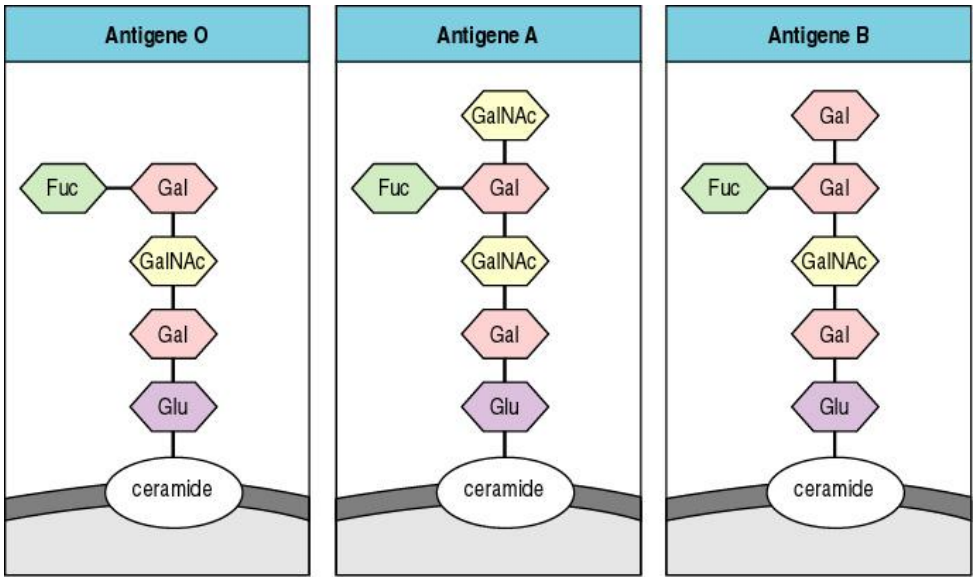


NON COMPATIBILE TRANSFUSION

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin



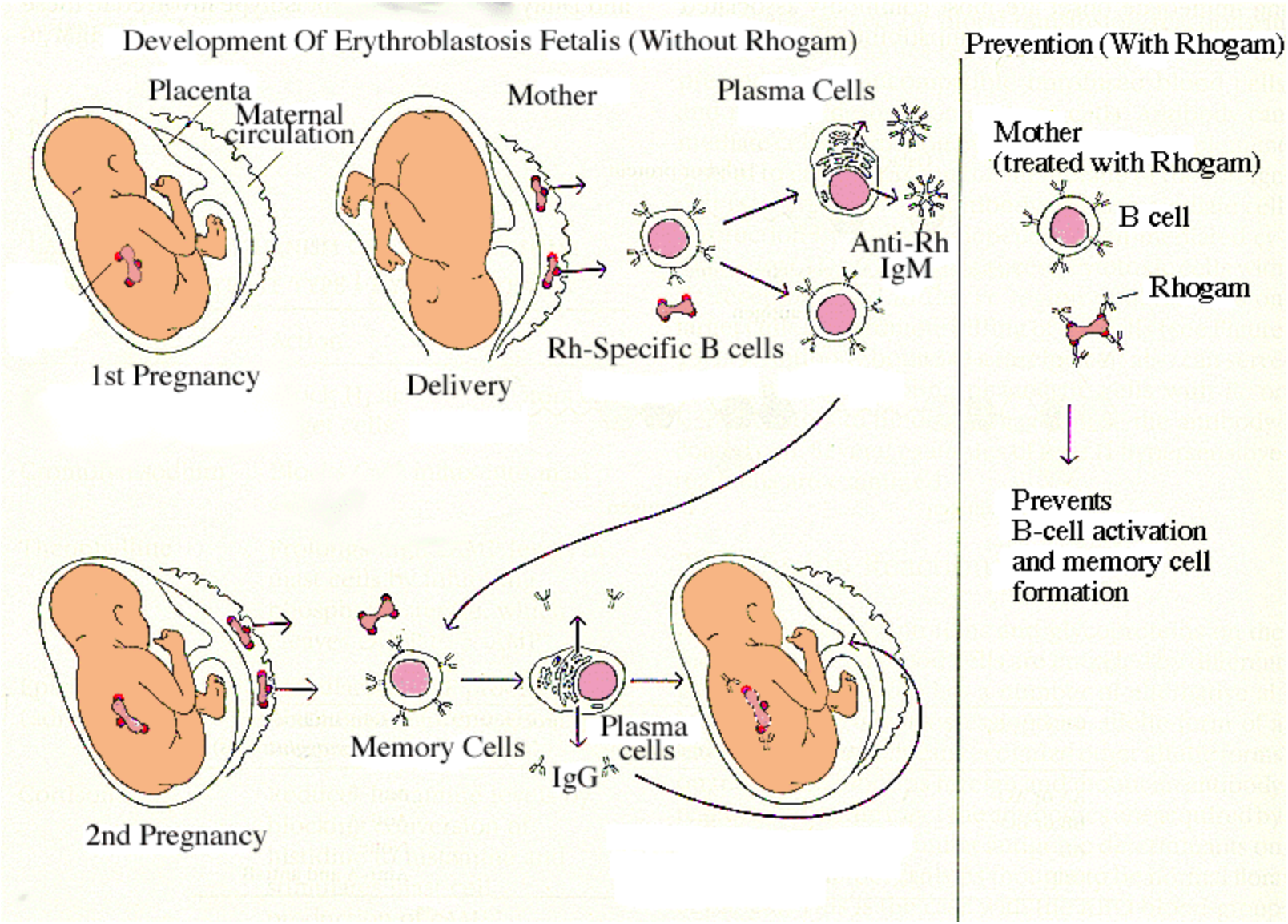


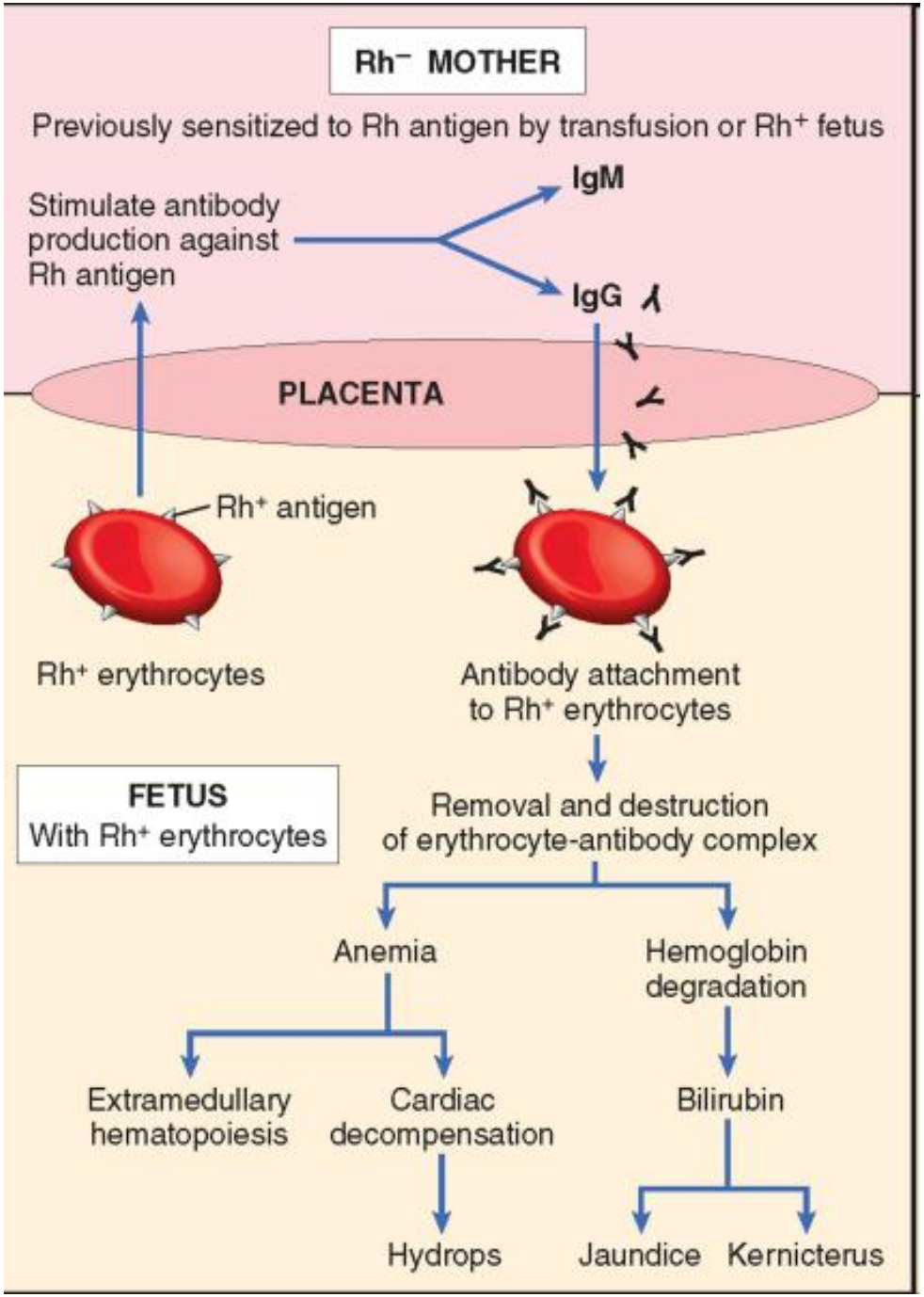
NEWBORN HEMOLYTIC DISEASE

Van der Schoot et al., 2008

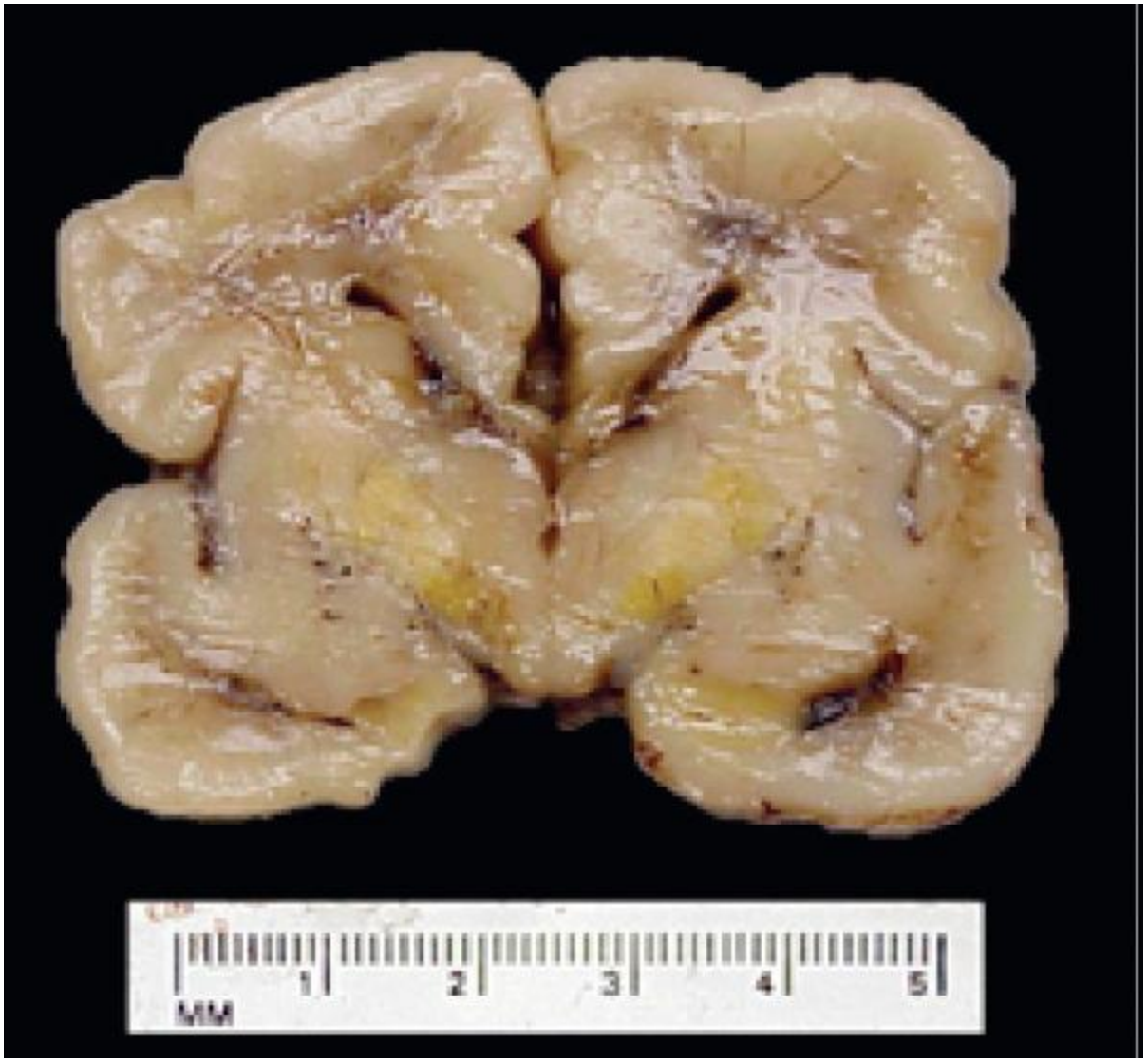
Rh Positive		Rh Negative	
0+	37%	0-	6%
A+	34%	A-	6%
B+	10%	B-	2%
AB+	4%	AB-	1%
85% positive		**15% negative**	

Macacus rhesus; 1940


Development Of Erythroblastosis Fetalis (Without Rhogam)
Placenta
Maternal circulation
1st Pregnancy
Mother
Delivery
Plasma Cells
Anti-Rh IgM
Rh-Specific B cells
2nd Pregnancy
Memory Cells
Plasma cells
IgG
Prevention (With Rhogam)
Mother (treated with Rhogam)
B cell
Rhogam
Prevents B-cell activation and memory cell formation

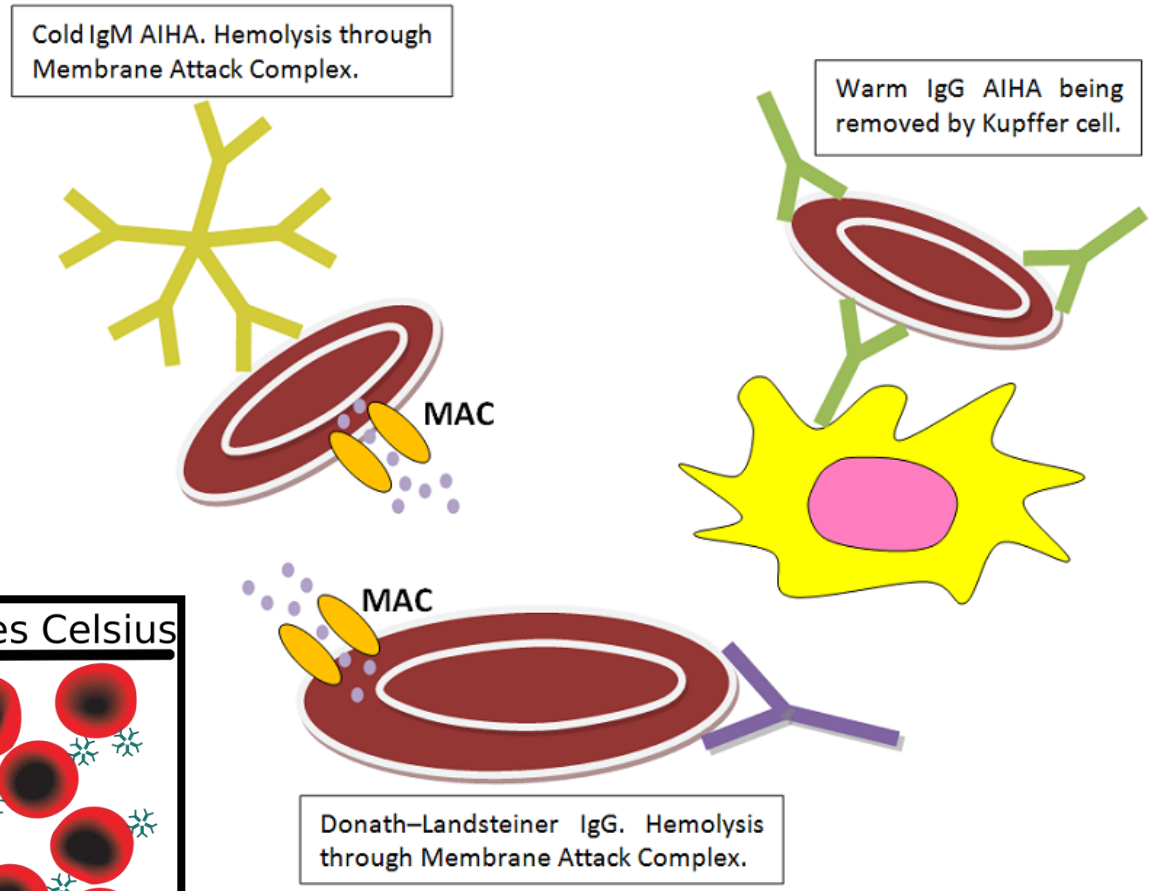


Elsevier, 2010

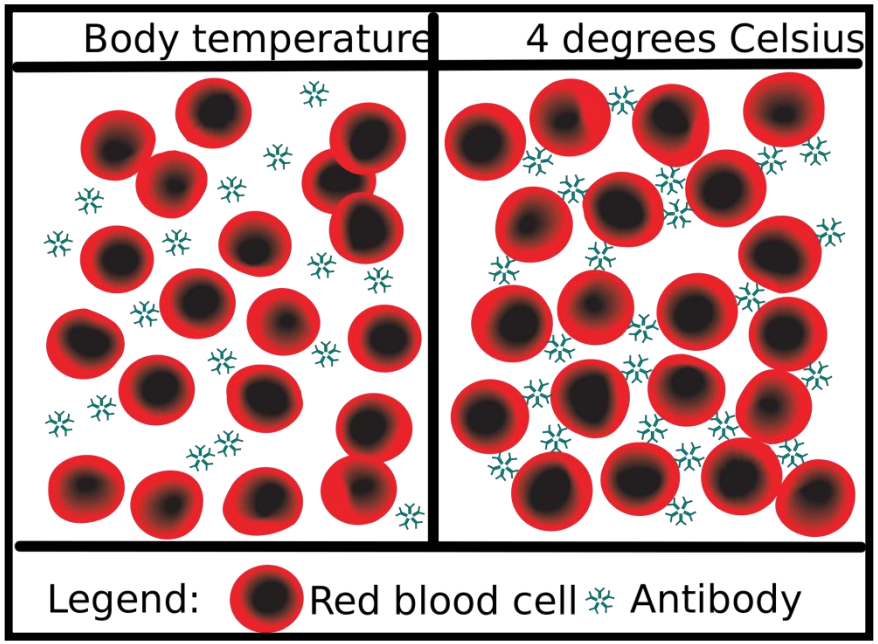


Elsevier, 2010

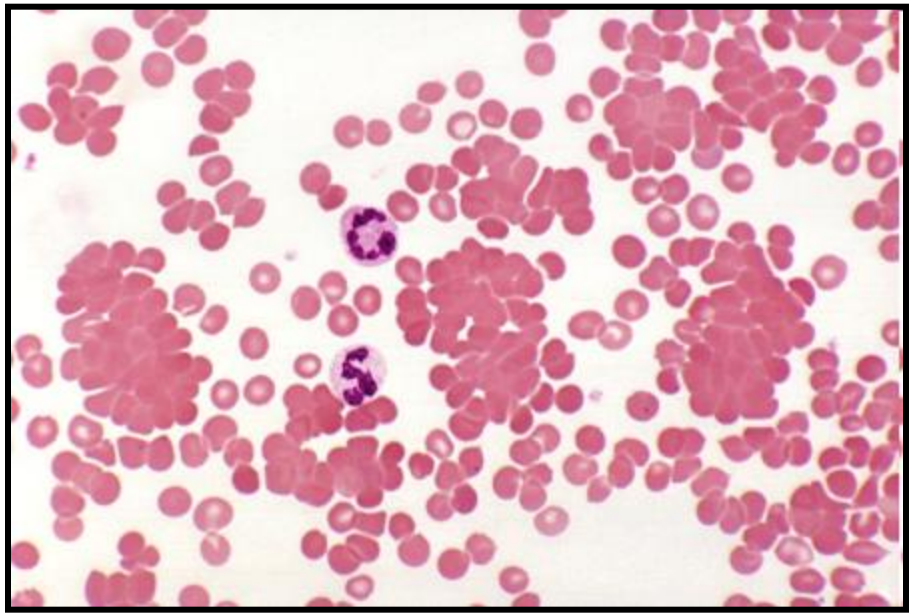
AUTOIMMUNE HEMOLYTIC ANEMIAS



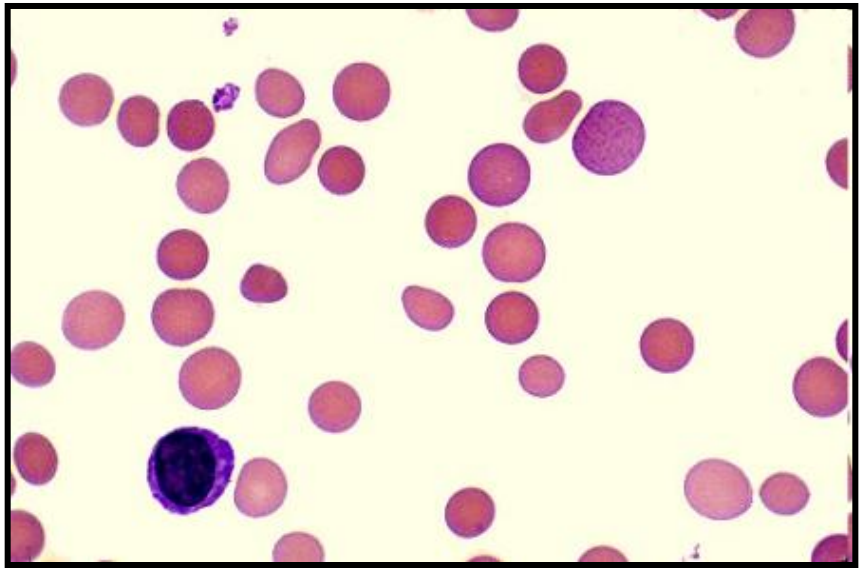
Autoimmune Hemolytic Anemia. © MedPrepOnline.Com

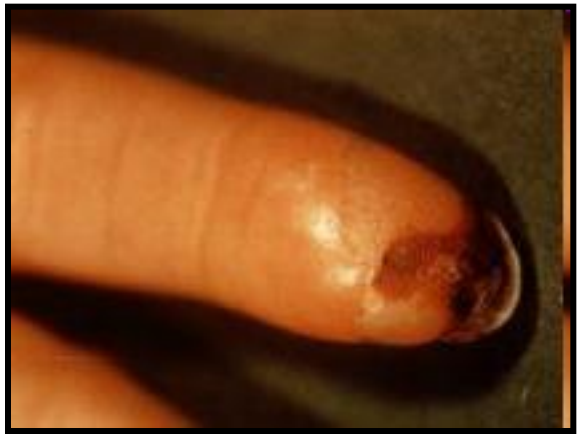


cold antibodies hemolytic anemia
agglutinin type (extravascular hemolysis)
hemolysin type (intravascular hemolysis; parossistic hemoglobinuria)



warm antibodies hemolytic anemia

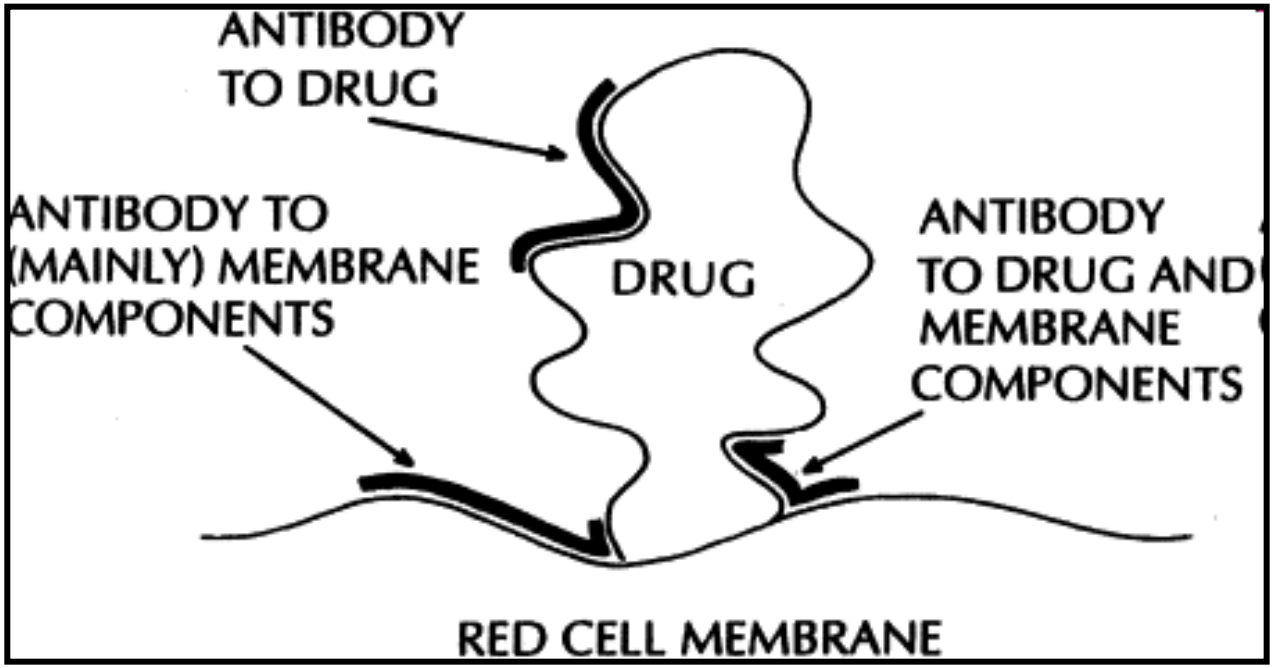




DRUG-INDUCED HEMOLYTIC ANEMIAS

Cephalosporins (3rd generation)
Diclofenac
α -Methyldopa
High-dose therapy with penicillin for > 10 days
Oxaliplatin
Rifampicin
Fludarabin
Levodopa
Quinidine
Mefenamic acid

Salama, 2009



Proposed unifying hypothesis of drug-induced antibody reactions. The thicker, darker lines represent antigen-binding sites on the Fab region of the drug-induced antibody. Drugs (haptens) bind loosely (or firmly) to cell membranes, and antibodies can be made to (a) the drug (producing in vitro reactions typical of a drug adsorption [penicillin-type] reaction); (b) membrane components, or mainly membrane components (producing in vitro reactions typical of autoantibody); or (c) part-drug, part-membrane components (producing an in vitro reaction typical of the so-called immune complex mechanism). Reprinted with permission.16 (Arndt and Garratty, 2005).