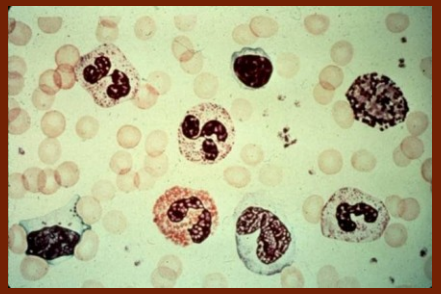
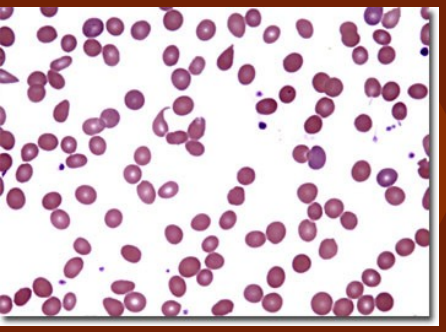
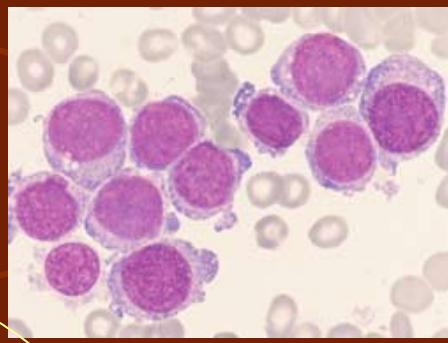
Hematology: Anemias

Stanislav Matoušek





HEMATOLOGY



Anemias and -penias
(not having enough elements)
thrombocytopenia

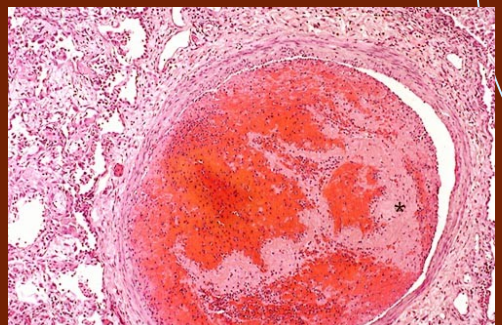
Leukemia etc.
(oncology – lympho- and myeloproliferative disorders)
E.g. chronic myeloid leukemia

Disorders of blood clotting

Primary hemostasis

Secondary hemostasis

Bleeding disorders/ thrombo-embolism



Clinical symptoms of hematologic disease

- Anemia
  - → signs of hypoxia – tiredness, weakness, dyspnea
  - → signs of low levels of hemoglobin - paleness
  - → cardiovascular symptoms – palpitation

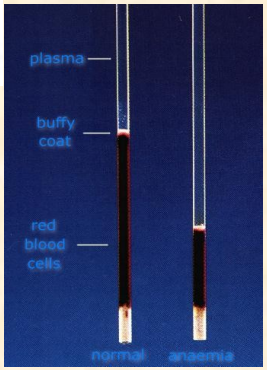
- Polycytemia → hyperviscose blood → risk of thrombosis

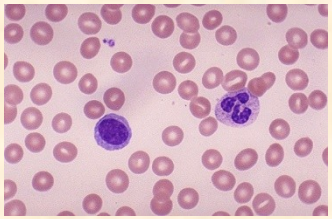
- Bleeding, spontaneous bleeding, unceasing bleeding

- Thrombosis → embolism – local symptoms of swelling or ischemia – DVT -, pulmonary embolism
- Frequent infections

Anemia

- Hb
  - M: < 135 g/L
  - F: < 120 g/L
- Hct
  - M: < 40 %
  - F: < 37 %
- Ery
  - M: < 4,3 * 10^{12} /L
  - F: < 3,9 * 10^{12} /L





Principal criterion: Hb< 120 g/L in w or < 135 in m

Pathophysiology of anemia symptoms

low hemoglobin concentration → paleness

↓

Deficient oxygen delivery into tissues

↓

Tissue hypoxia → sympaticus activated

↓ ↓

weakness,dyspnea

palpitations ← hyperkinetic circulation

Causes of hypoxia

- Altitude hypoxia – lack of O_2 in the inspired air = low pO_2
- Respiratory insufficiency– hypoxic hypoxia
- Lack of hemoglobin – transport hypoxia = anemia
- Circulatory disturbance – circulatory hypoxia
- Impaired oxidation in mitochondria – histotoxic hypoxia

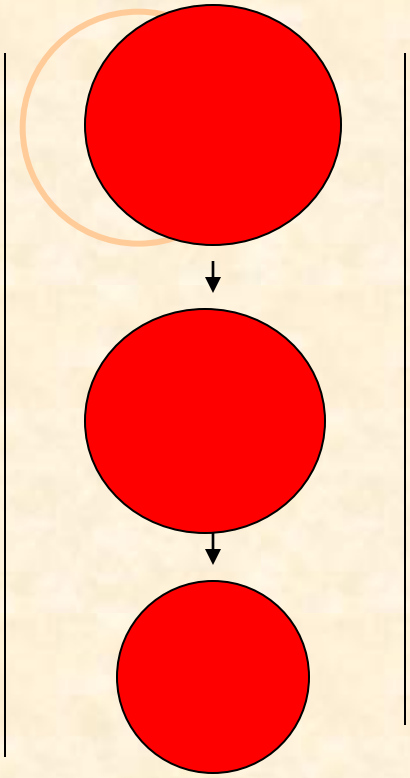
Laboratory tests:

- Principal:
  - Complete (full) blood count (CBC or FBC)
- Complementary:
  - Tests of iron metabolism
  - Erythropoietin levels
  - Detecting antibodies against RBC – Coombs test = antiglobuline test AGT
  - Osmotic fragility test
  - Historically: Ham's test (resistance in acidic environment)
  - Blood film/smear microscopic examination
  - **Bone marrow cytology/ aspiration (Sternal puncture)**

Anemias classified by RBC morfology (CBC)

- by MCV
  - microcytic - e.g. iron deficiency
  - normocytic - e.g. acute bleeding
  - macrocytic (megaloblastic) - pernicious
- by MCHC (color)
  - hypochromic – lack of iron
  - normochromic

BONE MARROW



Stem cells, growth factors
erythropoietin:

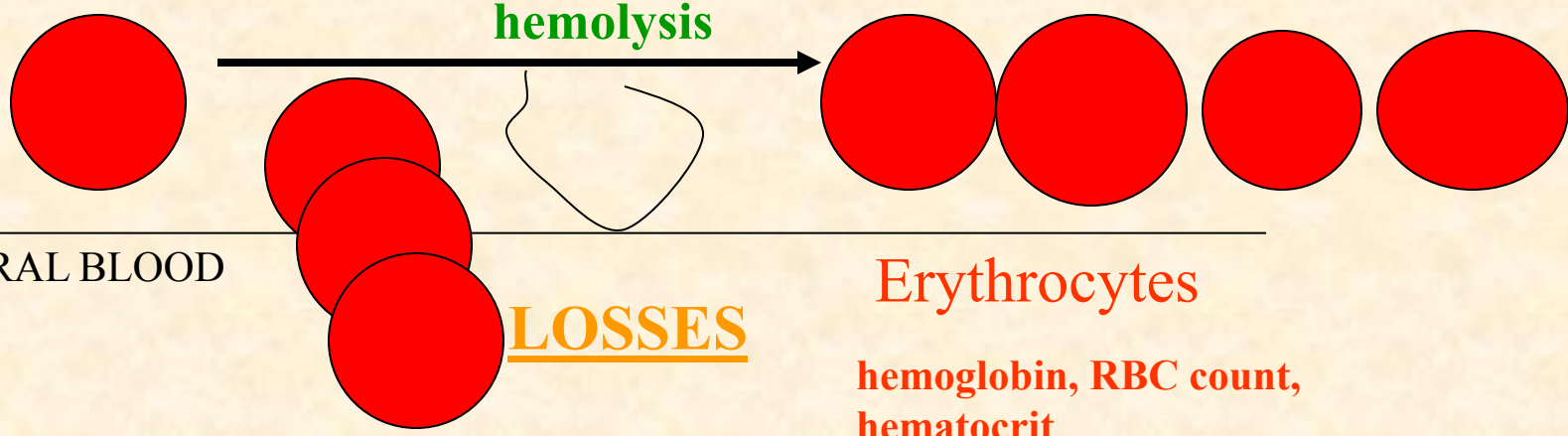
Cellular division, maturation:
DNA synthesis: vitamin B_{12}, folic acid

Hemoglobin synthesis: globin, porphyrine, Fe

Other factors

PRODUCTION

hemolysis



PERIPHERAL BLOOD

LOSSES

bleeding

Erythrocytes

hemoglobin, RBC count,
hematocrit
MCV, MCH, MCHC
shape etc.

Anemias by their etiology/patho

- **decreased production**
  - Stem cell failure or failure to differentiate
  - disorder in DNA synthesis
  - Disorder in hemoglobin synthesis
  - Lack of erytropoetin / renal failure
    - Complete loss of erythropoiesis – decrease of RBC count 10% / wk
- **increased destruction - hemolysis**
  - Defect of erythrocytes
  - Causes outside of RBC
- **increased loss - bleeding**
- **misdistribution and loss (hyperslenism, pooling in spleen)**

Reticulocyte count

- Daily replenishment rate
  - 0.5 – 1.5% of total RBC count
  - Mature during the 1st day in peripheral blood
- Criterion of bone marrow activity –
- Key test in distinguishing anemias
  - Reticulocytosis
    - Reaction of the BM to a blood loss (hemolytic anemias, severe bleeding)
    - Response to a correct anemia therapy (e.g. defic. B12 or Fe)
  - Reticulocytopenia
    - Defective erythropoiesis

Blood loss anemia

- **Acute** blood loss
  - shortly after massive blood loss Hb normal due to vasoconstriction
  - normochromic - normocytic
- **Chronic** blood loss
  - results in iron deficiency
- Excessive hemolysis (RBC destruction)

Excessive hemolysis (RBC destruction)

reticulocytosis, LDH is increased, unconjugated bilirubin accumulates

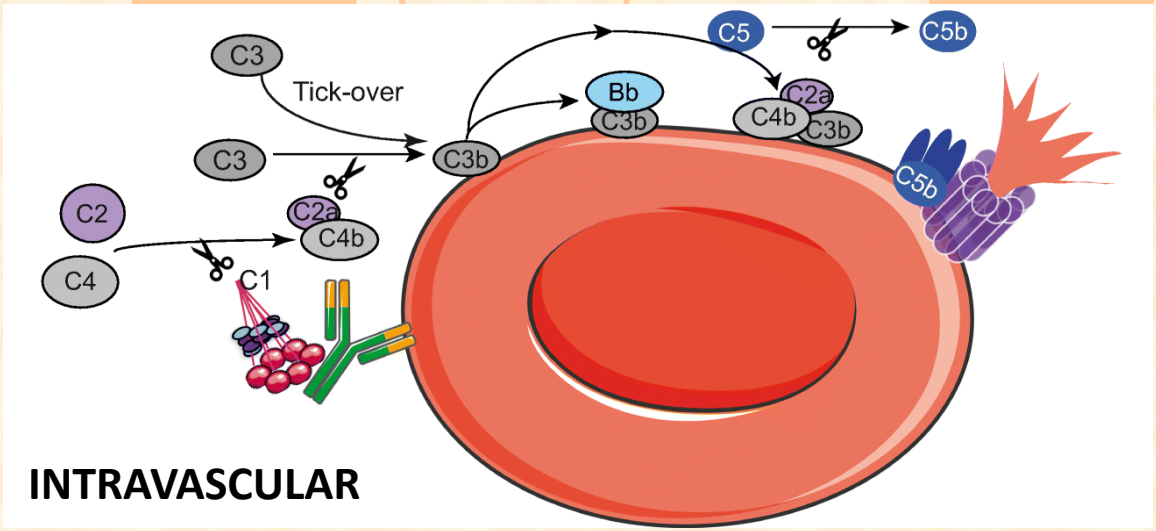
Extrinsic RBC defect (normocytic-normochromic RBC)

- Immunologic abnormalities (AIHA, PNH)
- Mechanical injury (trauma, infection)

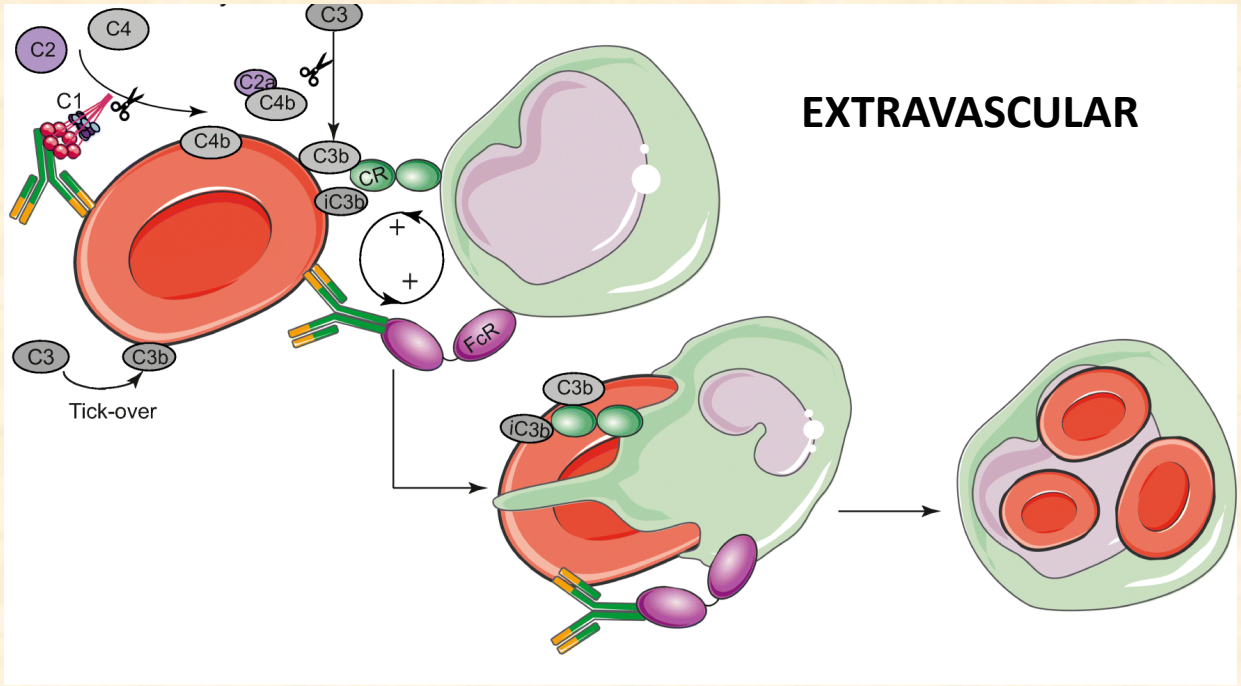
Intrinsic RBC defect

- Membrane alterations
  - congenital (spherocytosis, elliptocytosis)
  - Aquired (hypophosphatemia)
- Metabolic disorders (G6PD deficiency)
- Hemoglobinopaties (Sicle cell disease, Thalassemia)

Mechanisms of extravascular and intravascular hemolysis



paroxysmal nocturnal hemoglobinuria (PNH) and autoimmune hemolytic anemia (AIHA)



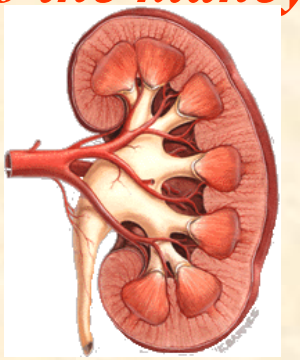
SYMPTOMS OF HEMOLYSIS

loss of red blood cells → loose Hb

intravascular

hemoglobinemia,
hemoglobinuria
hemosiderinuria

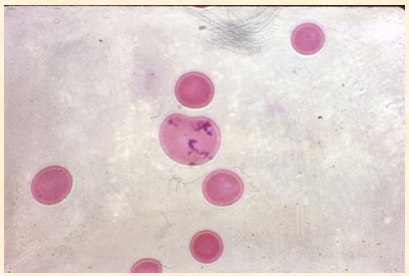
damage to the kidneys



anemia

BM activation

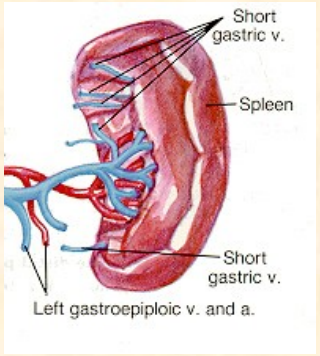
reticulocytosis



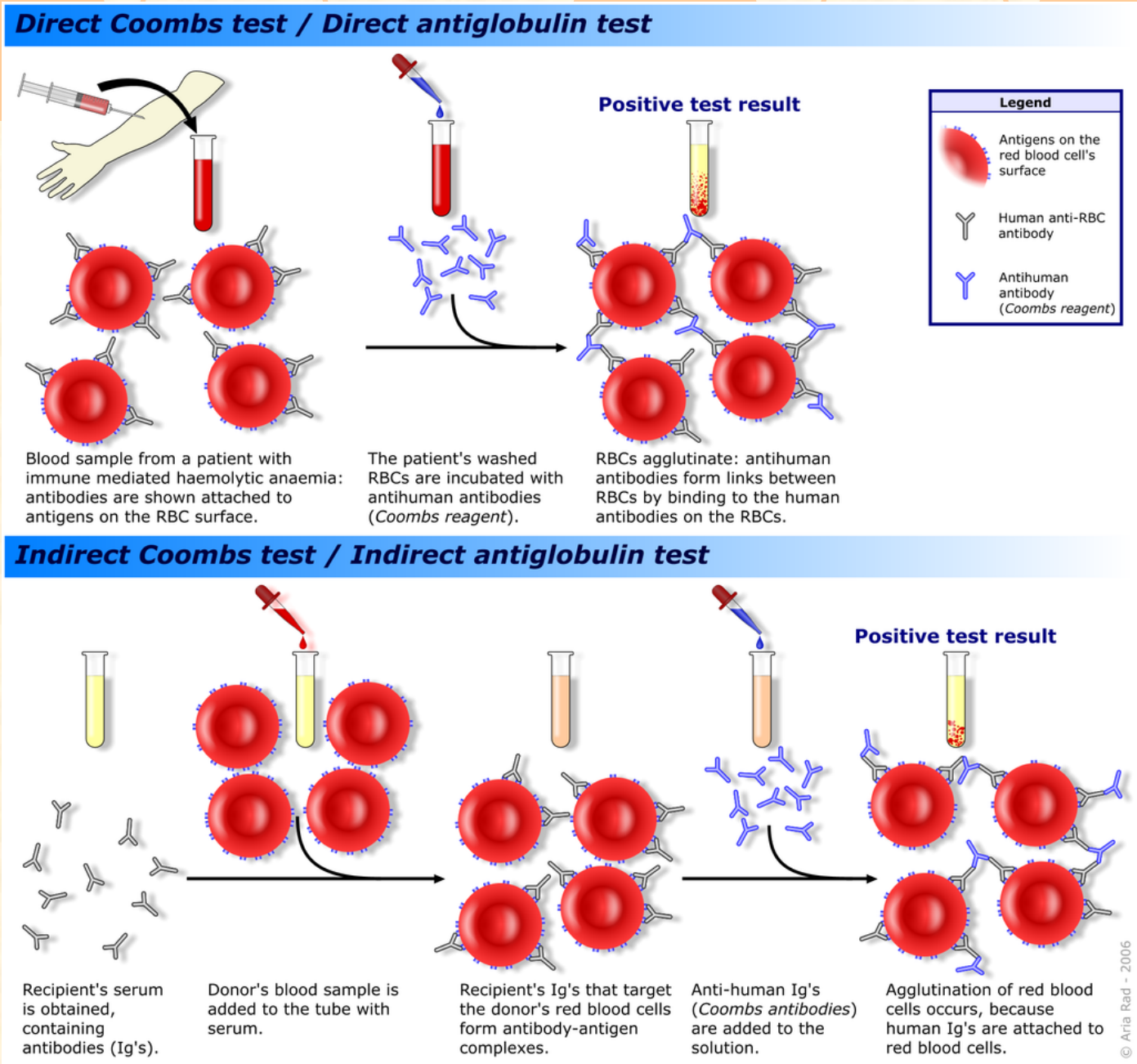
extravascular

increased prodution of ***bilirubin***
jaundice (icterus)

splenomegaly



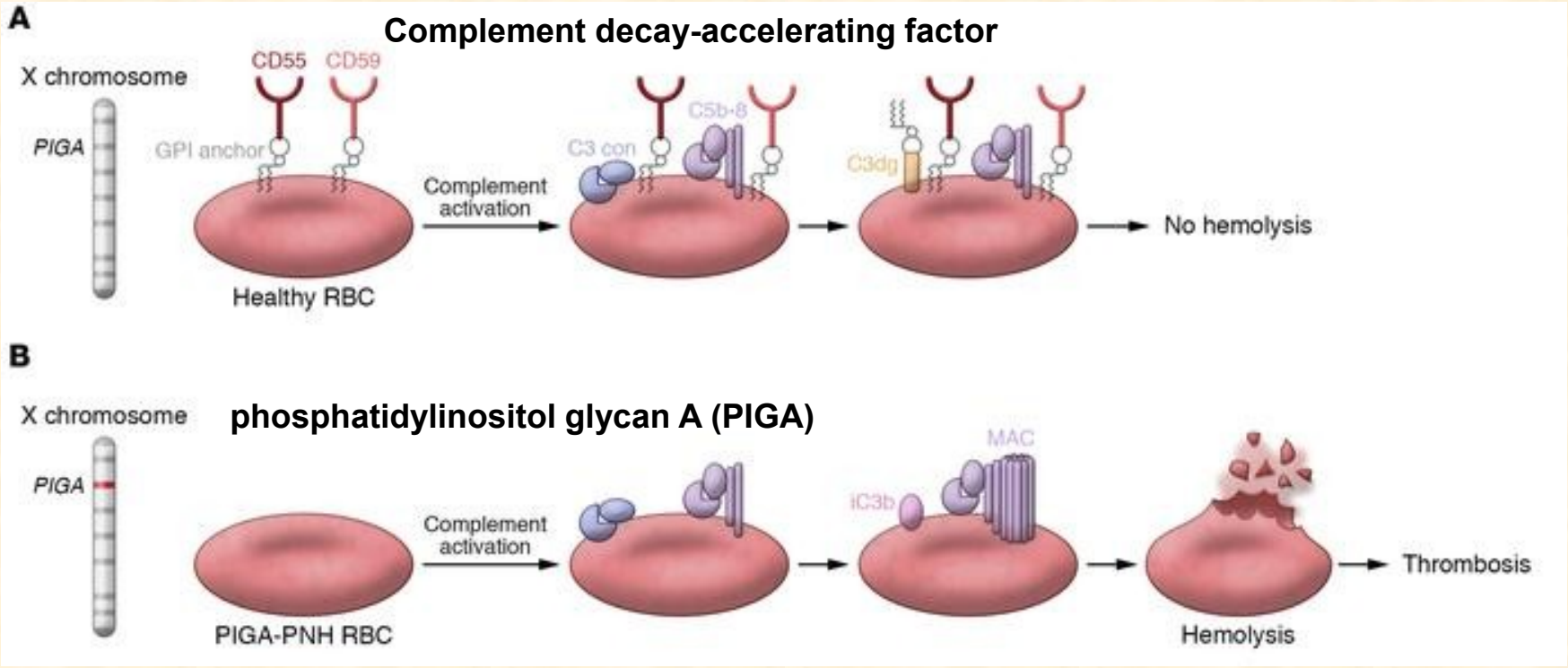
TESTS FOR HEMOLYSIS



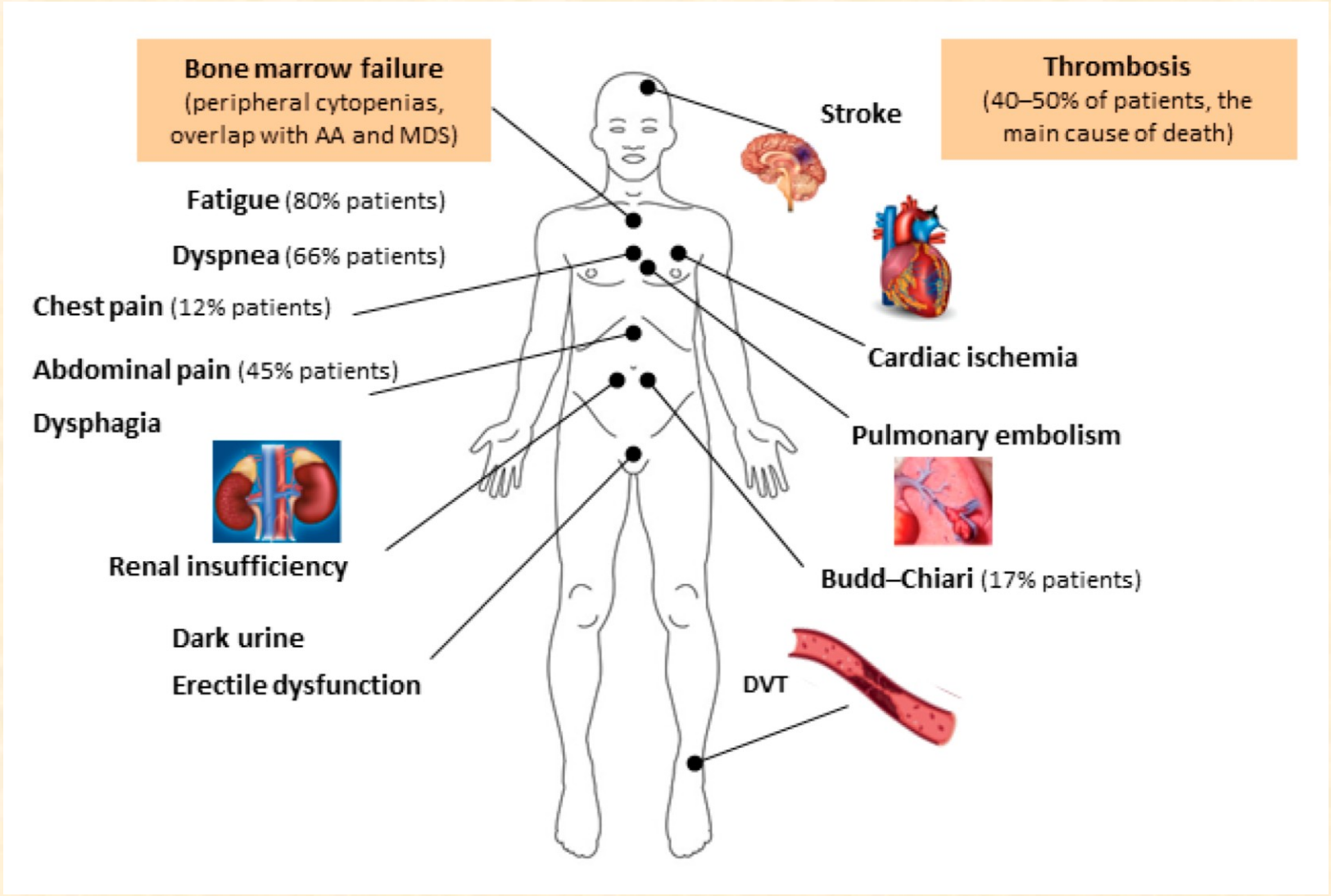
Paroxysmal nocturnal hemoglobinuria

life-threatening disease of the blood characterized by destruction of red blood cells by the complement system

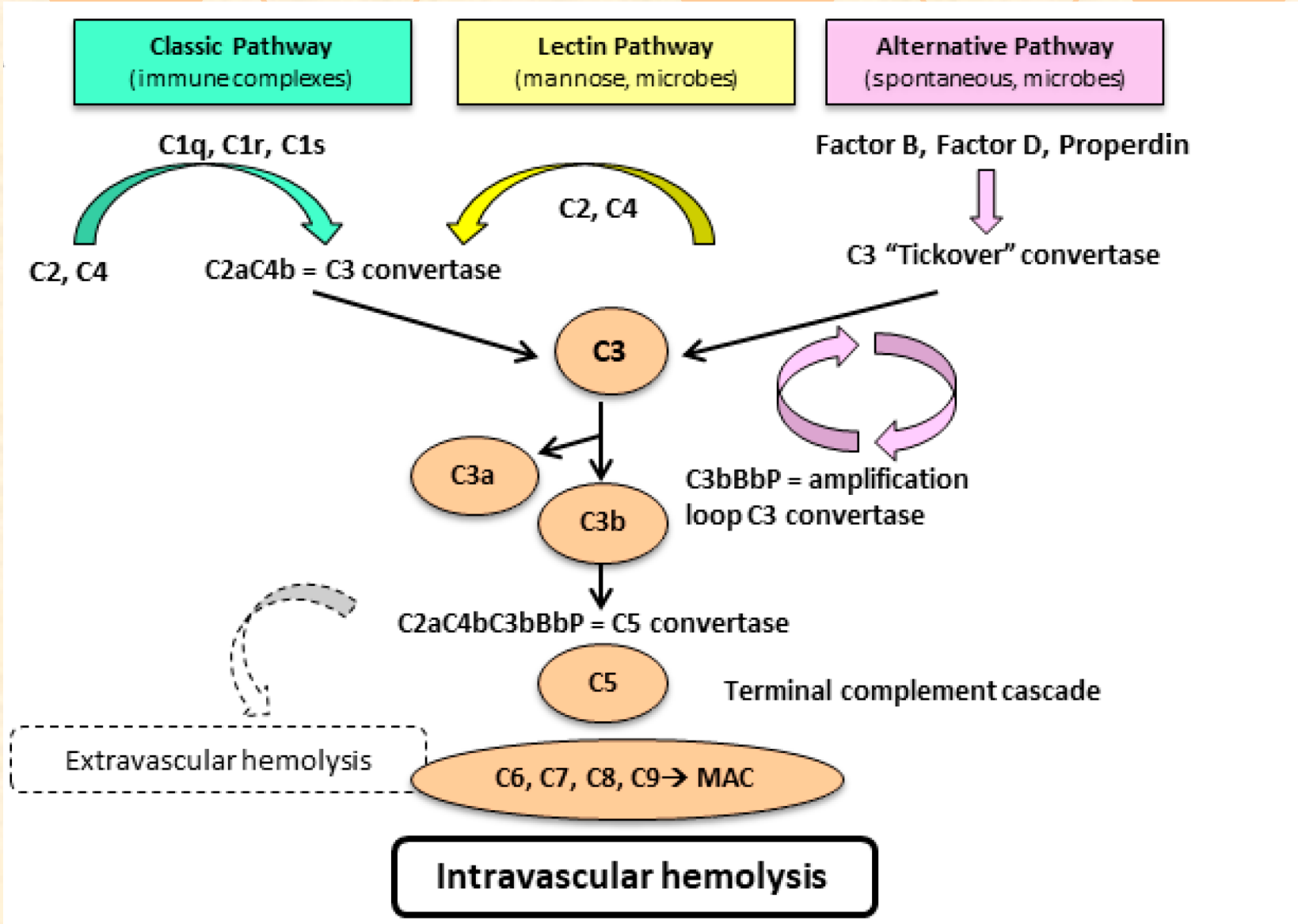
The main proteins that protect blood cells from destruction are **decay-accelerating factor (DAF/CD55)**, which disrupts formation of C3-convertase, and **protectin** (CD59/MIRL/MAC-IP), which binds the membrane attack complex and prevents C9 from binding to the cell.



Paroxysmal nocturnal hemoglobinuria



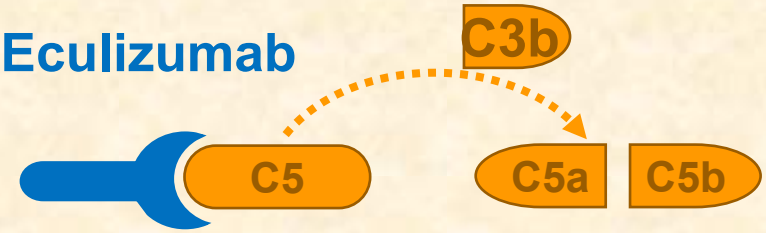
Paroxysmal nocturnal hemoglobinuria



PNH treatment

Eculizumab is humanized therapeutical antibody that binds C5 complement and prevents its cleavage by C3b. It is used to treat paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and neuromyelitis optica.

Eculizumab



Pegcetacoplan

- Pegcetacoplan binds to complement protein C3 and its activation fragment C3b
- regulating the cleavage of C3 and the generation of downstream effectors of complement activation

Direct antiglobulin (Coombs') test (DAT)

- Detection of antibodies to erythrocyte surface antigens
- AIHA
- Antiglobulin serum is added to washed RBC from the patient ------ agglutination indicates presence of immunoglobulins or complement components bound to RBC

TESTS FOR HEMOLYSIS

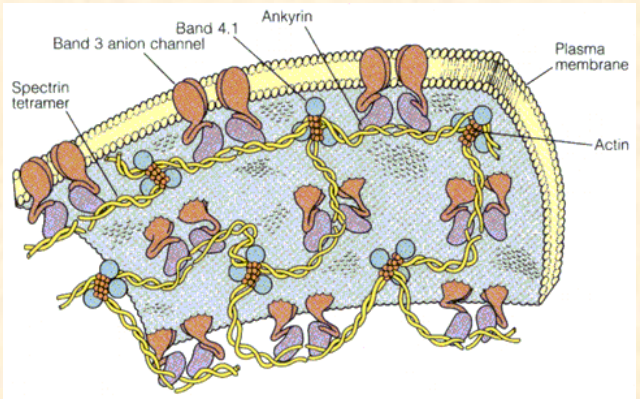
Test of osmotic resistence

RBC survive only in isotonic surrounding but have some toleration to its changes

RBC in some hemolytic states have decreased tolerance

Special tests

membrane properties (electrophoresis of proteins)
properties of hemoglobin
genetic tests



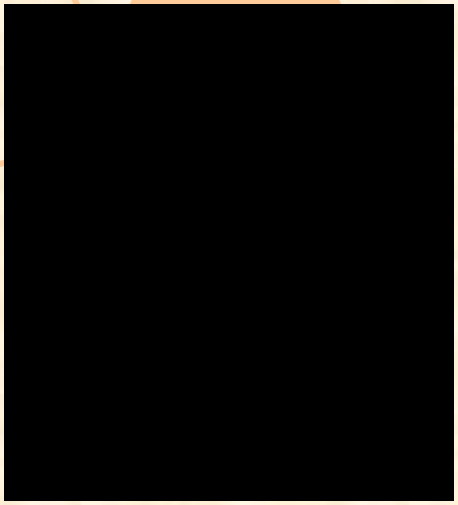
Deficient erythropoiesis

- **Iron deficiency**
  - microcytic-anisocytosis, ↓ reticulocytes
- **Vitamin B_{12} or Folate deficiency**
  - macrocytes-anisocytosis
- **BM failure -** chronic diseases, aplastic anemia, myelodysplasia, leukemia
  - normochromatosis-normocytosis
  - BM hypoplasia

Iron metabolism

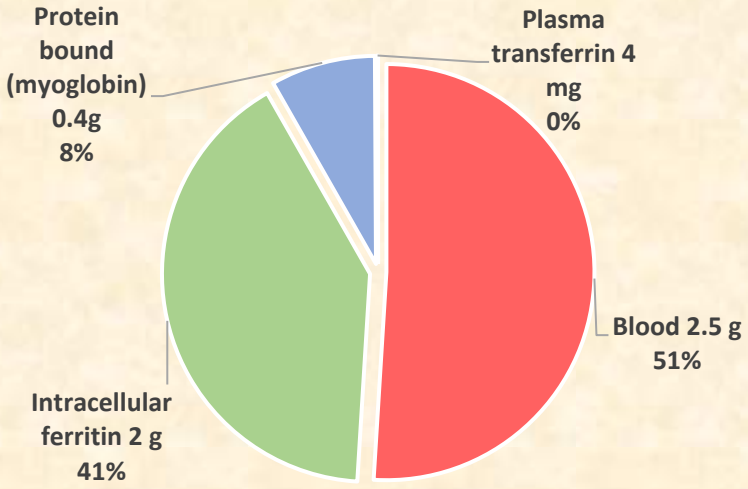
Iron is an essential bioelement for most forms of life, from bacteria to mammals due to its ability to mediate electron transfer.

Fe^{2+} (ferrous state) $\longleftrightarrow$ Fe^{3+} (ferric state)



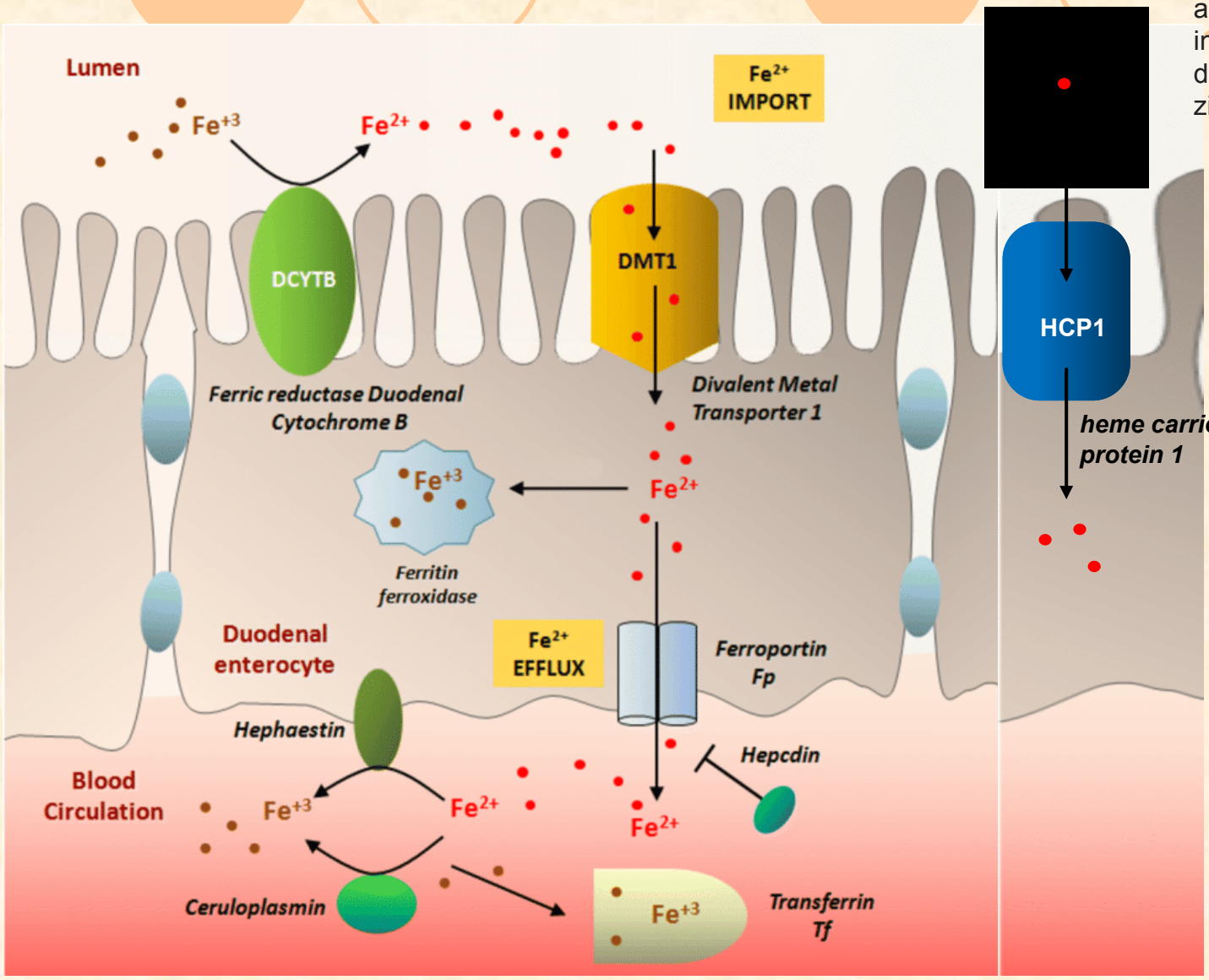
Structure of Heme b

Because of its toxicity, free soluble iron is kept in low concentration in the body



Iron transport

absorption of dietary iron is relatively low (5-35%)



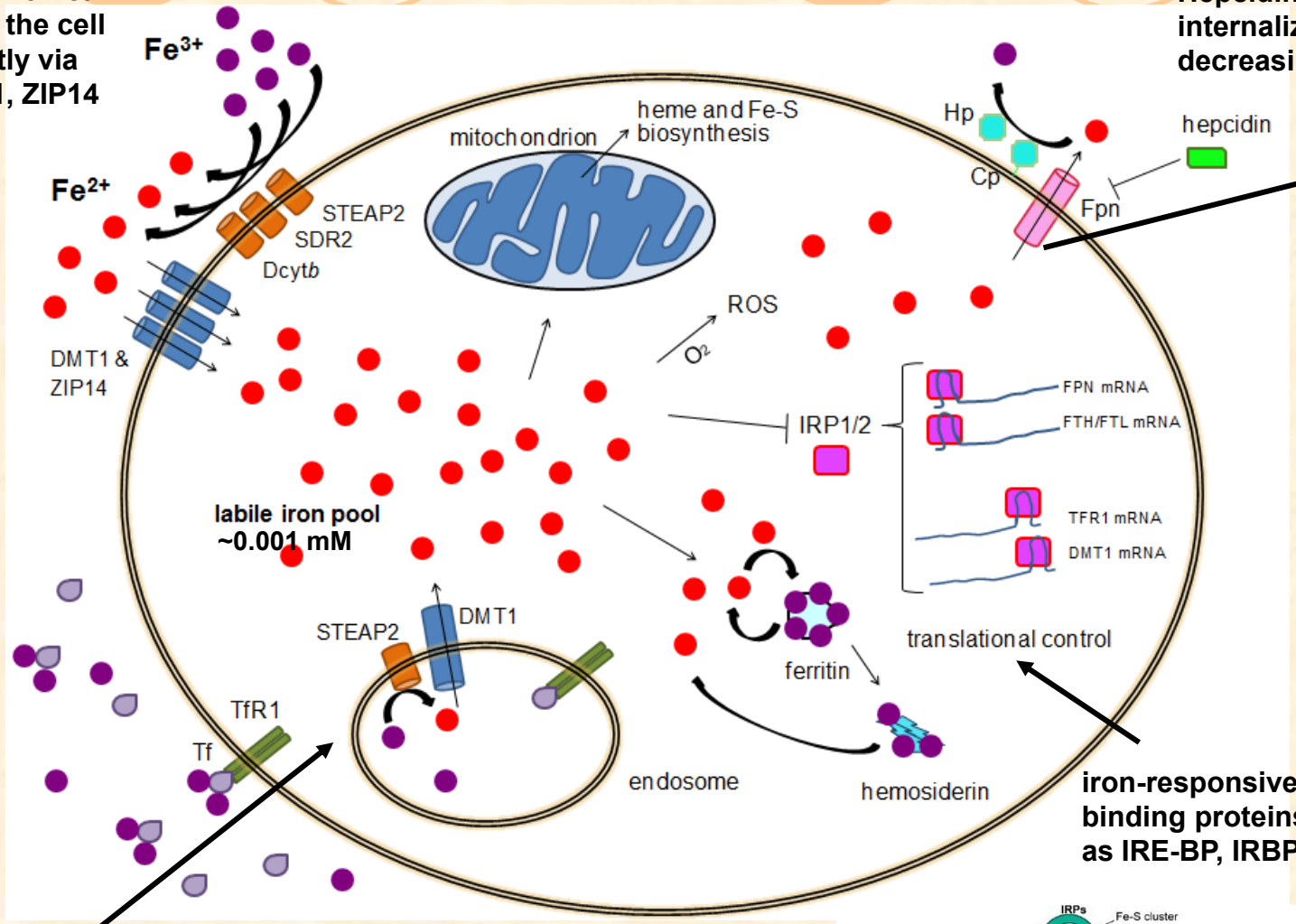
absorption from diet is enhanced in the presence of vitamin C and diminished by excess calcium, zinc, or manganese

enterocytes synthesize more Dcytb, DMT1 and ferroportin in response to iron deficiency

* there is no physiologic regulatory mechanism for excreting iron

Cellular iron homeostasis

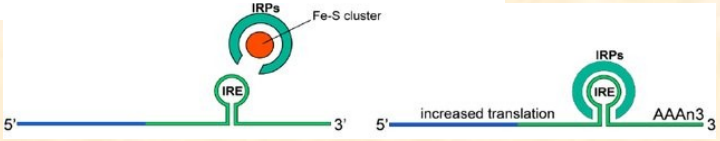
Alternatively,divalent iron can enter the cell directly via DMT1, ZIP14



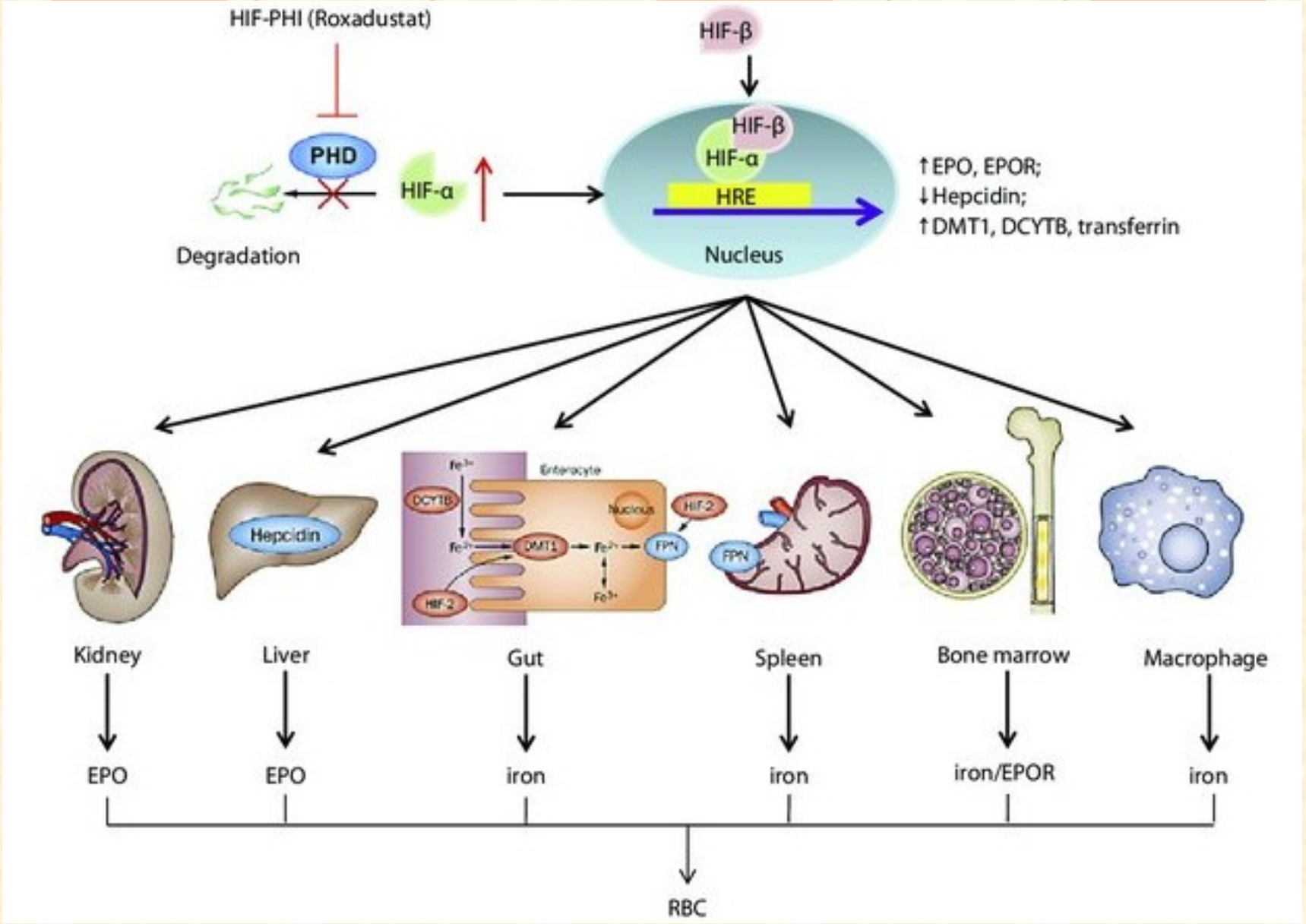
Hepcidin causes the internalization of ferroportin, decreasing iron export.

iron exporter ferroportin Fpn

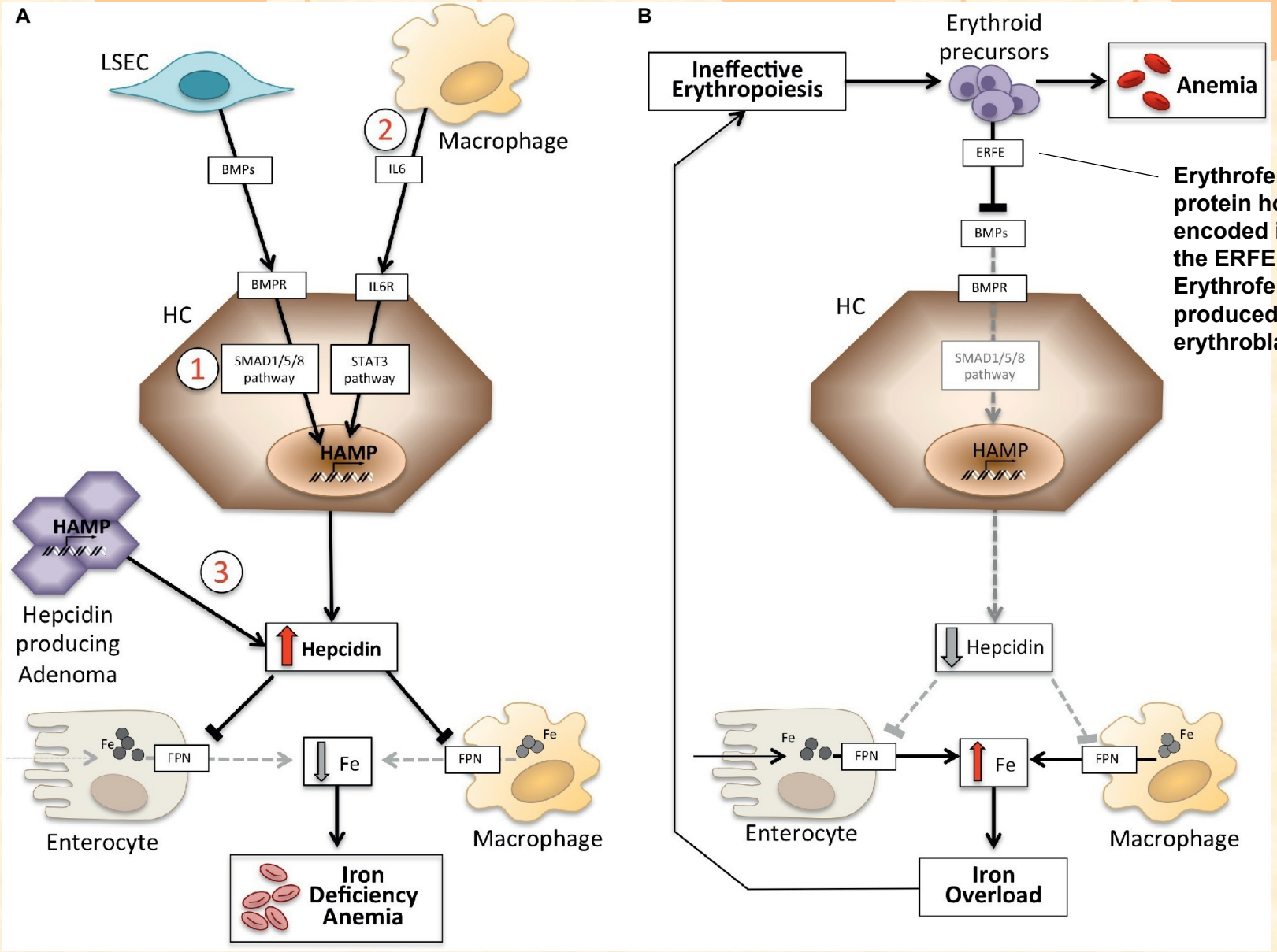
iron-responsive element-binding proteins, also known as IRE-BP, IRBP, IRP and IFR



Cellular uptake primarily through receptor-mediated endocytosis via transferrin receptor TFR1, TRF2 and GAPDH


HIF-PHI (Roxadustat)
PHD
HIF-α
Degradation
HIF-β
HIF-β
HIF-α
HRE
Nucleus
↑EPO, EPOR;
↓Hepcidin;
↑DMT1, DCYTB, transferrin
Hepcidin
Enterocyte
DCYTB
DMT1
HIF-2
FPN
Nucleus
Kidney
Liver
Gut
Spleen
Bone marrow
Macrophage
EPO
EPO
iron
iron
iron/EPOR
iron
RBC

HEPCIDIN



Erythroferrone is a protein hormone encoded in humans by the ERFE gene. Erythroferrone is produced by erythroblasts

Tests for iron

- iron concentration in serum (age , sex)
- TIBC (total iron binding capacity for Fe)
- transferrin saturation (N 20-55 %)
- serum ferritin
- serum (solubile) transferrin receptor (sTfR)

Tests of iron metabolism

Serum iron (SI)

- F: 600-1400 μg/L, 11-25μmol/L; M: 750-1500 μg/L, 13-27μmol/L
- Low in Fe deficiency and chronic disease
- High in hemolytic syndromes and iron overload

Total iron binding capacity (TIBC)

- 2500 – 4500 μg/L , 45-82 μmol/L
- High in Fe deficiency
- Low in chronic disease

Serum ferritin (30-300 ng/mL)

- Fe storage glycoprotein
- Closely correlates with total body Fe stores
- <12 ng/mL Fe deficiency
- Elevated in Fe overload, liver injury, tumors (Acute phase protein)

Tests for iron metabolism

Serum transferin receptor

- Increase in increased erythropoiesis and early Fe deficiency

RBC ferritin

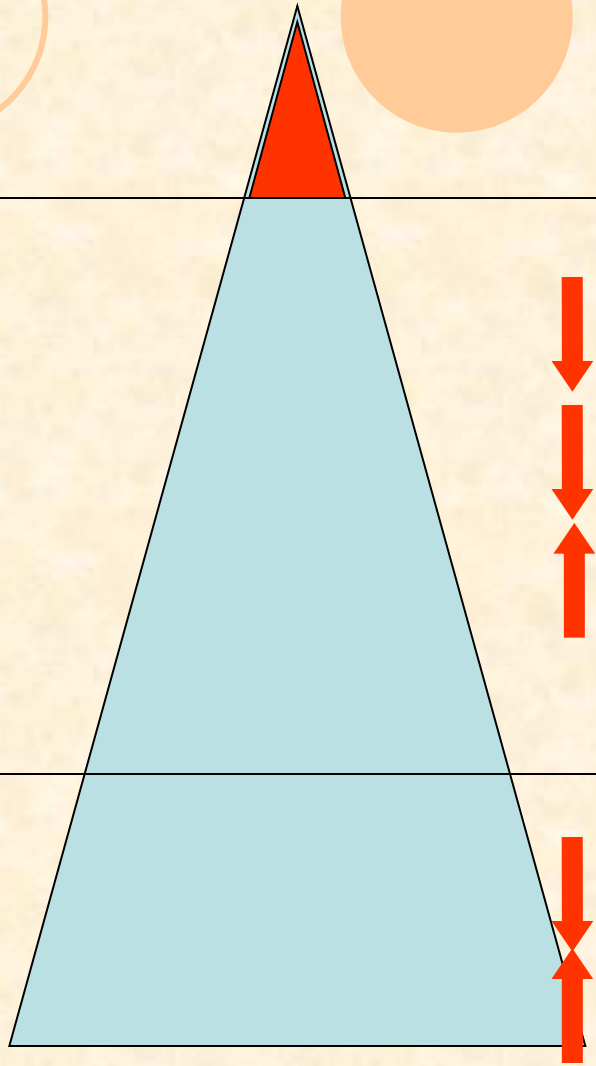
- storage status over the previous 3 month (Fe deficiency/overload)
- unaffected by liver function or acute illness

Free RBC porphyrin

- increased when heme synthesis altered

Manifest

anemia



Latent

iron deficiency erythropoiesis

serum iron

transferin satur.

transferin

Prelatent

no stores

serum ferritin

TIBC ~ transferin

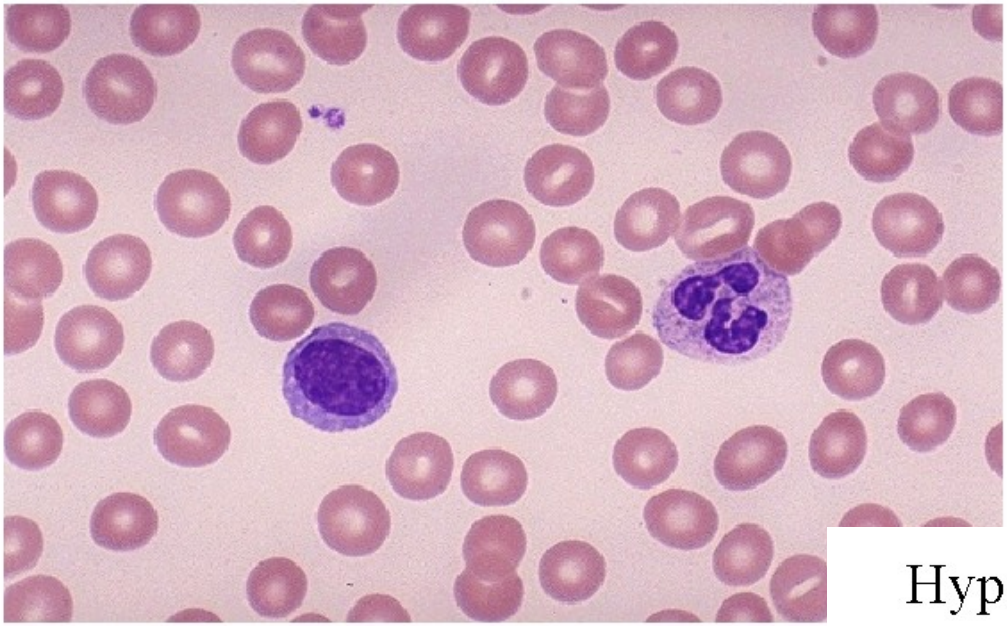
iron in BM

Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

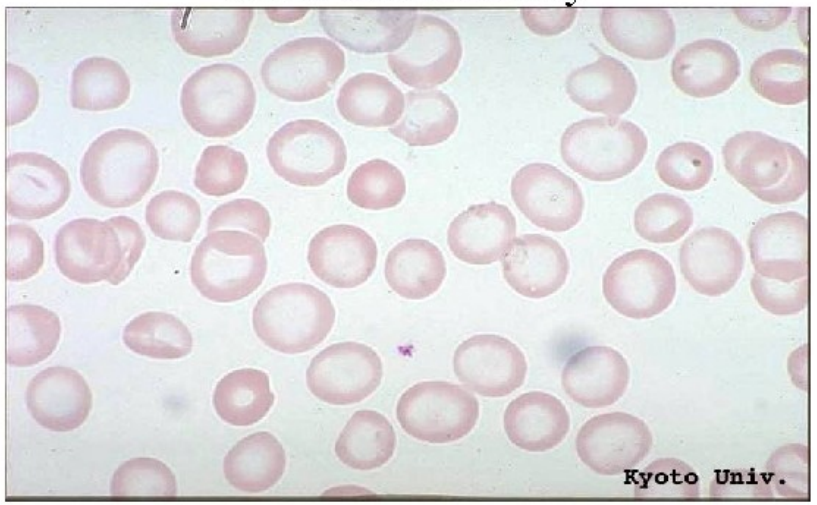
Iron deficiency	Responsive to iron therapy
Chronic inflammation	Unresponsive to iron therapy
Thalassemia major	Reticulocytosis and indirect bilirubinemia
Thalassemia minor	Elevation of fetal hemoglobin, target cells, and poikilocytosis

Lead poisoning	Basophilic stippling of RBCs
Sideroblastic	Ring sideroblasts in marrow
Hemoglobinopaties	Hemoglobin electrophoresis

Normal Smear



Hypochromic/Microcytic Anemia
Iron Deficiency



Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

	Serum Iron	Total Iron-Binding Capacity (TIBC)	Bone Marrow Iron	Comment
Lead poisoning	N	N	++	Basophilic stippling of RBCs
Sideroblastic	⬆	N	++++	Ring sideroblasts in marrow
Hemoglobinopaties	N	N	++	Hemoglobin electrophoresis

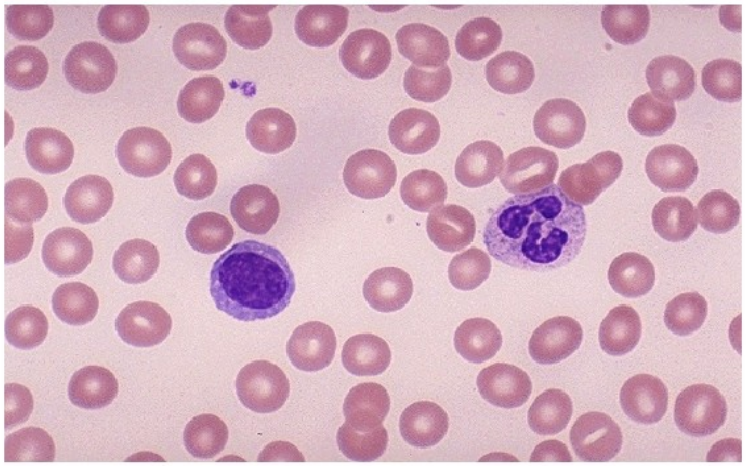
Microcytic Hypochromic Anemia (MCV<83; MCHC<31)

	Serum Iron	Total Iron-Binding Capacity (TIBC)	Bone Marrow Iron	Comment
Iron deficiency	⬇	⬆	0	Responsive to iron therapy
Chronic inflammation	⬇	⬇	++	Unresponsive to iron therapy
Thalassemia major	⬆	N	++++	Reticulocytosis and indirect bilirubinemia
Thalassemia minor	N	N	++	Elevation of A of fetal hemoglobin, target cells, and poikilocytosis

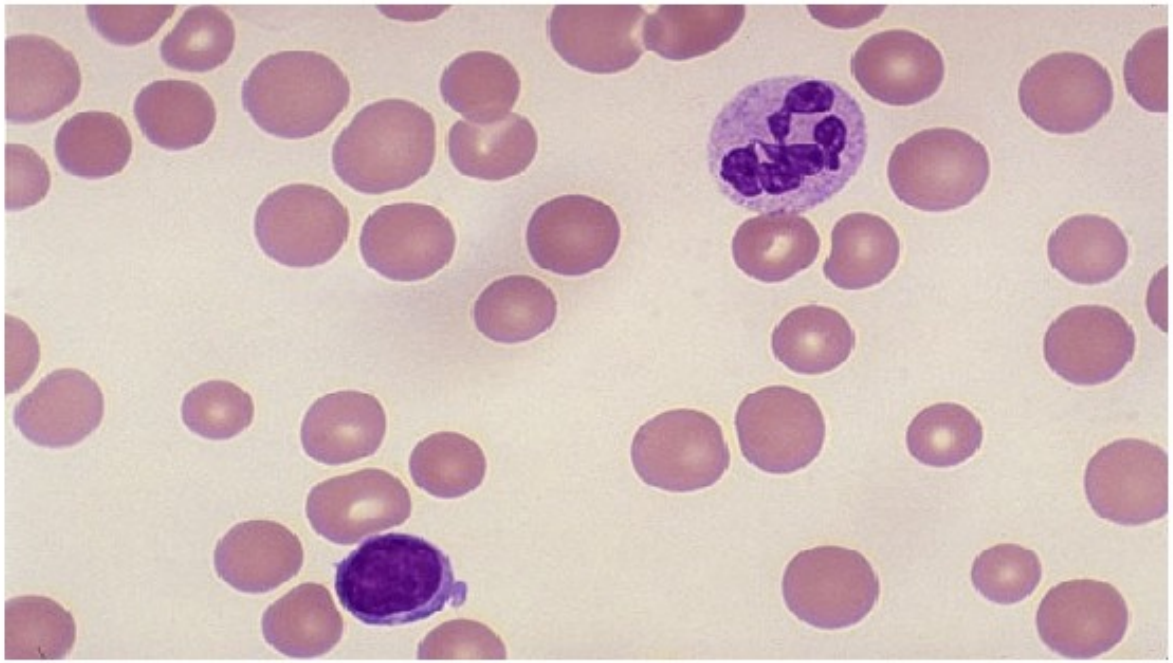
Macrocytic Anemia (MCV, >95)

Megaloblastic bone marrow	Deficiency of vitamin B-12
	Deficiency of folic acid
	Drugs affecting DNA synthesis
	Inherited disorders of DNA synthesis
Nonmegaloblastic bone marrow	Liver disease
	Hypothyroidism and hypopituitarism
	Accelerated erythropoiesis (reticulocytes)
	Hypoplastic and aplastic anemia
	Infiltrated bone marrow

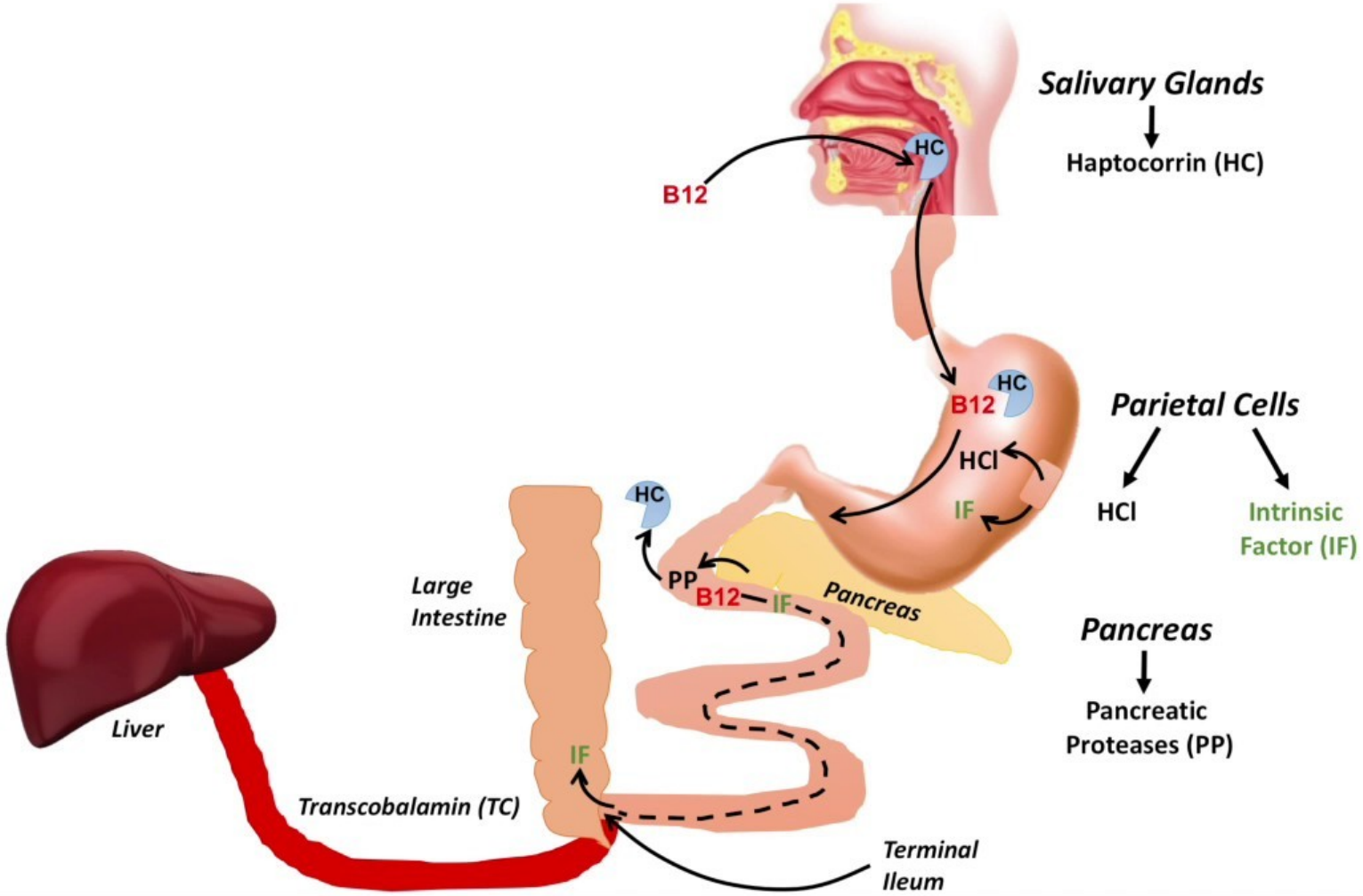
Normal Smear



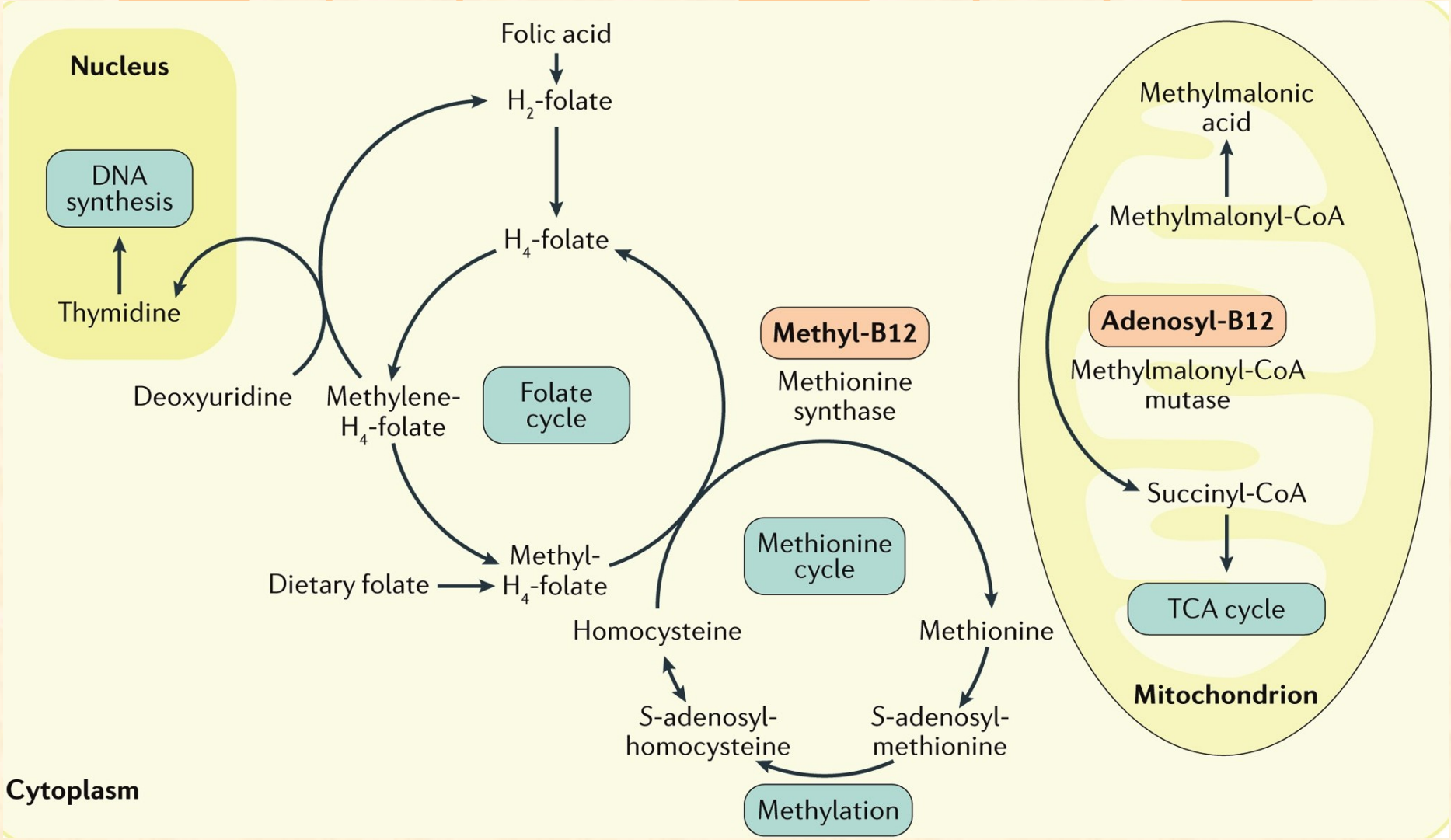
Megaloblastic Anemia
B-12 Deficiency



Absorption of vit. B12



The role of vit. B12 and folate



Lack of vit. B12 - causes

- Not enough ingestion – strict vegans if they do not take care
- Autoimmune inflammation of gastric mucosa (atrophic gastritis) leading to deficiency in intrinsic factor
- Diseases of terminal ileum (celiac disease, Crohn disease)

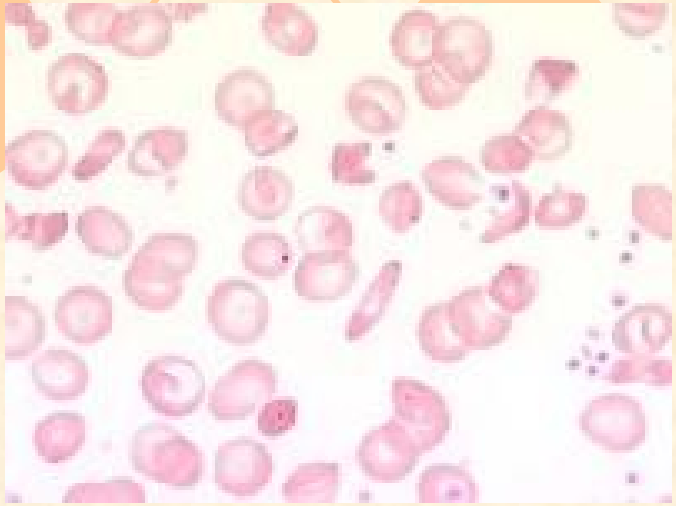
Blood smear

- **Morphology** of blood elements
  - Anisocytosis = variation in size
  - Poikilocytosis = variation in shape (schistocytes=RBC fragments; ovalocytes; spherocytes)

Various Forms of RBCs

Spherocyte	Loss of central pallor, stains more densely, often microcytic. Hereditary spherocytosis and certain acquired hemolytic anemias.
Target cell	Hypochromic with central "target" of hemoglobin. Liver disease, thalassemia, hemoglobin D, postsplenectomy.
Elliptocyte	Oval to cigar shaped. Hereditary elliptocytosis, certain anemias (particularly vitamin B-12 and folate deficiency).
Schistocyte	Fragmented helmet- or triangular-shaped RBCs. Microangiopathic anemia, artificial heart values, uremia, malignant hypertension.
Stomatocyte	Slitlike area of central pallor in erythrocyte. Liver disease, acute alcoholism, malignancies, hereditary stomatocytosis, and artifact.
Sickle cell	Elongated cell with pointed ends. Hemoglobin S and certain types of hemoglobin C and I.

Sicle Cell Disease



- Hemoglobin (Hb) S arises from a mutation coding of valine instead of glutamine in position 6 of the hemoglobin beta chain.
- The resulting hemoglobin has the physical properties of forming polymers under deoxy conditionsUnder deoxy conditions.
- Hb S forms a gel-like substance containing hemoglobin crystals called tactoids.