Blood

Approach to hematological disorders

History	Physical findings	Blood findings	
Weakness Fatigue Exertional dyspnea	Pallor Icterus	Anemia	Approach to a patient with anemia
Recurrent infections Unexplained fever	Spleenomegaly Enlarged lymph nodes	Leukocytosis Leukopenia Abnormal leukocytes	Disorders of phagocytic and immune system
Bleeding Bruising	Petechiae Ecchymosis Hemarthrosis Mucosal bleeding	Thrombocytopenia Prolonged bleeding time Abnormal prothrombin or partial thromboplastin time	Approach to bleeding disorders

Blood: Physical characteristics

Average amount: 8% body weight

(70 kg man- 5.6L)

Specific gravity: 1055-1065 (viscosity is 5 times that of water)

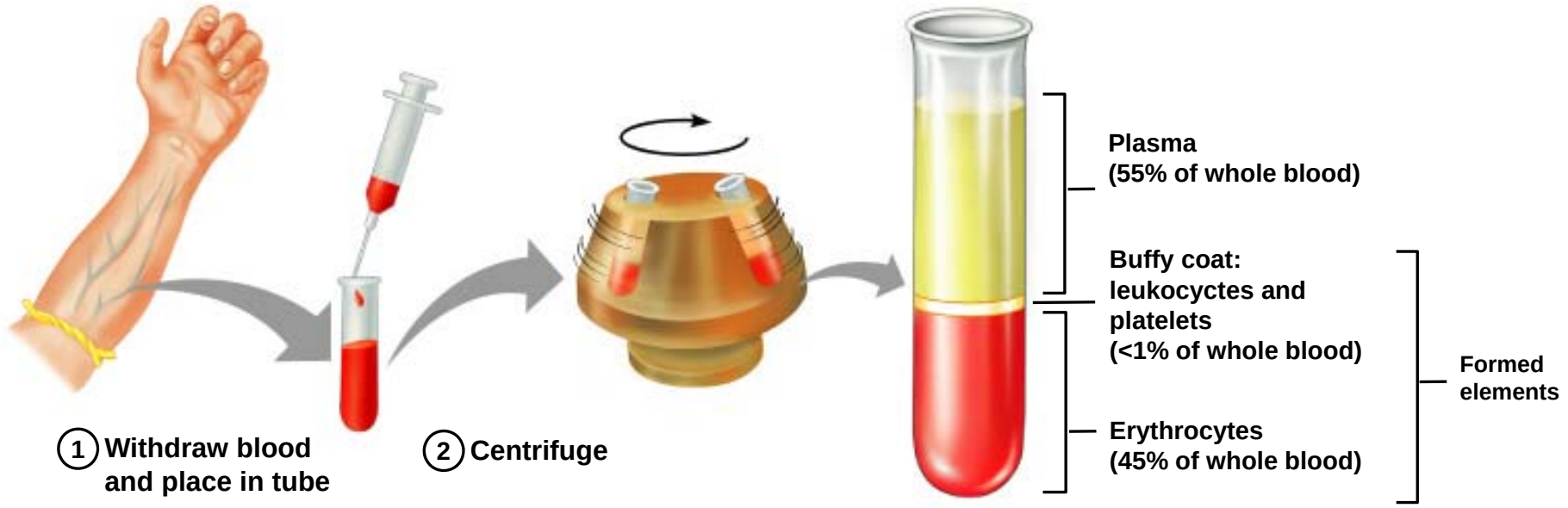
pH: 7.35-7.45

Osmolarity: 300 mOsm

Salanity: 0.9%

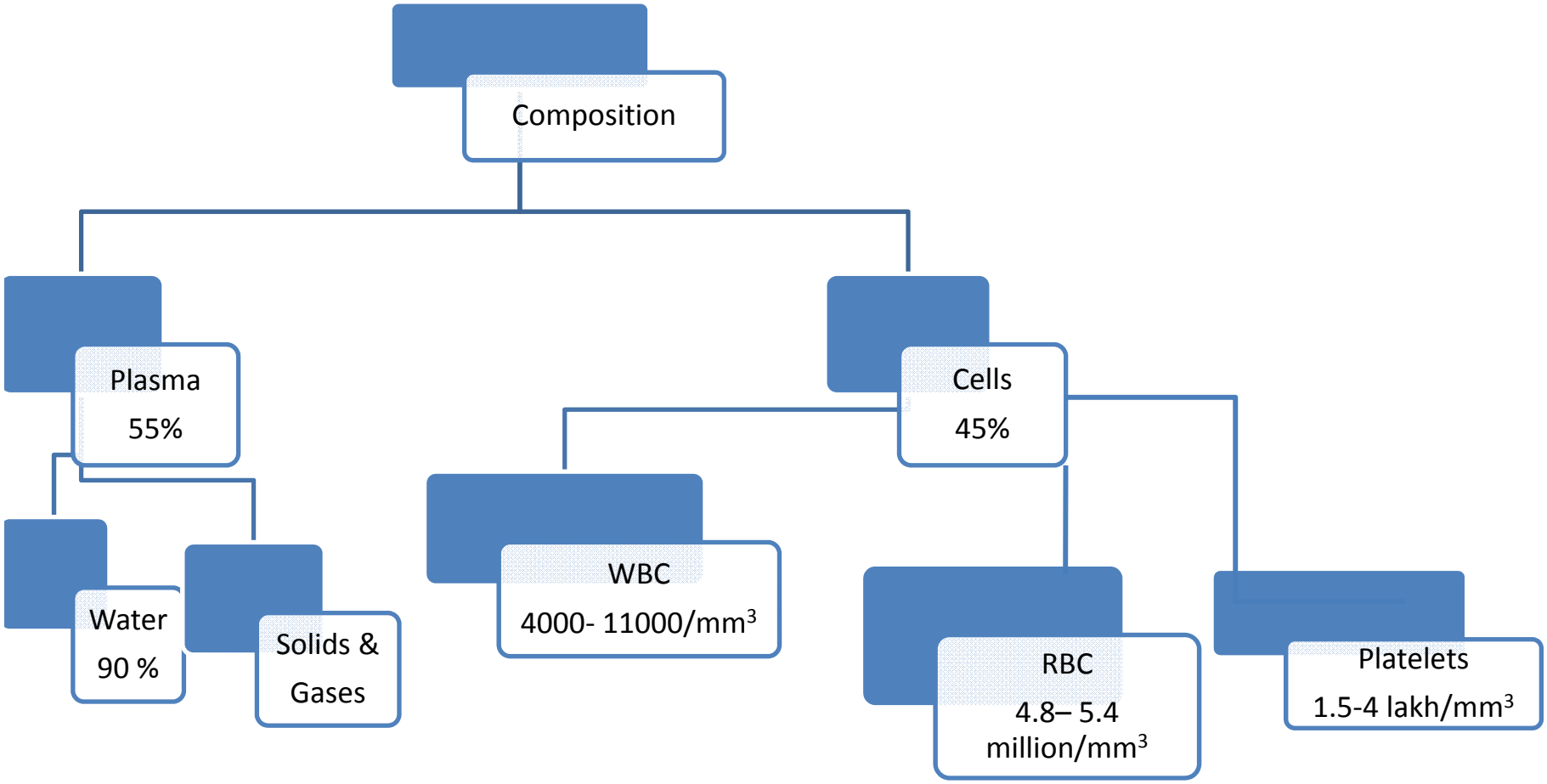
Colour: Bright red to deep red

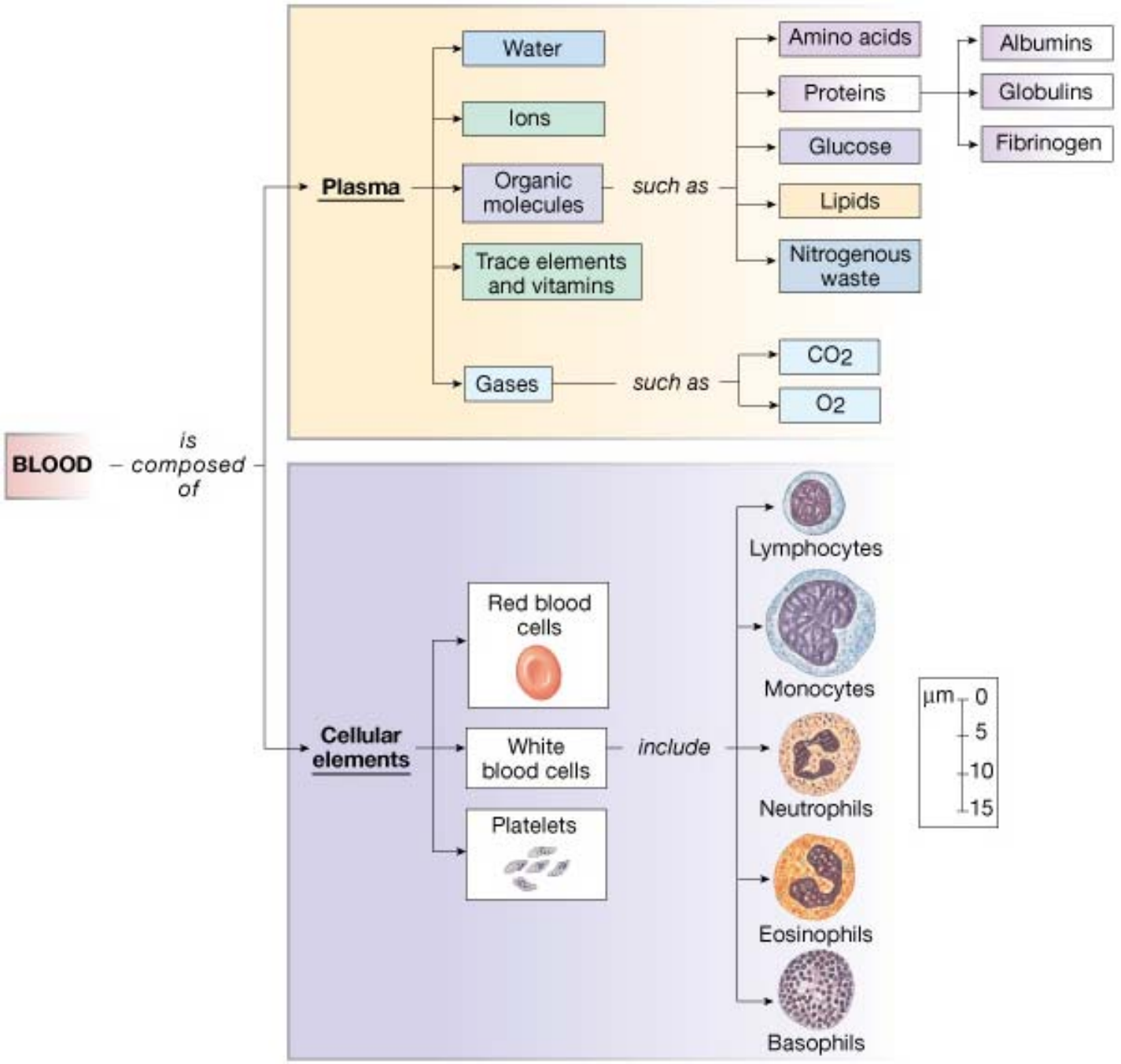
Components of Whole Blood



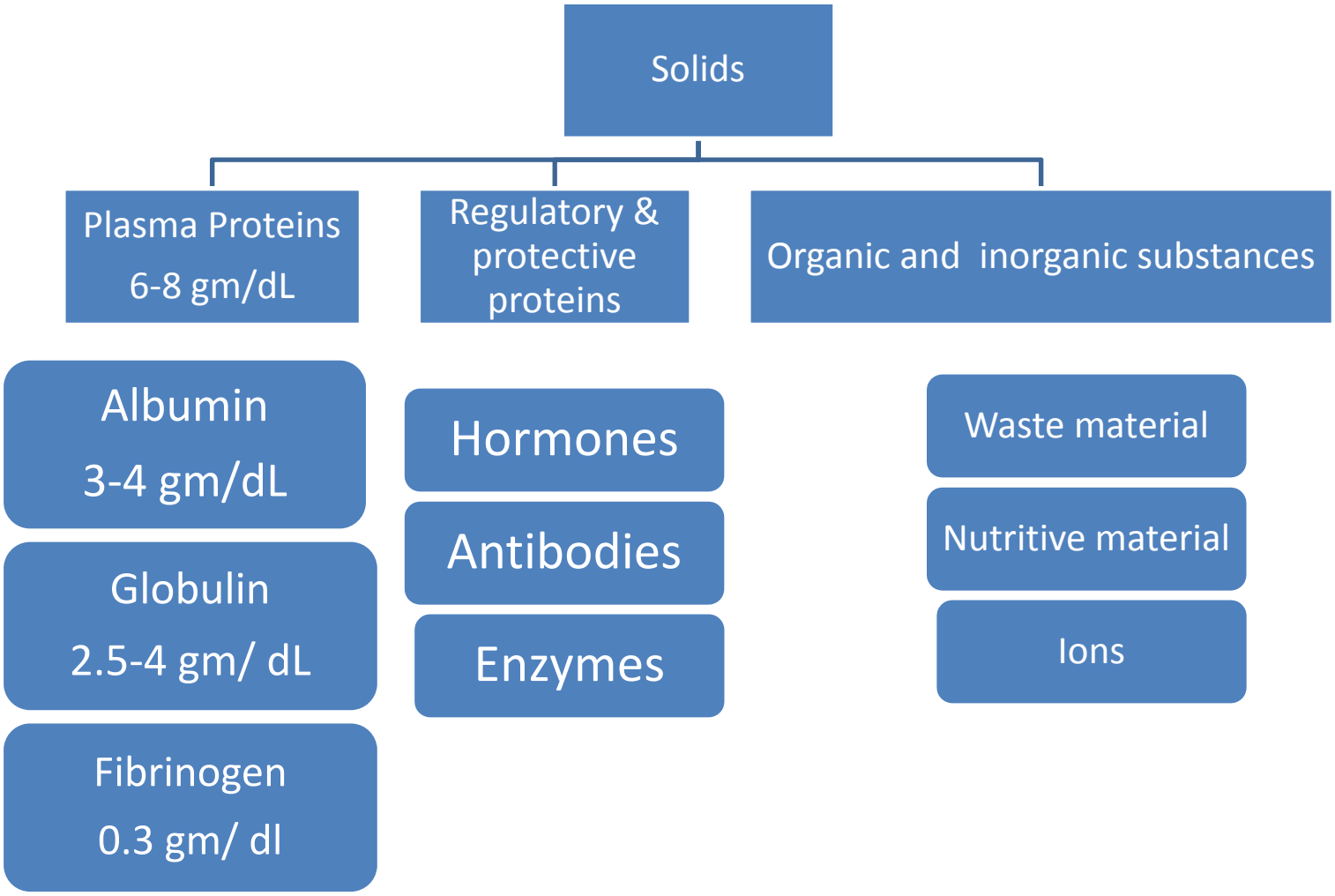
- Hematocrit
  - Males: 47% ± 5%
  - Females: 42% ± 5%

Components of blood




BLOOD
is composed of
Plasma
Water
Ions
Organic molecules
Trace elements and vitamins
Gases
such as
Amino acids
Proteins
Glucose
Lipids
Nitrogenous waste
Albumins
Globulins
Fibrinogen
such as
CO_2
O_2
Cellular elements
Red blood cells
White blood cells
Platelets
include
Lymphocytes
Monocytes
Neutrophils
Eosinophils
Basophils
µm
0
5
10
15

Components of plasma (90% water rest solids)



Functions of blood

Distributive

- Carries O_2 (from lungs) and nutrients (from GIT and body stores) to all cells
- Carries wastes from all cells to elimination sites (lungs for CO_2, liver for bilirubin and kidneys for nitrogenous wastes)
- Carries hormones (chemical signals) from endocrine organs to target tissues.

Functions of blood…

Regulatory functions

- Body T° by absorbing and distributing heat
- pH by virtue of its many buffers
- Maintains adequate fluid volume in the body

Protective functions

- Prevents blood loss by initiating clotting mechanisms in response to blood vessel damage
- Prevents infection via WBCs and plasma immune proteins

Separation of plasma proteins

- Solubility
  - Salting in
  - Salting out
- Molecular size
  - Dialysis
  - Gel filtration chromatography
  - Ultracentrifugation
  - Sodium dodecyl sulfate (SDS) polyacramide gel electrophoresis

Separation of plasma proteins

- Molecular charge
  - Ion exchange chromatography
  - High performance liquid chromatography
  - Electrophoresis

- Specific binding of the protein to a specific substance
  - Affinity/ absorption chromatography
  - Precipitation by antibodies

Separation procedures

- Protein solubility

➢ Salting out

Adding divalent salts eg. Ammonium sulphate

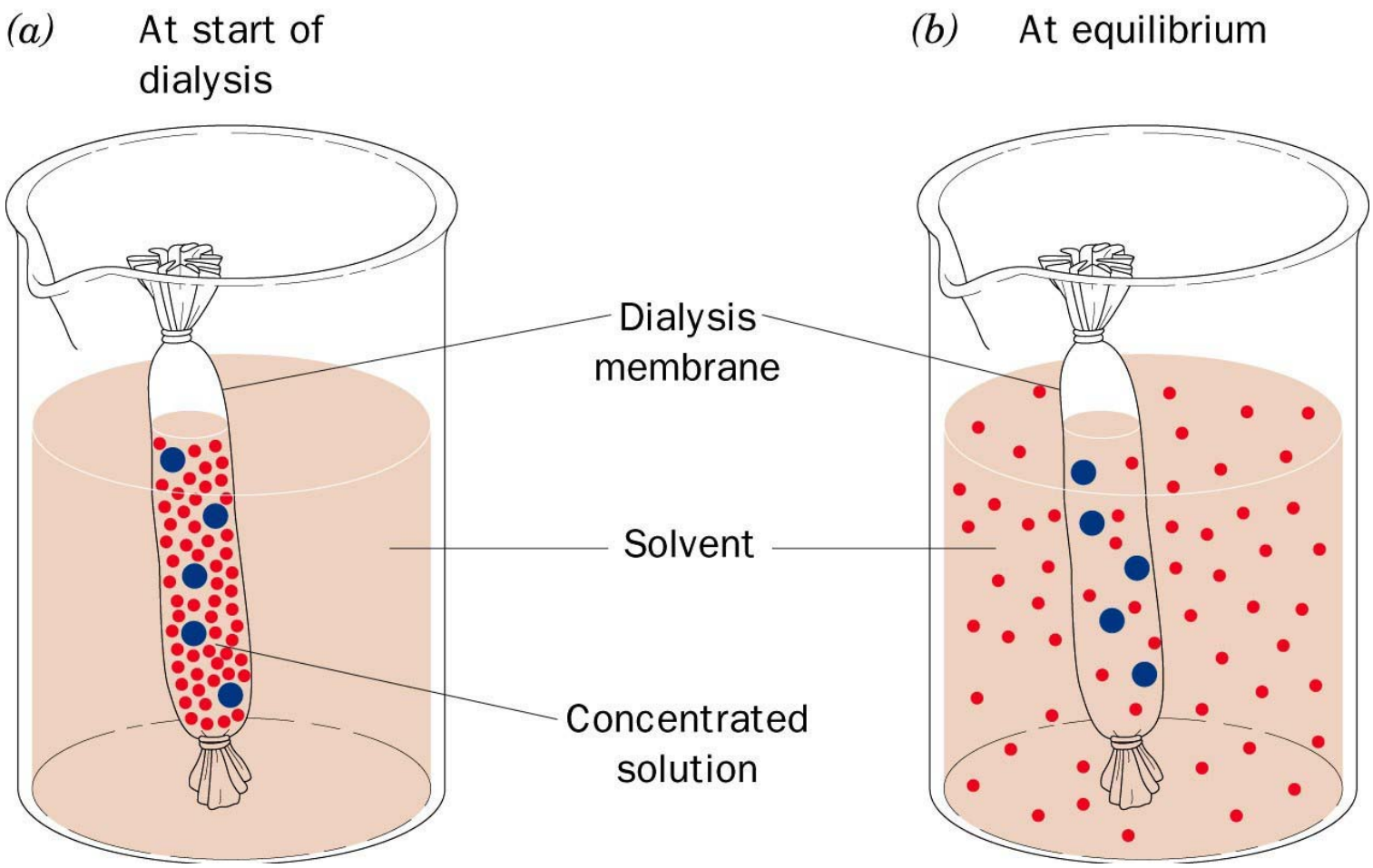
➢ Salting in

Dialysis against a solution with low salt concentration

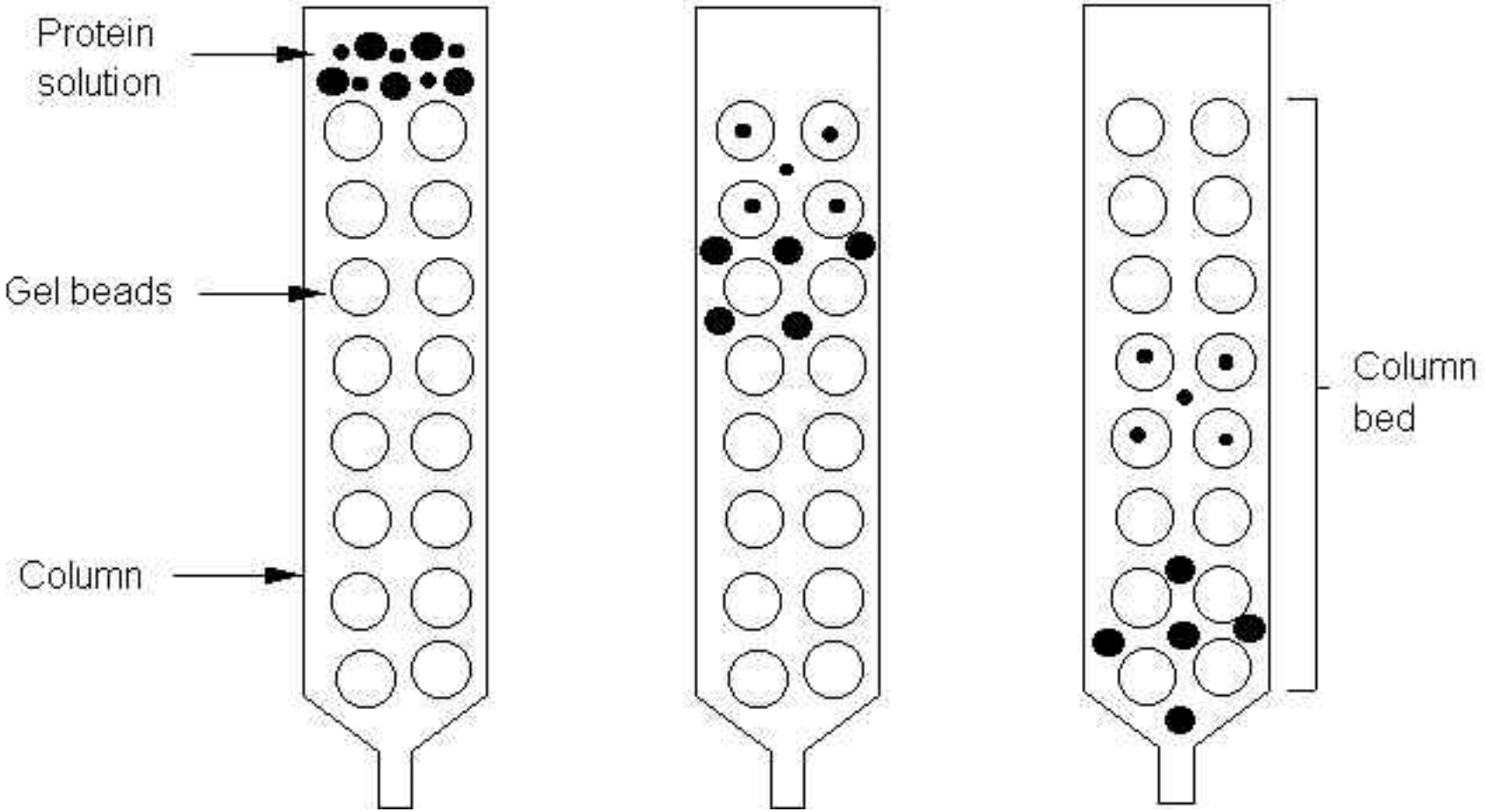
Separation procedures…

- Molecular size
  - Dialysis
  - Gel filtration chromatography
  - Ultracentrifugation
  - Sodium dodecyl sulfate (SDS) polyacramide gel electrophoresis

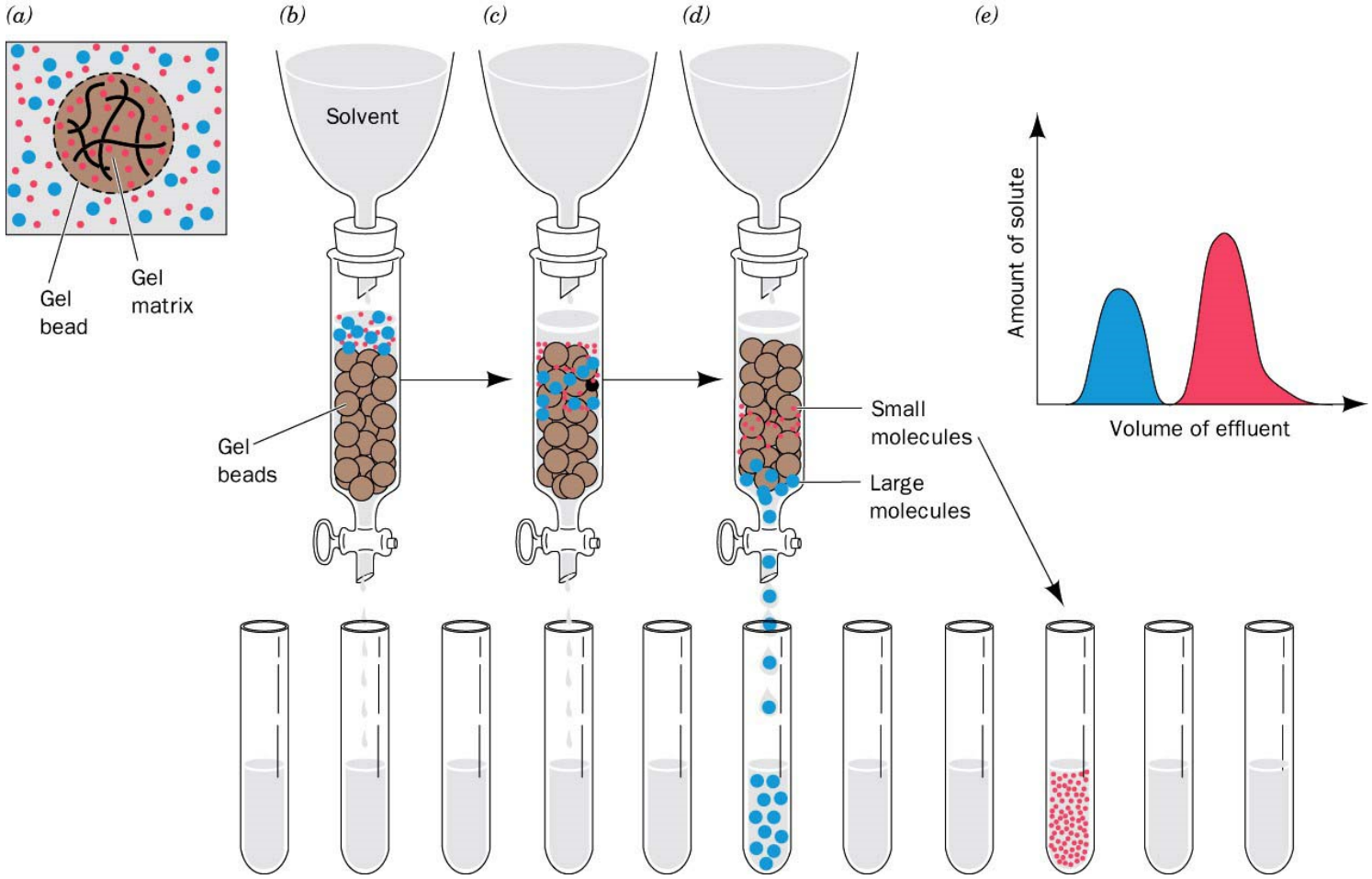
Dialysis


(a) At start of dialysis
(b) At equilibrium
Dialysis membrane
Solvent
Concentrated solution

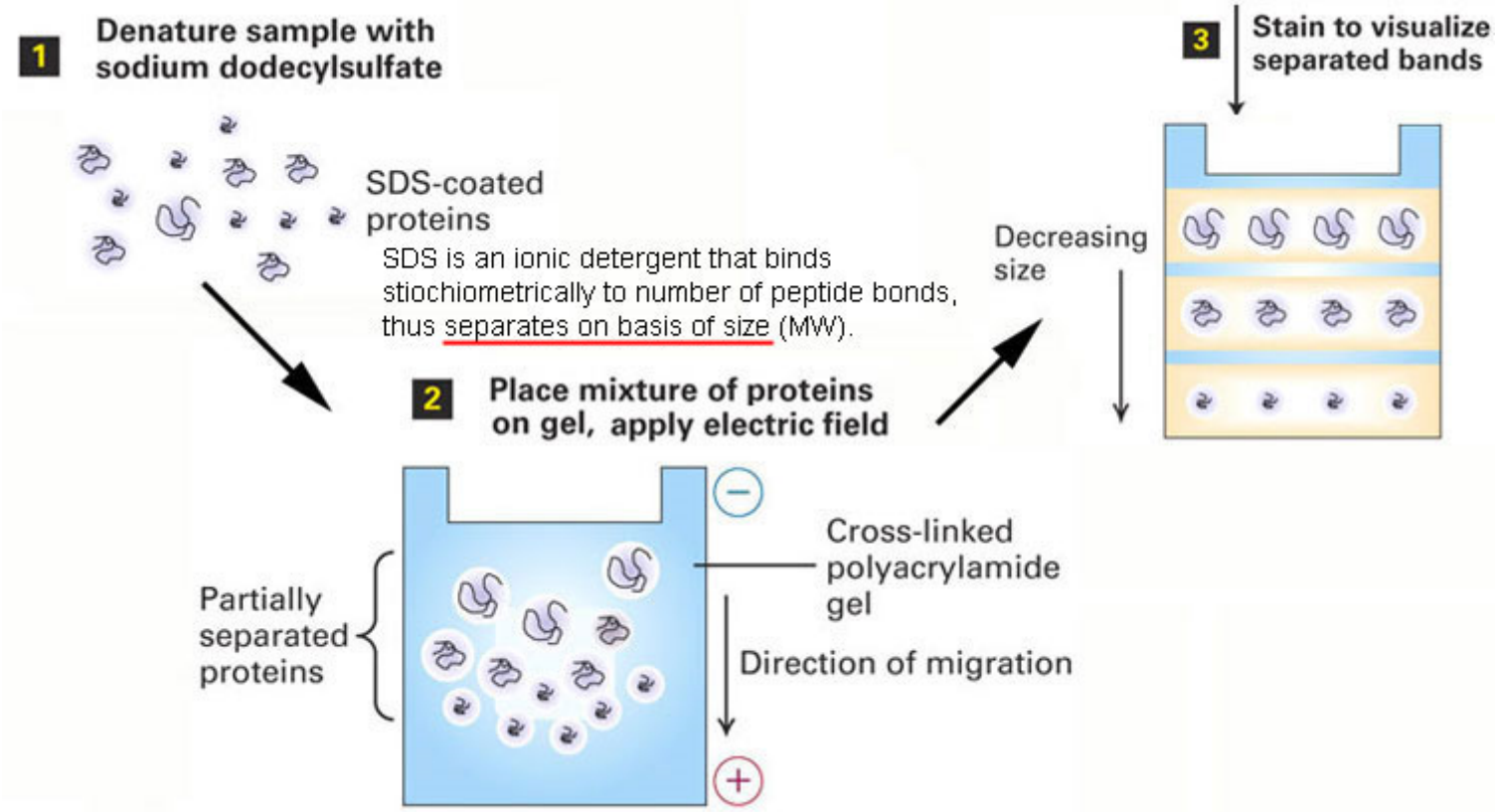
Gel filtration chromatography



Gel filtration chromatography…



SDS-PAGE electrophoresis



Separation procedures…

- Molecular charge
  - Ion exchange chromatography
  - High performance liquid chromatography
  - Electrophoresis

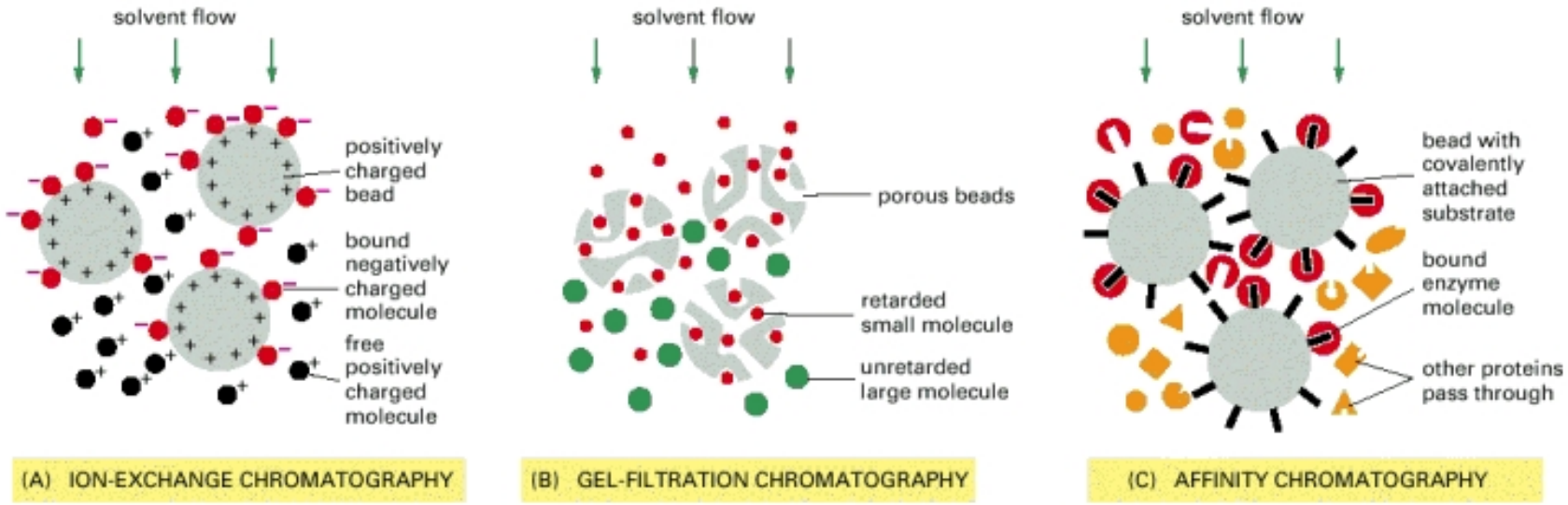
Separation procedures…

- Specific affinity binding
  - ➢ Affinity/ absorption chromatography
  - ➢ Precipitation by antibodies

Column chromatography

- Gel filtration
- Ion exchange
- Affinity

Chromatography: Matrices



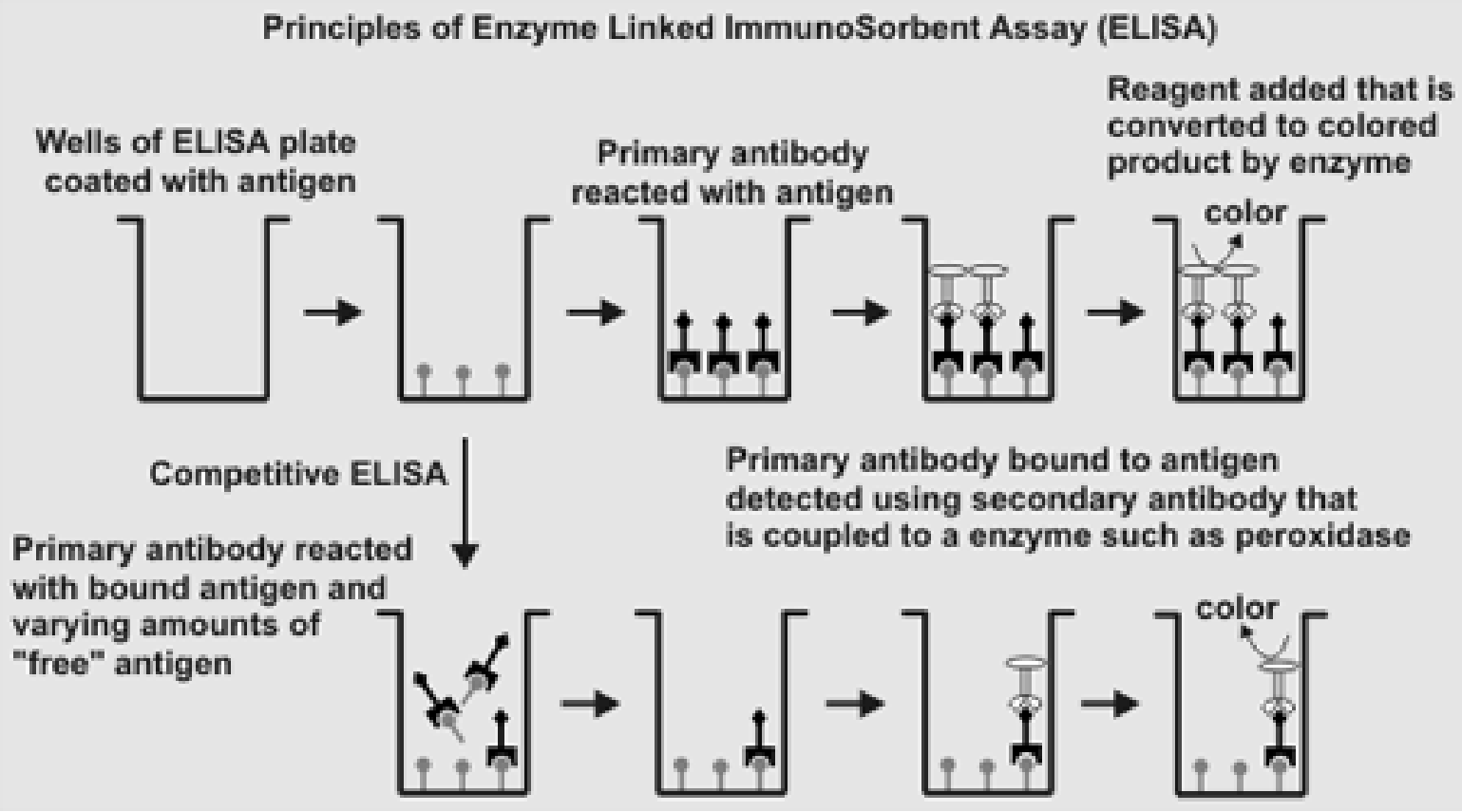
Separation procedures…

- Immunoelectrophoresis
- ELISA(Enzyme linked immunosorbant assay)

Immune electrophoresis

- This is a double immunodiffusion technique
- Sample is placed in a well on a glass slide coated with agarose or cellulose acetate, and electrophoresed to separate the proteins according to their charge
- A trough is cut in the agarose parallel to the axis of the electrophoresed proteins into which is placed monospecific antibodies against IgG, IgM, IgA, or kappa or lambda light chains
- The slide is incubated for 18 to 24 hours to allow the antibodies to diffuse from the trough into the agarose, forming precipitin arcs with their respective antigens
- The relative size of the precipitin arcs is proportional to the quantity of immunoglobulin or light chains

ELISA


Principles of Enzyme Linked ImmunoSorbent Assay (ELISA)
Wells of ELISA plate coated with antigen
Primary antibody reacted with antigen
Reagent added that is converted to colored product by enzyme
color
Competitive ELISA
Primary antibody reacted with bound antigen and varying amounts of "free" antigen
Primary antibody bound to antigen detected using secondary antibody that is coupled to a enzyme such as peroxidase
color

ELISA

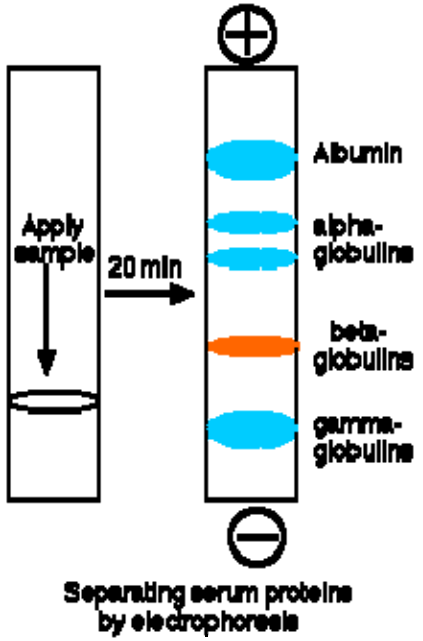
- Binding the antigen to the surface of the wells of a ELISA plate
- Blocking any non-specific antibody binding sites with a generic protein (BSA.

  ovalbumin, gamma-globulin, skimmed milk)
- Adding primary antibody solution to each well to allow specific antigen- antibody recognition/binding to occur
- Removing unbound primary antibody.

ELISA…

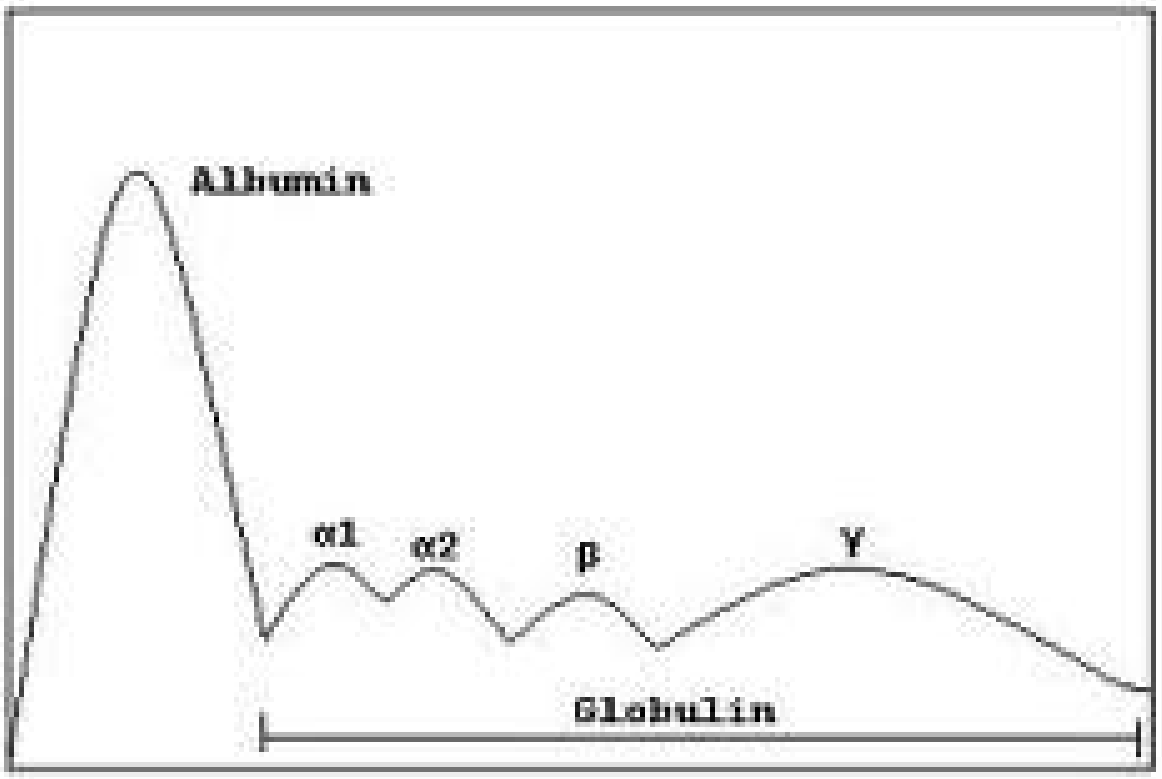
- Adding a secondary antibody (e.g. goat anti-mouse anti IgG that is covalently linked to a protein such as horse radish peroxidase or alkaline phosphatase). This antibody binds to the primary antigen that remained bound to antigen after washing the wells
- Remove unbound secondary antibody
- Determine the amount of secondary antibody bound to the wells by adding a chemical reagent that is converted by the peroxidase or phosphatase to a colored product whose absorbance is measured using a ELISA plate reader

Separation of proteins by electrophoresis



Separating serum proteins by electrophoresis

Plasma protein fractions



Plasma protein fractions…

- α1 zone: α1 anti trypsin, TBG, HDL
- α2 zone: α2 macroglobulin,caeruloplasmin, VLDL, haptoglobin
- β zone: Transferrin, LDL, fibrinogen, C3 & C4 complement
- γ zone: Immunoglobulins, Factor VIII,

  C-reactive proteins, α feto protein

Origin of plasma proteins

In embryo

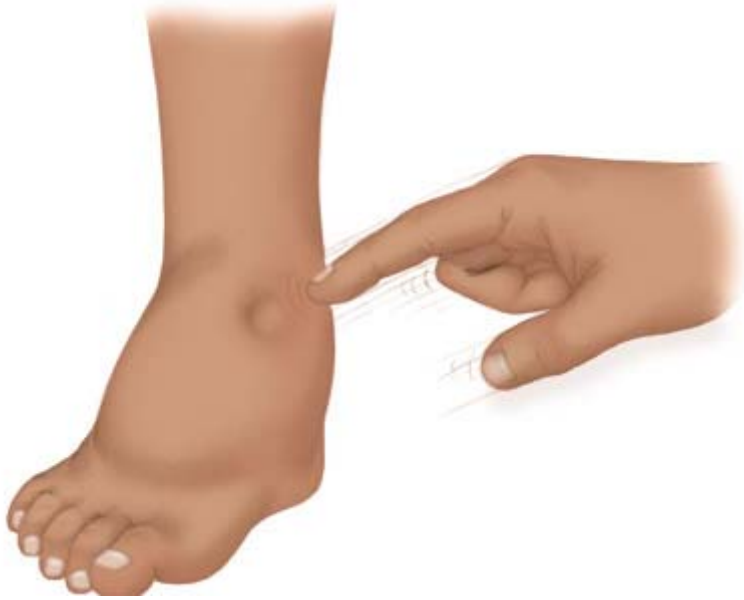
Mesenchymal cells

In adults

Liver: albumin, α and β globulin , fibrinogen

B lymphocytes in lymph nodes, bone marrow: γ globulins(immunoglobulins)

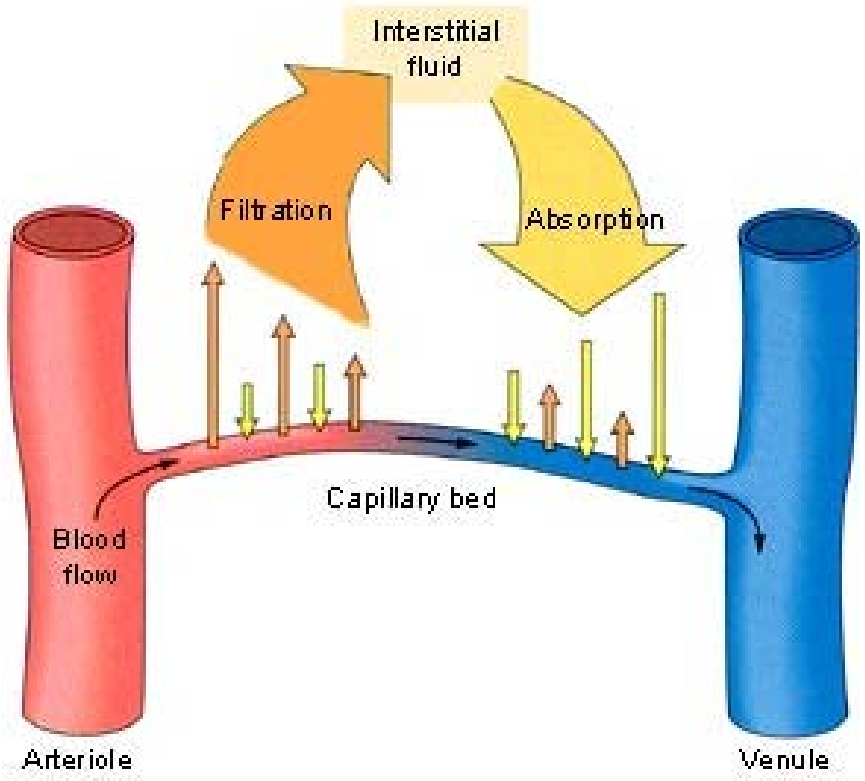
Albumin



Albumin

- 60% of total plasma proteins(3.4-4.7 g/dL)
- 40% intravascular and 60 % extra vascular
- T1/2 = 19 days
- Molecular weight= 66000 Da
- Shape ellipsoid
- Catabolism: receptor mediated trans cytosis and then pinocytosis by tissue cells

Functions of albumin



Exerts colloidal osmotic pressure of 25 mmHg (80%)

Functions of albumin…

- Regulates blood volume & body fluid balance
- Viscosity: One of the determinants of resistance to blood flow
- Blood pressure maintenance
- Protein reserve

Functions of albumin…

- Binding to various ligands helps in transport of free fatty acids, bilirubin, calcium
- Secondary carrier for thyroxin, cortisol & heme
- Drug binding eg. Sulfonamides, penicillin G, dicumarol, aspirin
- Buffering action: Helps maintain pH of blood

Variations in plasma albumin

- Increase
- Secondary to burns , dehydration
- Decrease
- Infants and newborns
- Pregnancy
- Hepatitis
- Cirrhosis
- Nephrosis
- Protein losing enteropathies

Alpha Globulins

- Alpha 1 antitrypsin
- Antithrombin III
- Antiplasmin
- Caeruloplasmin
- Haptoglobin
- Progesterone binding globulin
- Retinol binding proteins
- Transcortin

Beta globulins

- Beta 2 microglobulin
- Hemopexin
- Plasminogen
- Sex hormone binding globulin
- Transferrin

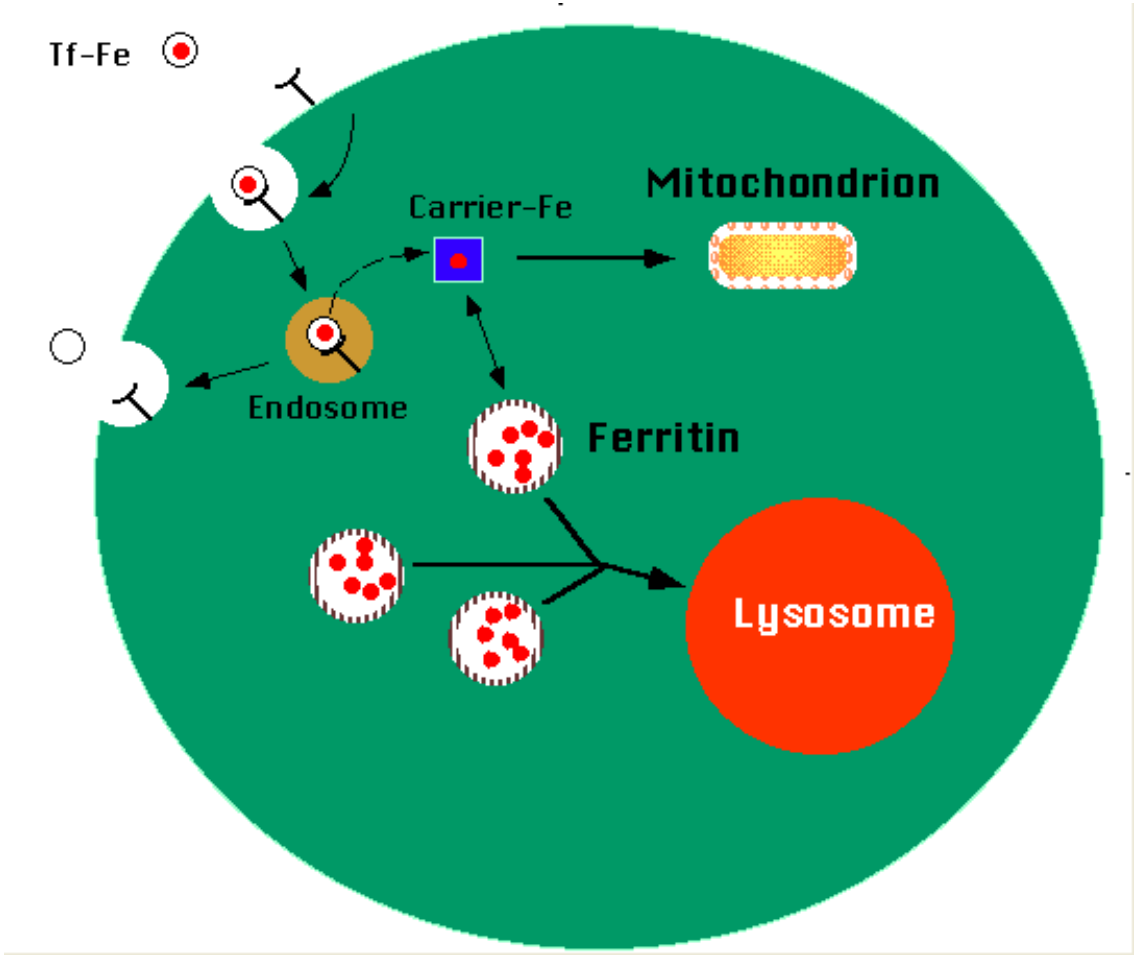
Functions of alpha & beta globulins

- TBG: Carrier protein for thyroid hormone in blood
- α2 macroglobulin: Inhibitor of serum endoprotease
- Caeruloplasmin: Transports copper
- Haptoglobin: Transports free hemoglobin
- Transferrin: Transports iron
- Fibrinogen: Precursor of fibrin
- C3 & C4 : Proteins of complement ssystem
- α feto protein: Osmotic regulation, carrier protein

Transferrin

- Transport of iron: from catabolism of heme and from food (gut) to the sites where iron is required, i.e. to the bone marrow and other organs
- 2 moles of Fe^{3+} per 1 mol of transferrin

Receptor mediated transferrin endocytosis



Ceruloplasmin

- Carries 90% of copper in plasma (copper – cofactor for a variety of enzymes);
- 1 molecule binds 6 atoms of copper
- binds copper more tightly than albumin that carries other 10%
  of copper
- albumin may be more important in copper transport (donates copper to tissues more readily)

Wilson’s disease

- accumulation of copper in liver, brain, kidneys
- Liver disease, neurologic symptoms

Haptoglobin

- Binds free hemoglobin and delivers it to the reticuloendothelial cells
- complex Hb-Hp is too large to pass through glomerulus prevention of loss of free Hb

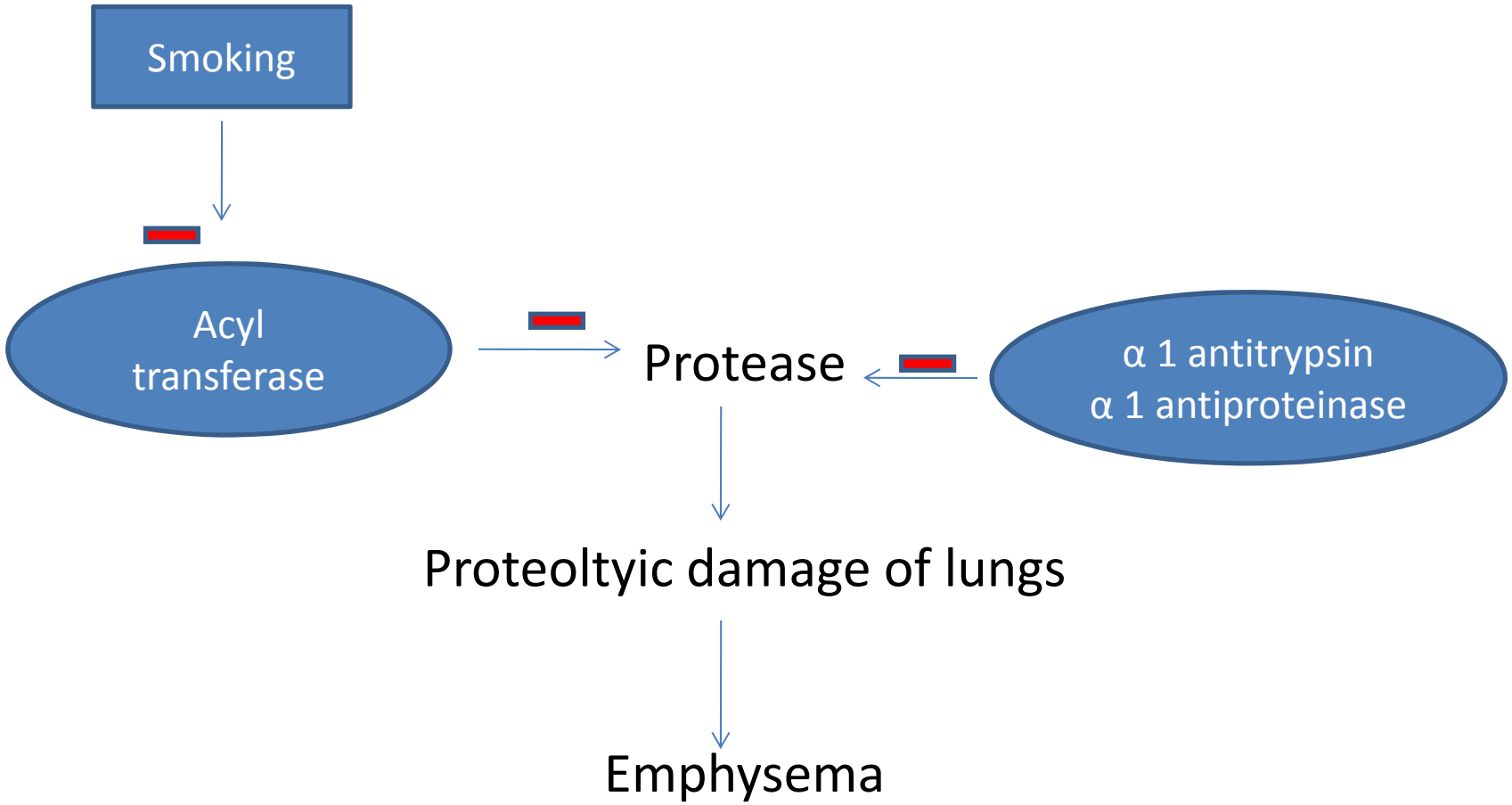
- Free Hb passes through glomeruli, enters tubules and precipitates leading to kidney damage

Act as antioxidants

- transferrin
- ferritin
- ceruloplasmin
- haptoglobin
- hemopexin (binds heme and transfers it to the liver)
- remove Fe 2+ and thus prevent the Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH\cdot + OH^-$

α_1- ANTITRYPSIN
(α_1-antiproteinase)

- Principal plasma inhibitor of serine protease (inhibits trypsin, elastase)
- Deficiency has a role in emphysema – proteolytic damage of the lung
- methionine involved in AT binding to proteases is oxidized by smoking
- AT no longer inhibits proteases
- increased proteolytic damage of the lung, particularly devastating in patients with AT-deficiency


Smoking
Acyl transferase
Protease
α 1 antitrypsin
α 1 antiproteinase
Proteoltyic damage of lungs
Emphysema

- Functions of gamma globulins
- Functions of fibrinogen

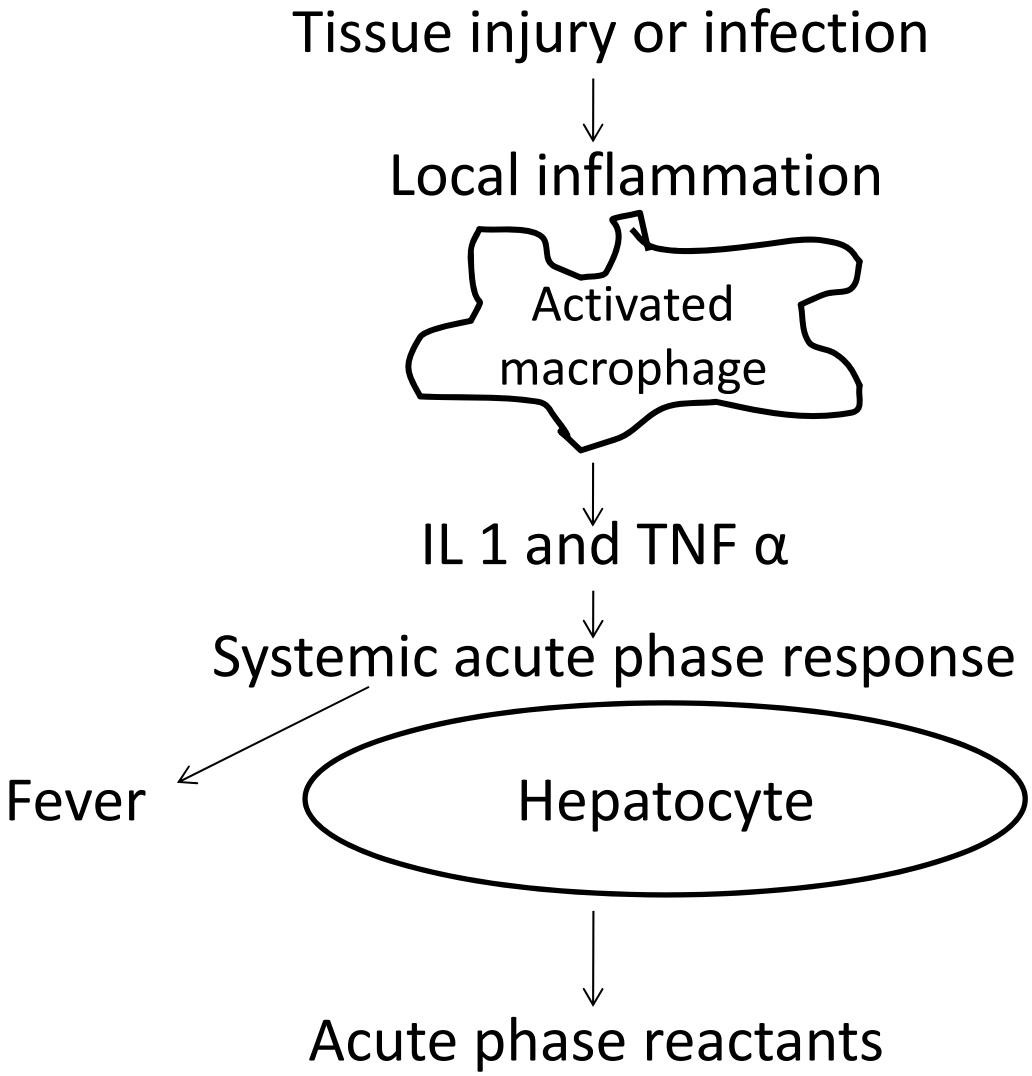
Variations in gamma globulins

- Increase
  - TB
  - Leukemia
  - Cirrhosis and acute hepatitis
  - Nephritis
- Decrease
  - Immune deficiency

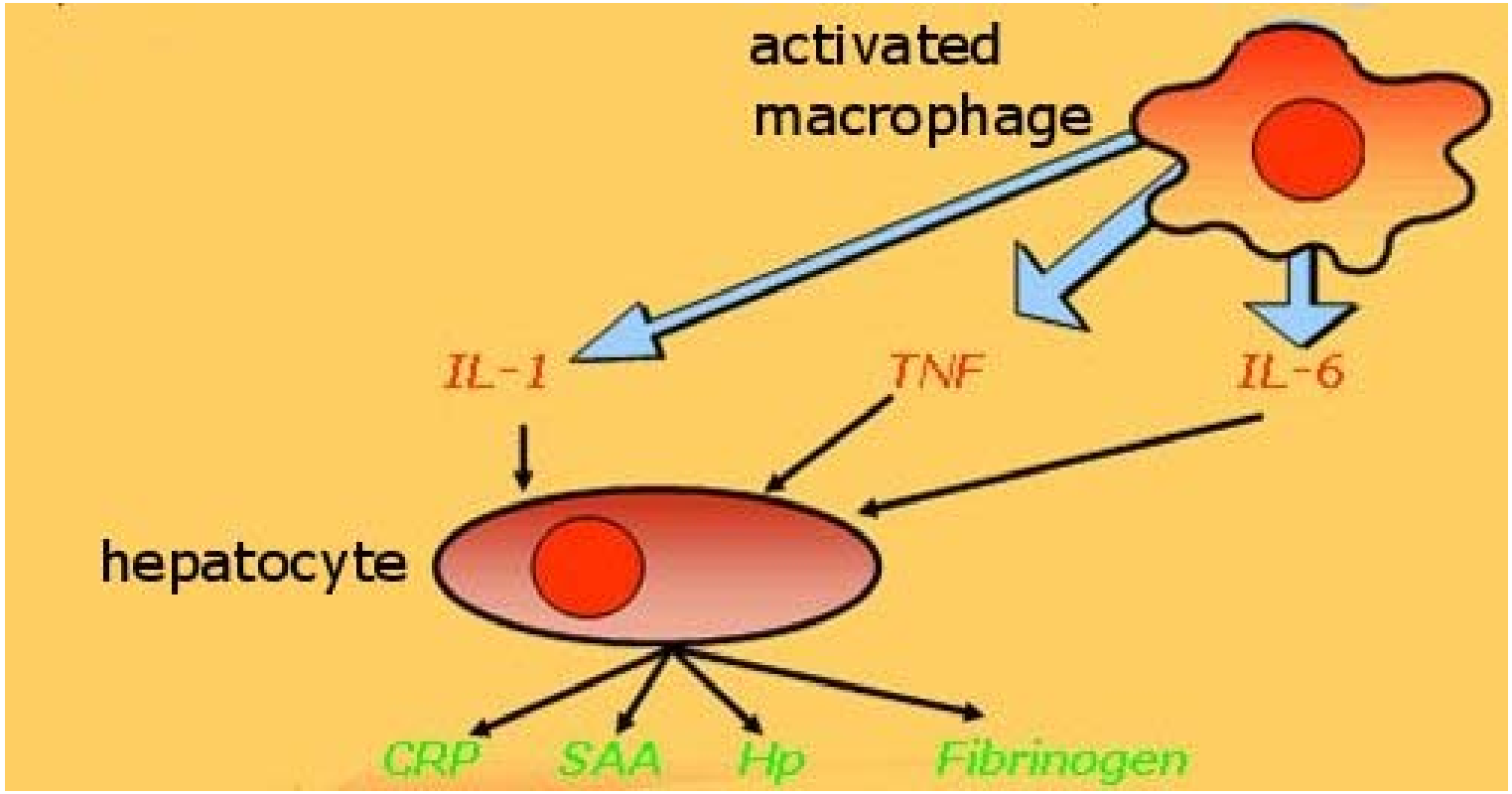
Variations in fibrinogen

- Increase
  - Pregnancy
  - Menstruation
  - Malaria
  - Tissue injury
- Decrease
  - Congenital
  - Carcinoma prostate
  - Intravascular coagulation

Acute phase proteins



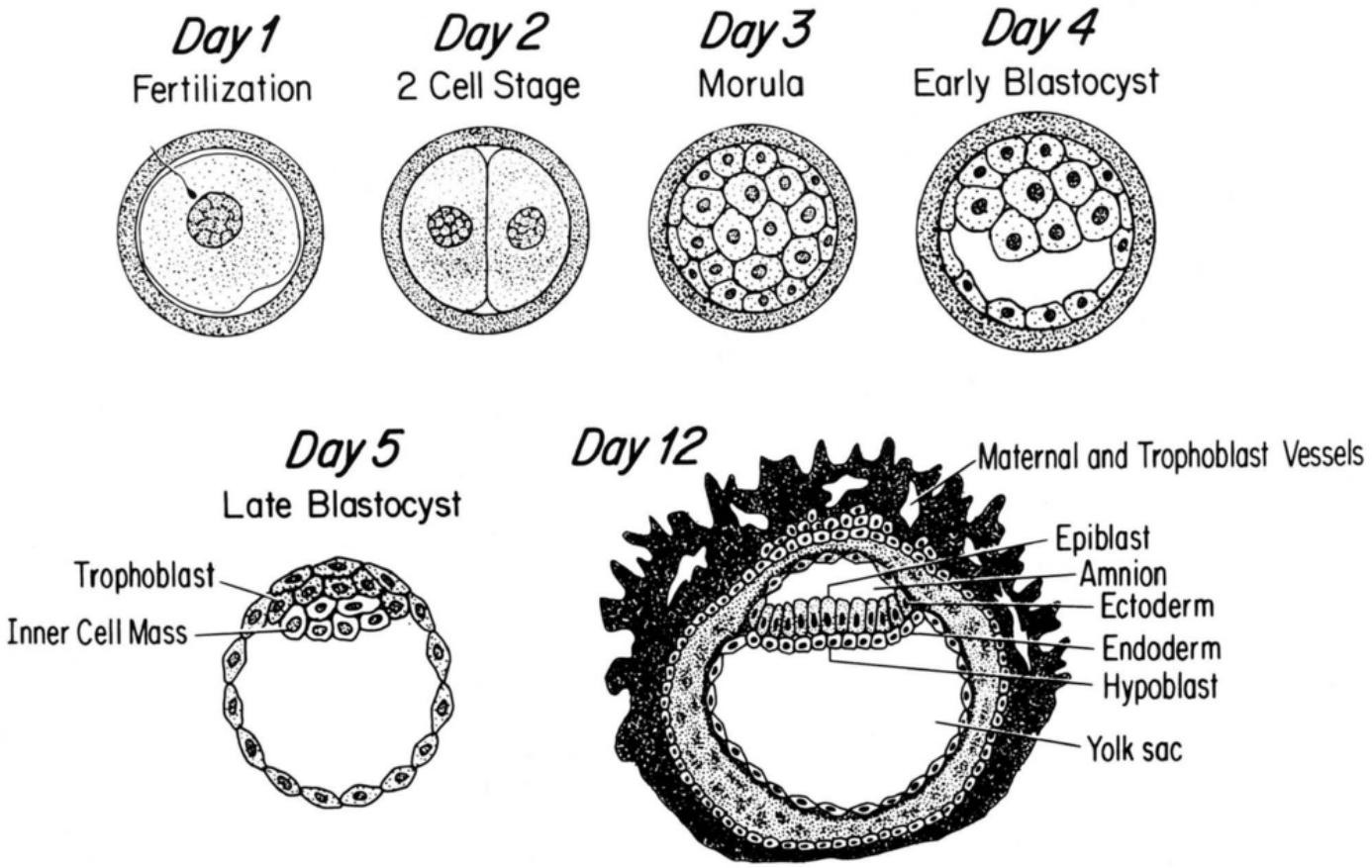
Acute phase reactant response



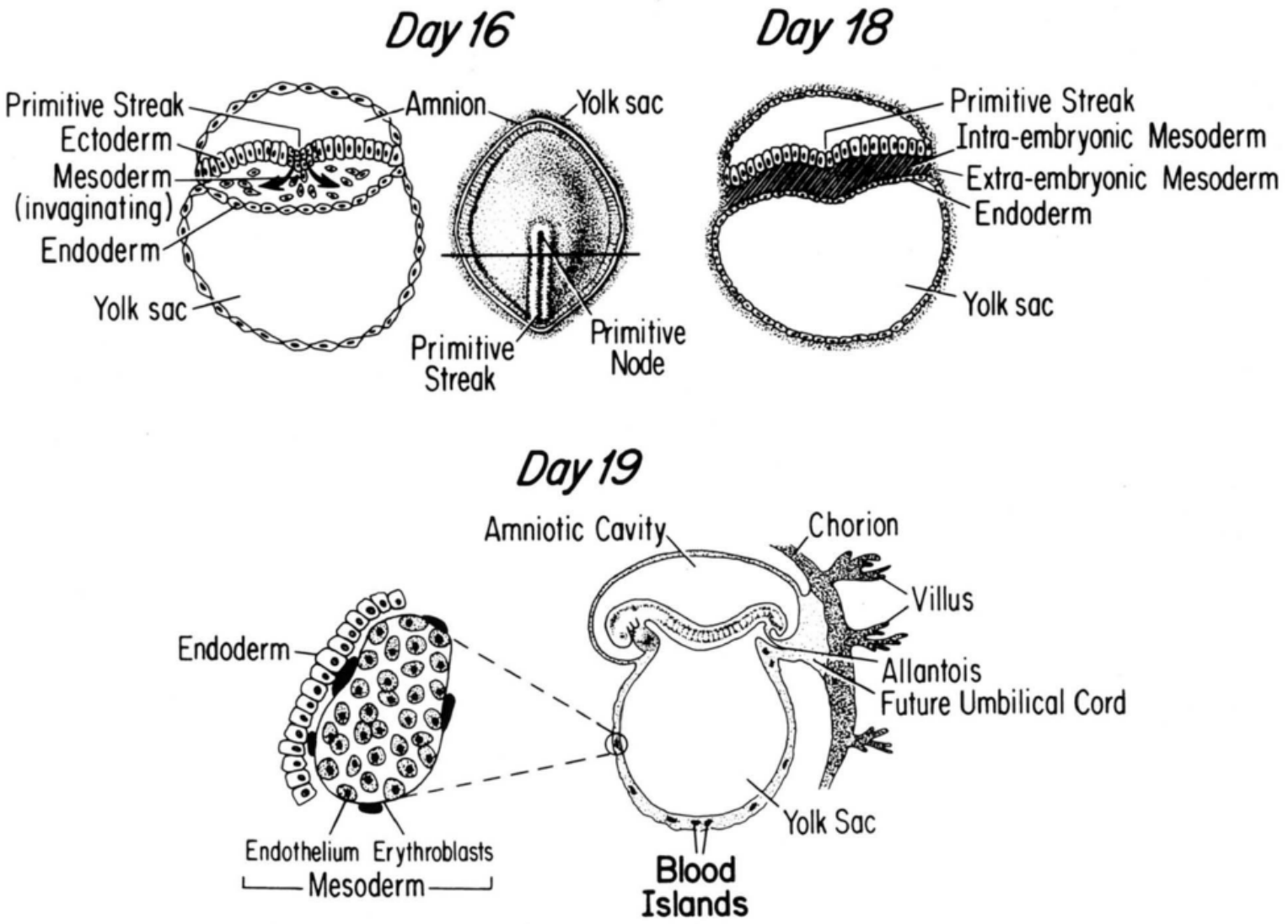
Acute phase proteins

- α1 antitrypsin
- Fibrinogen
- Complement
- Haptoglobin
- C-reactive protein

Mammalian embryonic blood formation



Human embryo at various stages

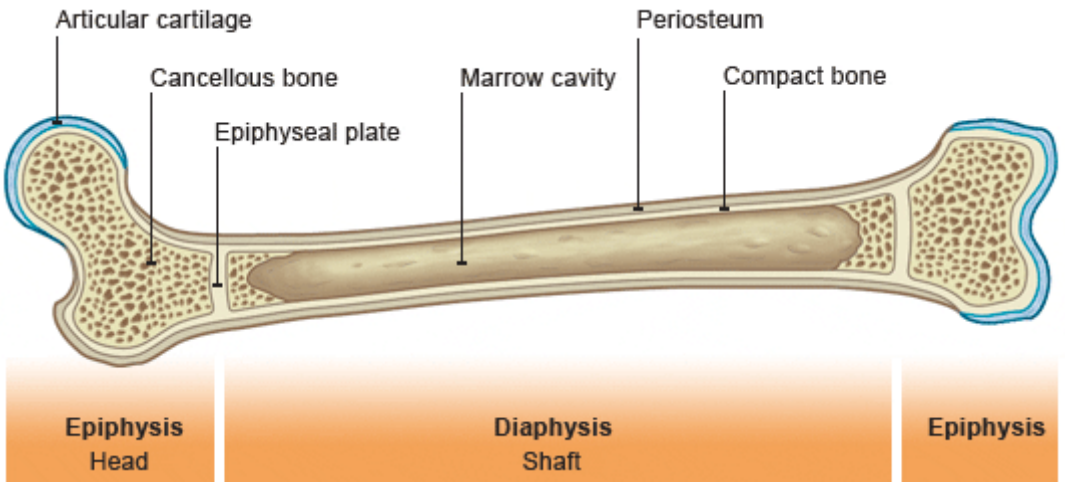

Day 16
Primitive Streak
Ectoderm
Mesoderm
(invaginating)
Endoderm
Yolk sac
Amnion
Yolk sac
Primitive
Streak
Primitive
Node
Day 18
Primitive Streak
Intra-embryonic Mesoderm
Extra-embryonic Mesoderm
Endoderm
Yolk sac
Day 19
Amniotic Cavity
Chorion
Villus
Allantois
Future Umbilical Cord
Endoderm
Yolk Sac
Endothelium Erythroblasts
Mesoderm
Blood
Islands

Formation of cellular elements of blood

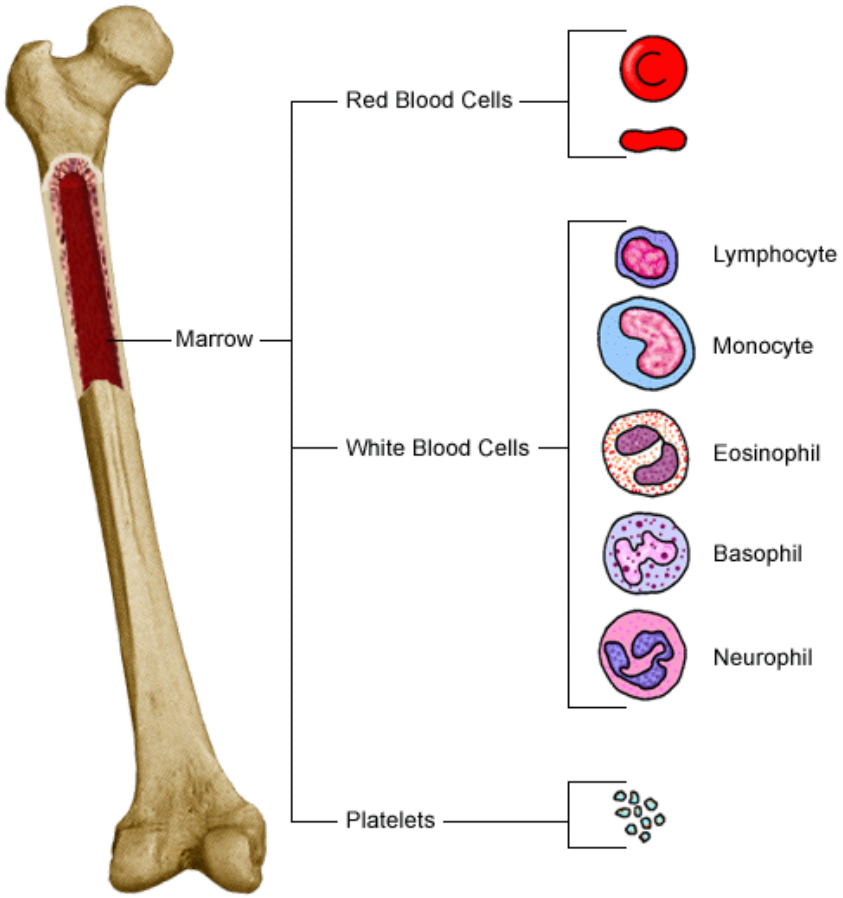
In fetus: Extramedullary hematopoiesis(3 stages)

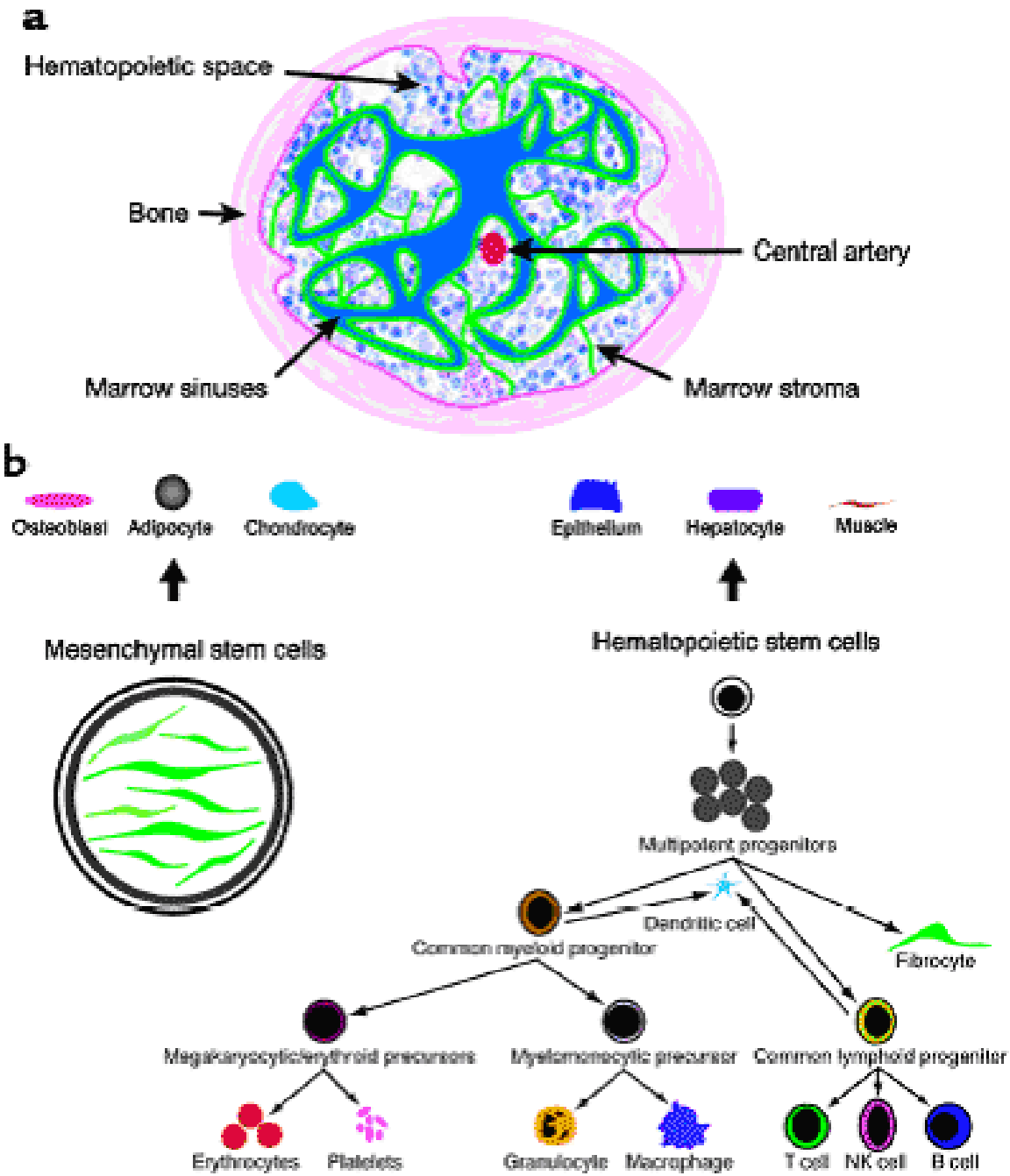
- **Mesoblastic**: (16-19 days): Clusters of mesenchymal cells in yolk sac ends by 12 weeks
- **Hepatic** (Second trimester) **Liver**, spleen and lymph node
- **Myeloid**(Last month of gestation+ after birth): Bone marrow

Bone marrow is the site of synthesis of all formed elements of blood after birth

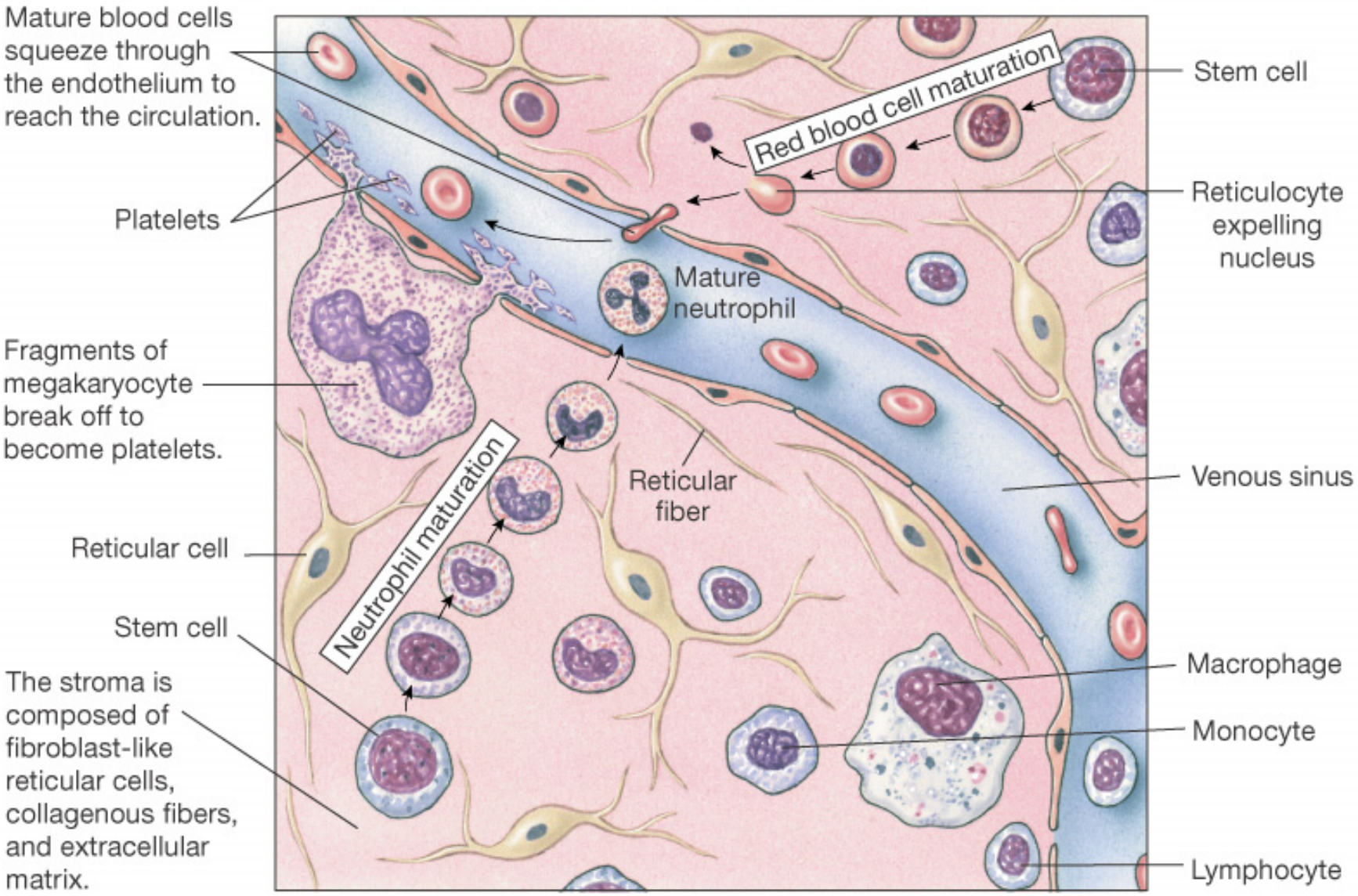


Red bone marrow




a
Hematopoietic space
Bone
Central artery
Marrow sinuses
Marrow stroma
b
Osteoblast
Adipocyte
Chondrocyte
Epithelium
Hepatocyte
Muscle
Mesenchymal stem cells
Hematopoietic stem cells
Multipotent progenitors
Dendritic cell
Common myeloid progenitor
Fibrocyte
Megakaryocytic/erythroid precursors
Myelomonocytic precursor
Common lymphoid progenitor
Erythrocytes
Platelets
Granulocyte
Macrophage
T cell
NK cell
B cell

(c) Bone marrow consists of blood cells in different stages of development and supporting tissue known as the **stroma** (mattress).



Formation of cellular elements of blood

Upto 5 yrs: all bone marrow

Upto 20 yrs: bone marrow of membranous + ends of long bones

After 20 yrs: bone marrow of membranous bone

Granulocyte vs erythroid development

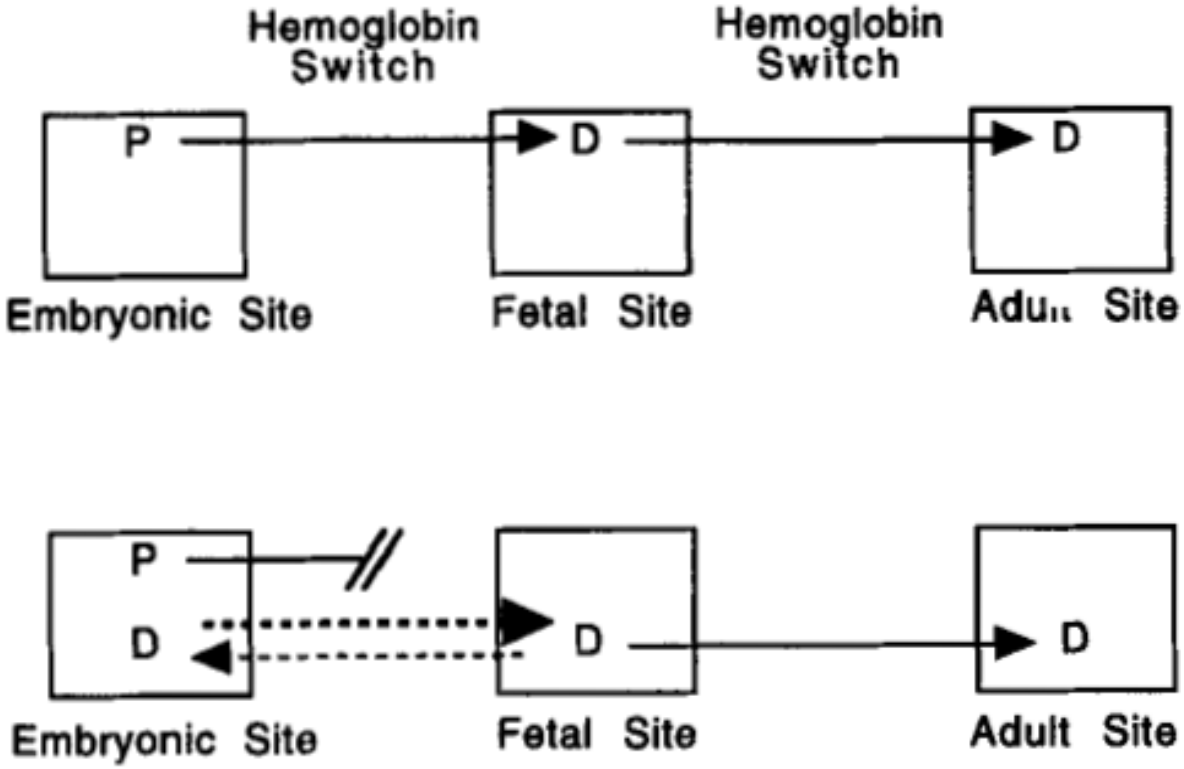
- Development of granulocytic system lags behind
- Number of mature neutrophils stored in the marrow is less

Sensitivity of newborns to bacterial sepsis

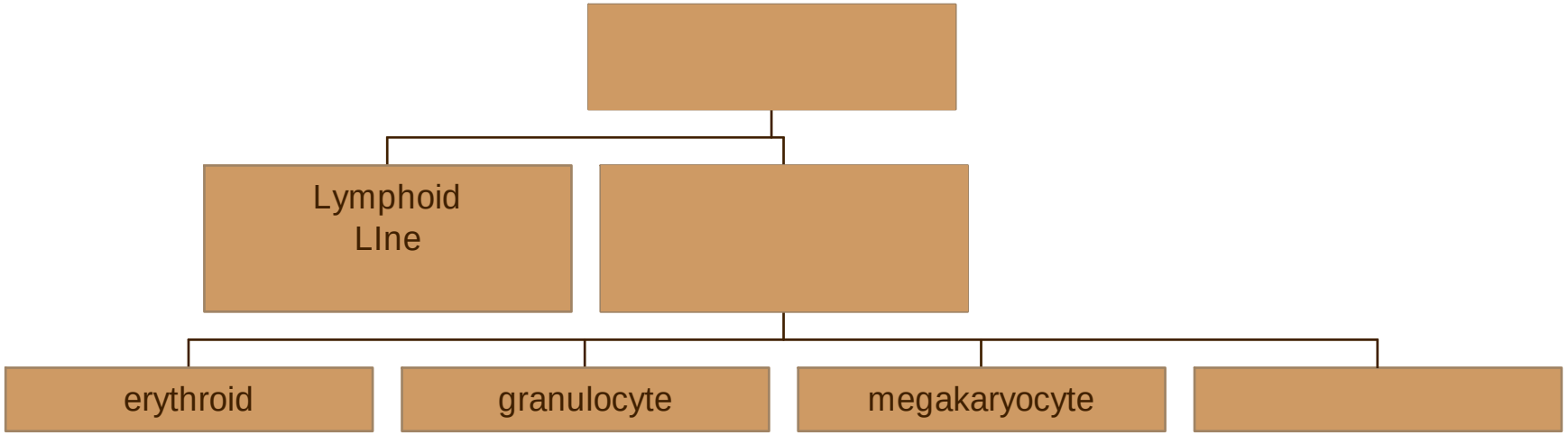
Classical studies in developmental biology have used amphibians to examine embryogenesis and the general principles of embryonic development are maintained in higher organisms.

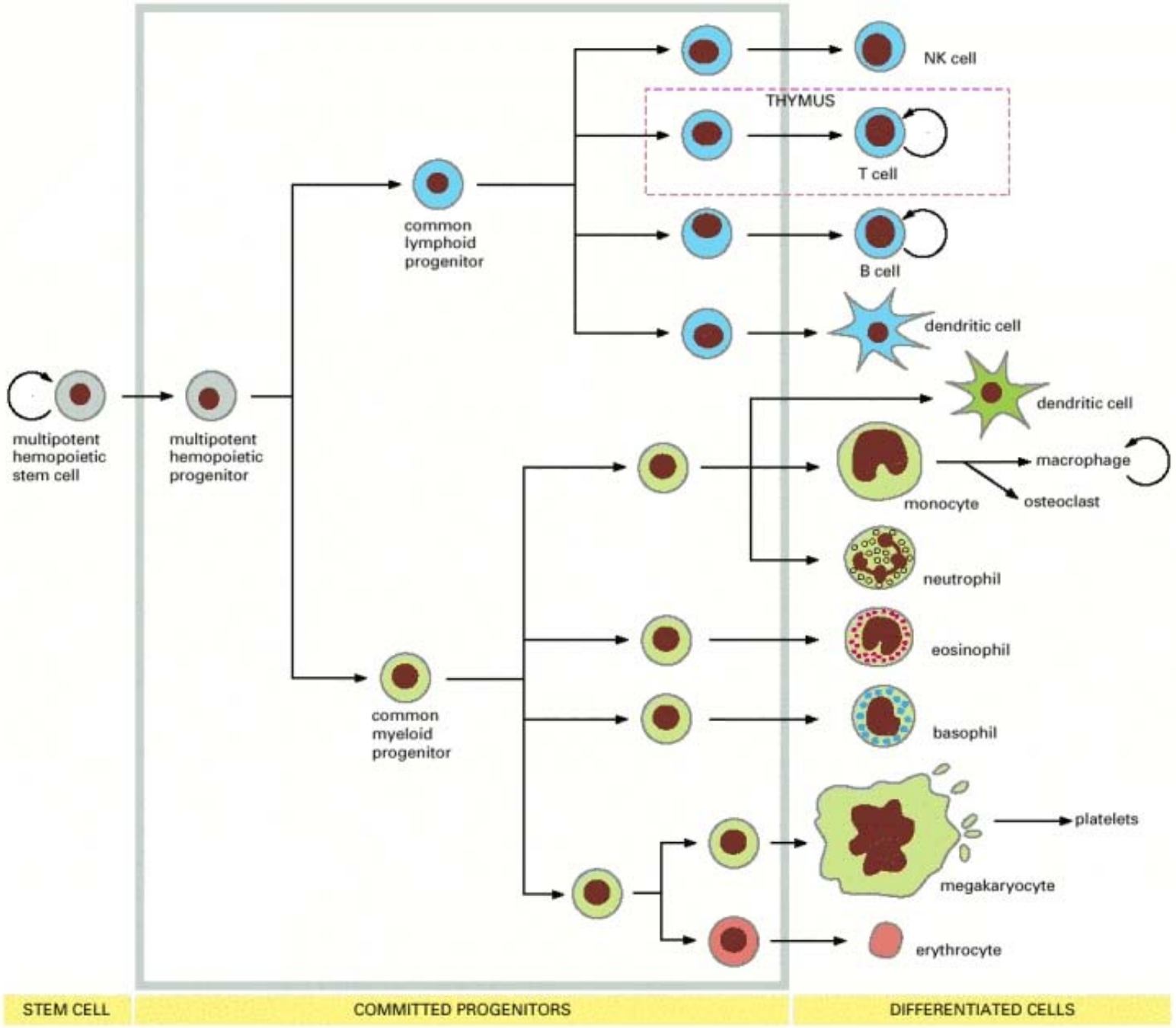
- 1924(Maximow) postulated that blood cells were derived from a single class of progenitors
- 1938(Downey) added the concept that progenies of pleuripotent cells were progressively more committed to a single lineage
- 1961(Till & Mc Culloch) demonstrated that single cells were capable of establishing nodules of hemopoitic growth in spleens of irradiated mice & that such colonies displayed multilineage differentiation

Developmental models for hematopoiesis

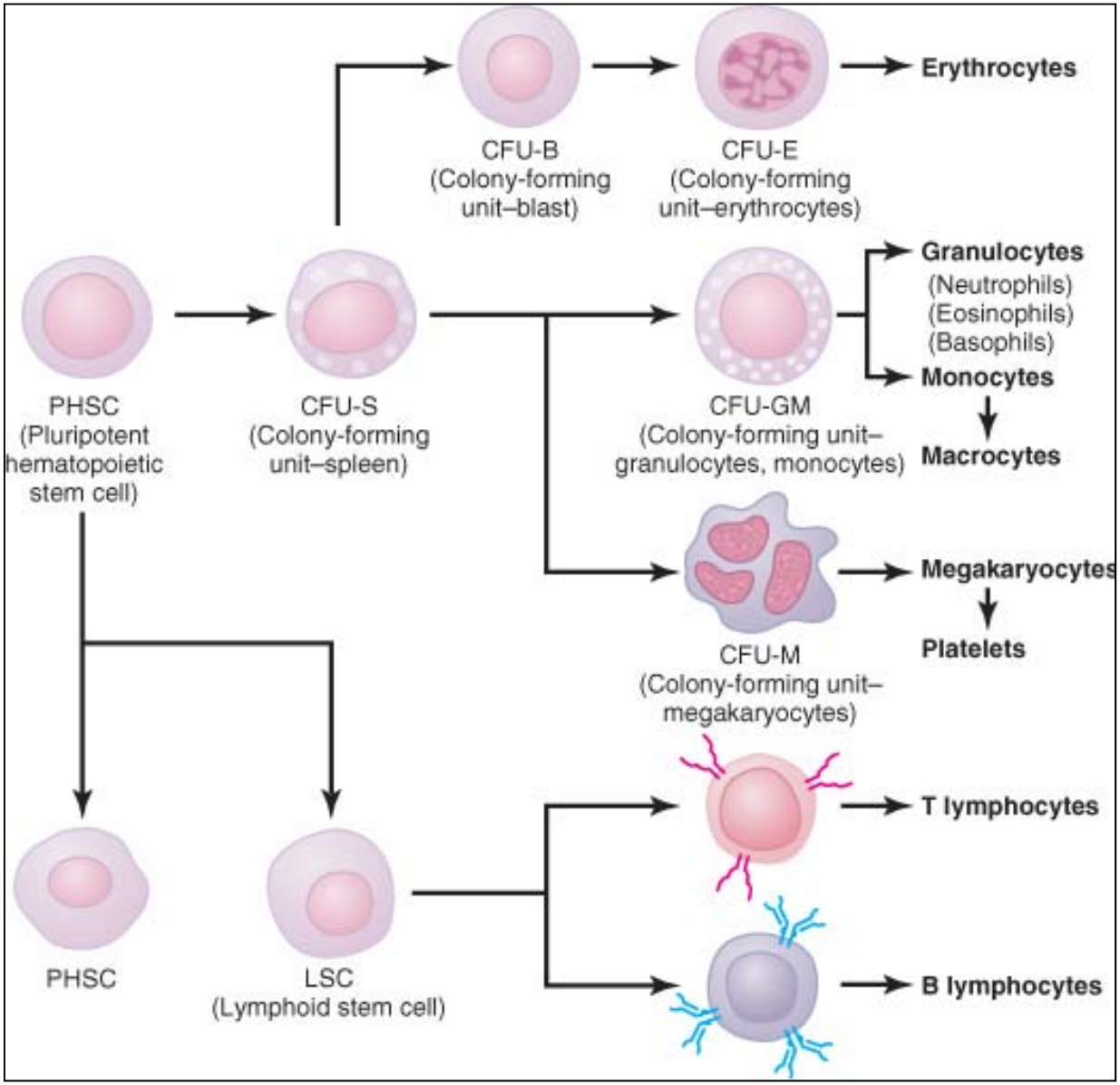


Terminology of stem cells

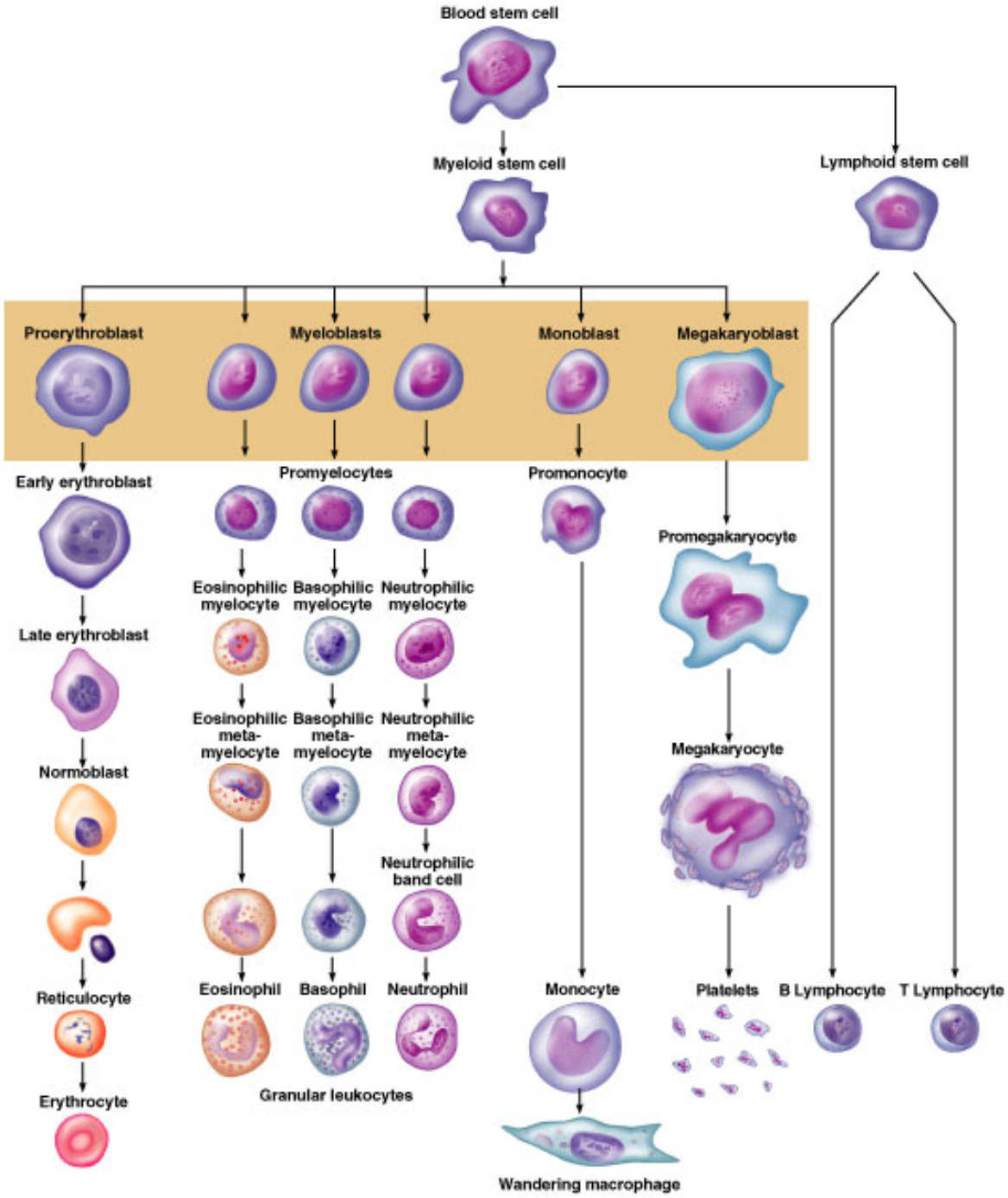



multipotent hemopoietic stem cell
multipotent hemopoietic progenitor
common lymphoid progenitor
common myeloid progenitor
THYMUS
NK cell
T cell
B cell
dendritic cell
dendritic cell
macrophage
monocyte
osteoclast
neutrophil
eosinophil
basophil
platelets
megakaryocyte
erythrocyte
STEM CELL
COMMITTED PROGENITORS
DIFFERENTIATED CELLS

Hematopoiesis



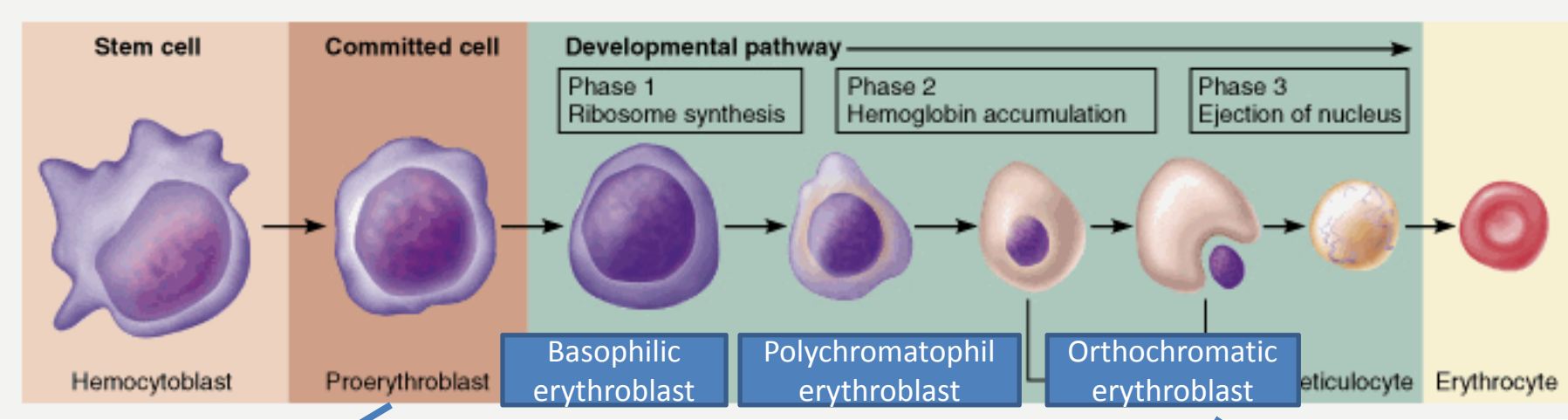
Stages in differentiation blood cells Hematopoiesis



Erythropoiesis: Formation of RBCs

This development takes about 7 days and involves three to four mitotic cell divisions, so that each stem cell gives rise to 8 or 16 cells.

Erythropoiesis



Megaloblast

15-20µ m
Nucleus: Big
Hb: absent

Early normoblast

Nucleus: size ↓
Nucleoli: absent
Hb: absent

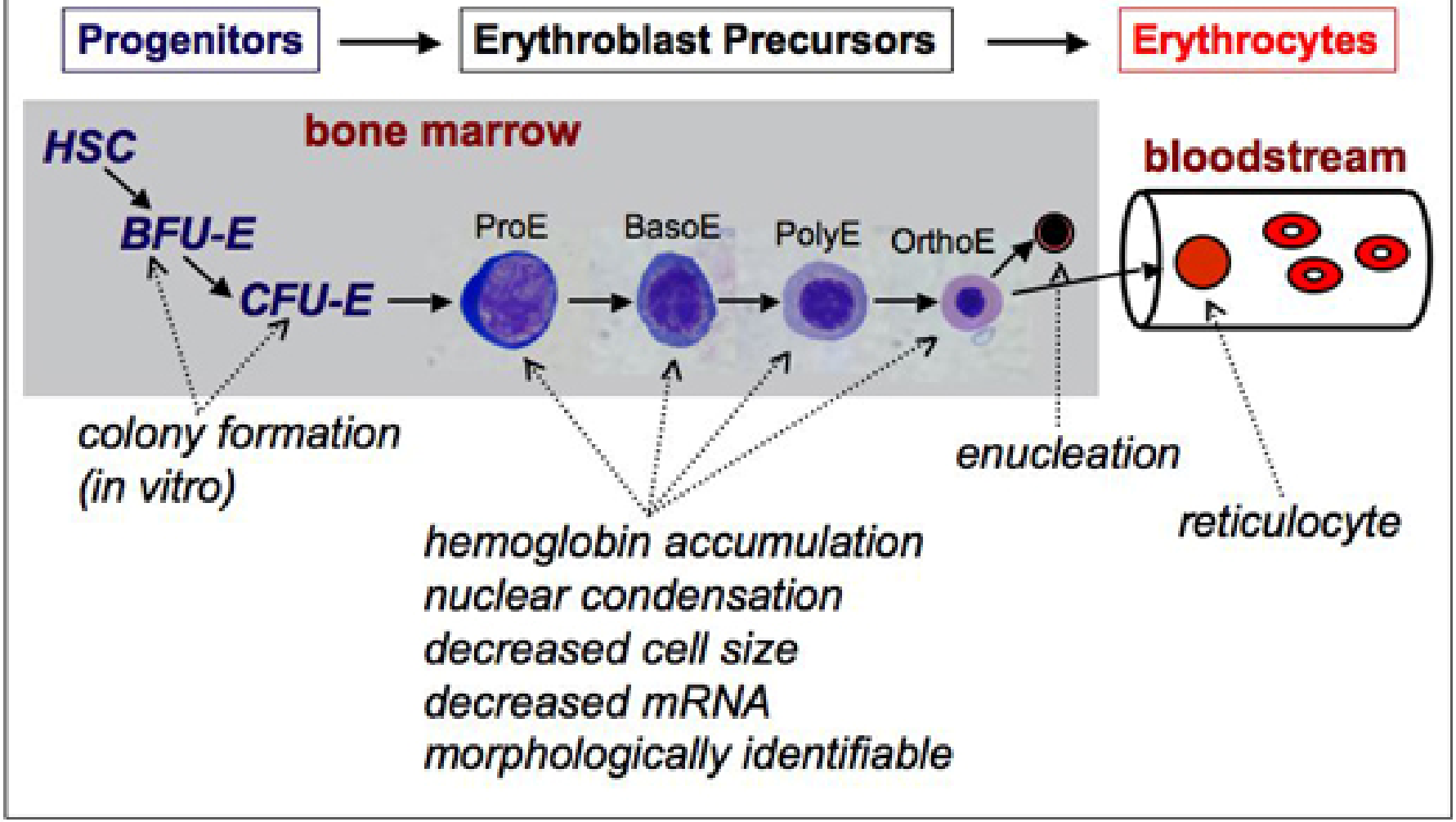
Intermediate normoblast

10-14 µ m
Nucleus: Size ↓
Hb: Starts appearing

Late normoblast

7-10 µ m
Nucleus: cart wheal- pyknotic
Hb: increases

Erythropoiesis



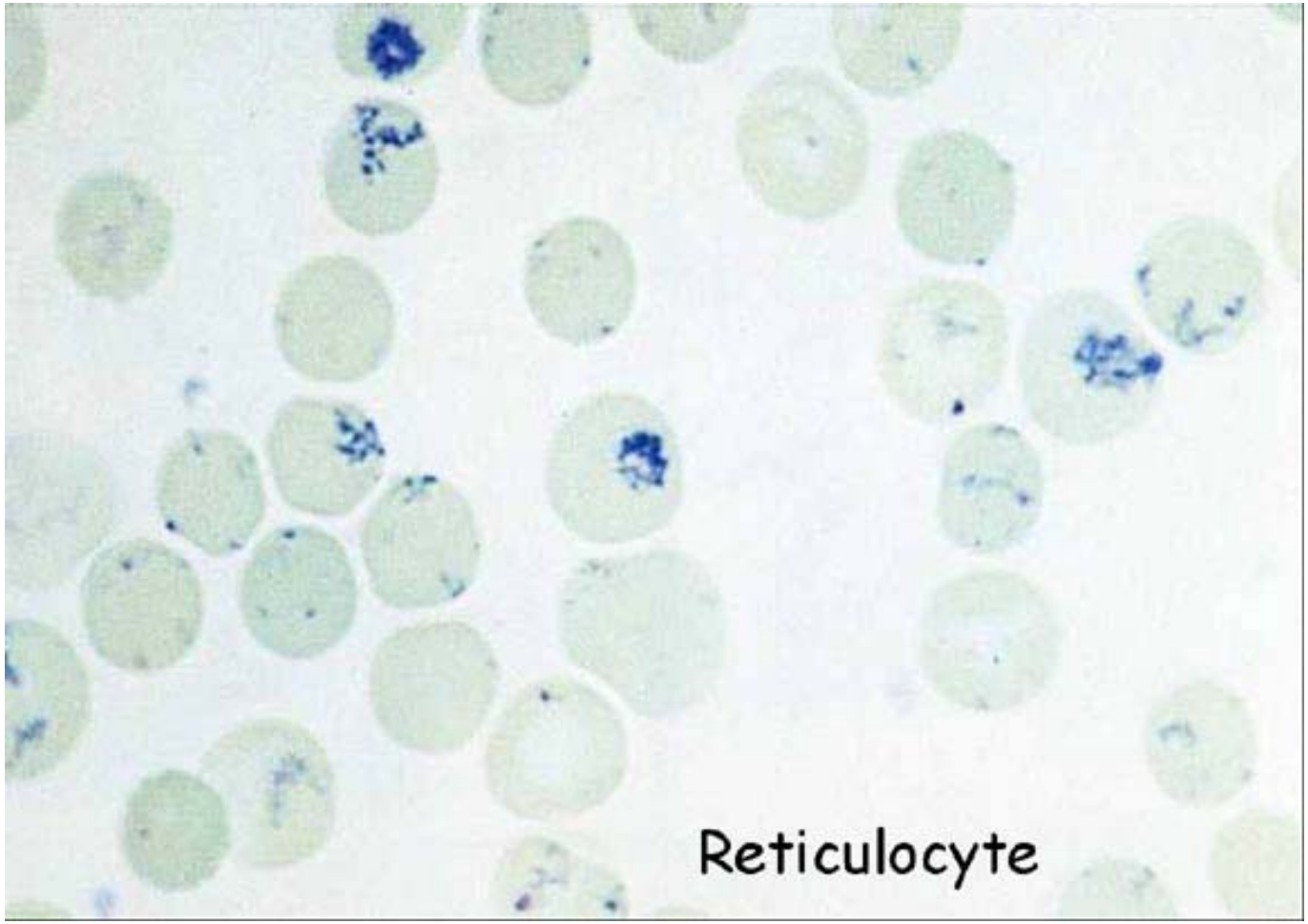
Q

- Why does the cytoplasm become more eosinophilic as RBCs mature

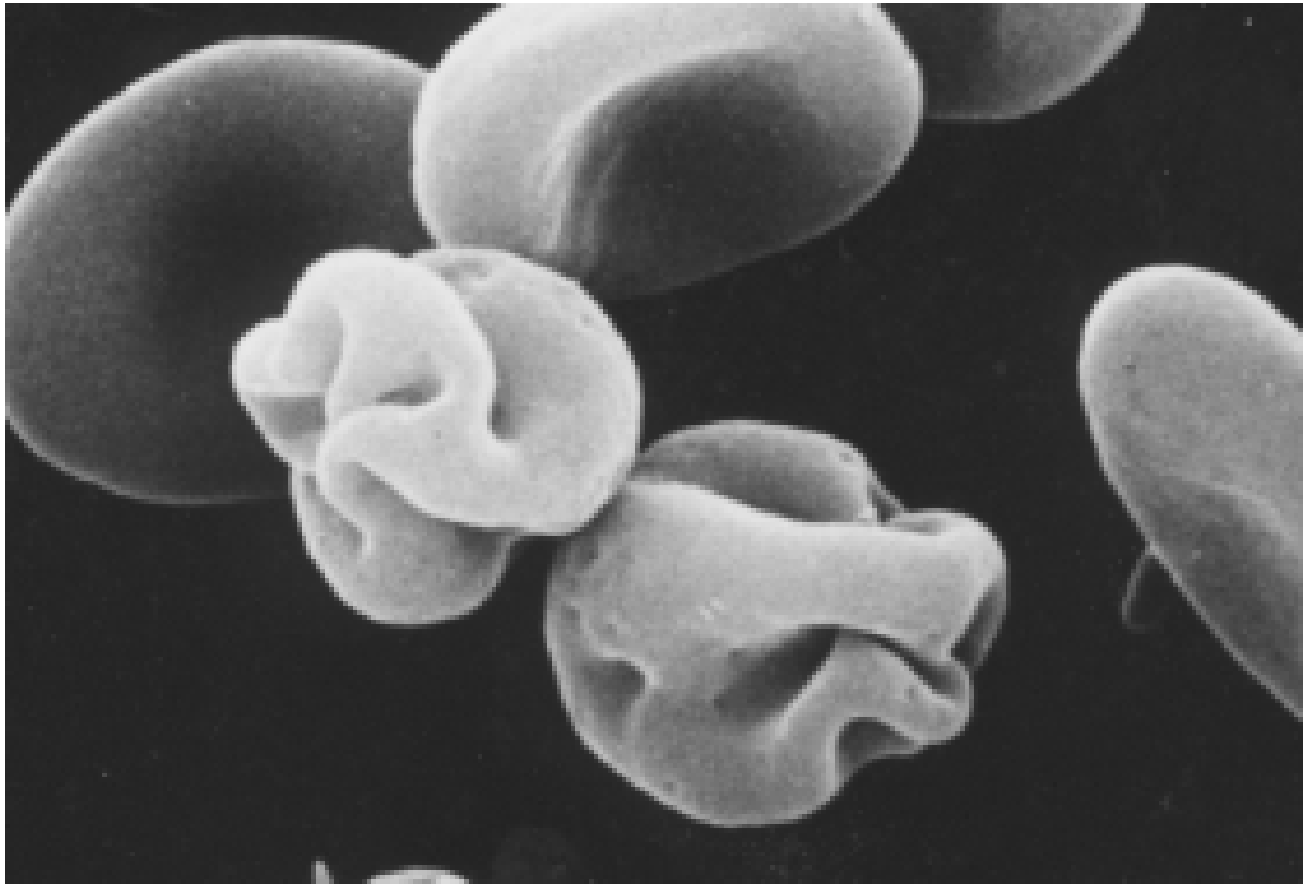
M:E ratio = 3:1(2.3)

In acclerated erythropoiesis the ratio

- Increases
- Decreases
- Doesn’t change
- None of the above


Reticulocyte

Reticulocyte



Reticulocyte

- Why the name?
- Size: 8 microns
- Shape: irregular & polylobulated
- More adhesive
- Contain ribosomes, mitochondria & golgi complex
- Produce 30% of total hemoglobin
- Reticulocytes have transferrin receptors

Percentage in circulation

In newborn: 30-40%

In infants up to first week of life: 2-6%

In children & adults: 0.2-2.0 %(Ave. 1%)

Absolute count: 20000- 90000/ mm^3

Reticulocytes VS mature RBC

- Size: 8 µm
- Shape:Polylobulated
- Adhesiveness more
- RNA and ribosomes present
- Transferrin receptors present
- Hemoglobin synthesis

- Size: 7 µm
- Shape: Biconcave disc
- Adhesiveness less
- RNA and ribosomes absent
- Transferrin receptors absent
- No more hemoglobin synthesis

Reticulocytosis

Physiological causes: Newborn, high altitude

Pathological causes:

During t/t of deficiency anemias

After hemorrhage

Reticulocytopenia

Aplastic anemia

Post spleenectomy

Q

- How do reticulocytes differ from mature erythrocytes?

Erythrocytes (RBCs)

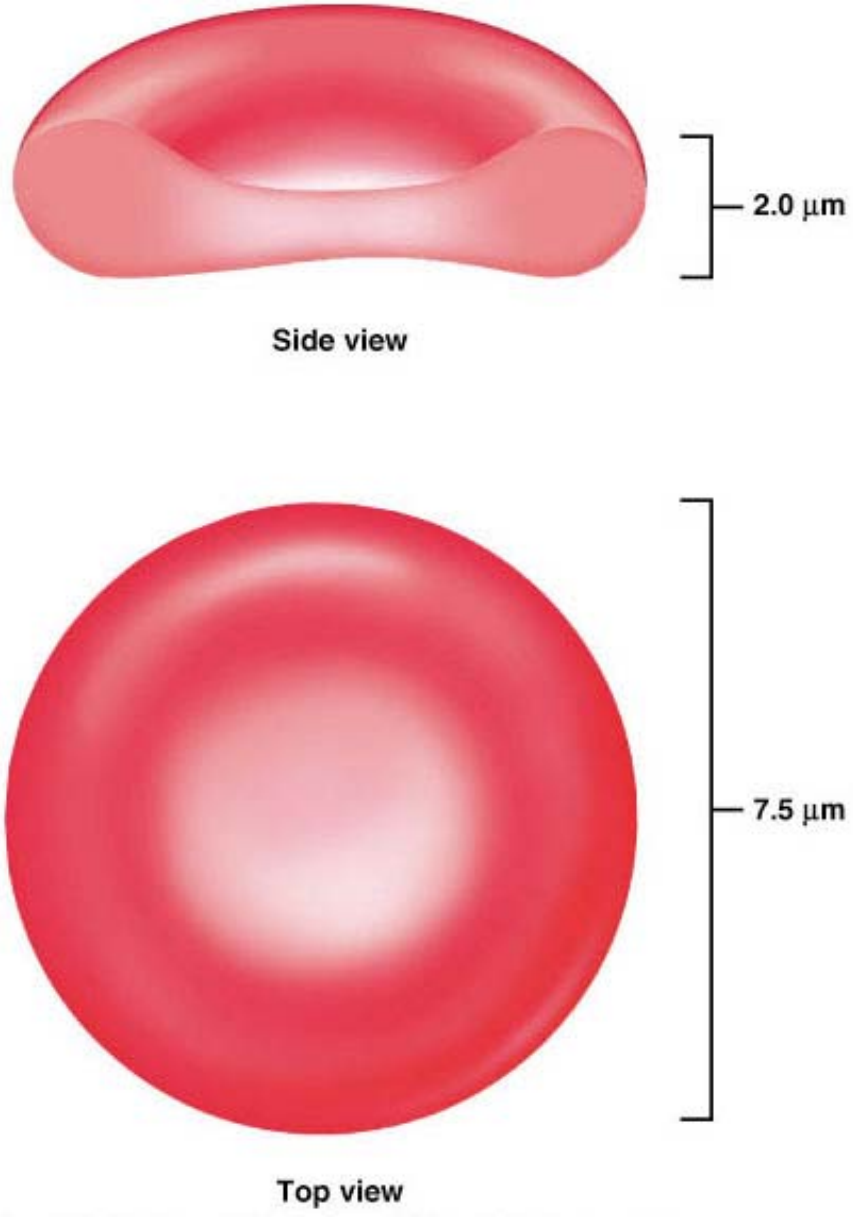
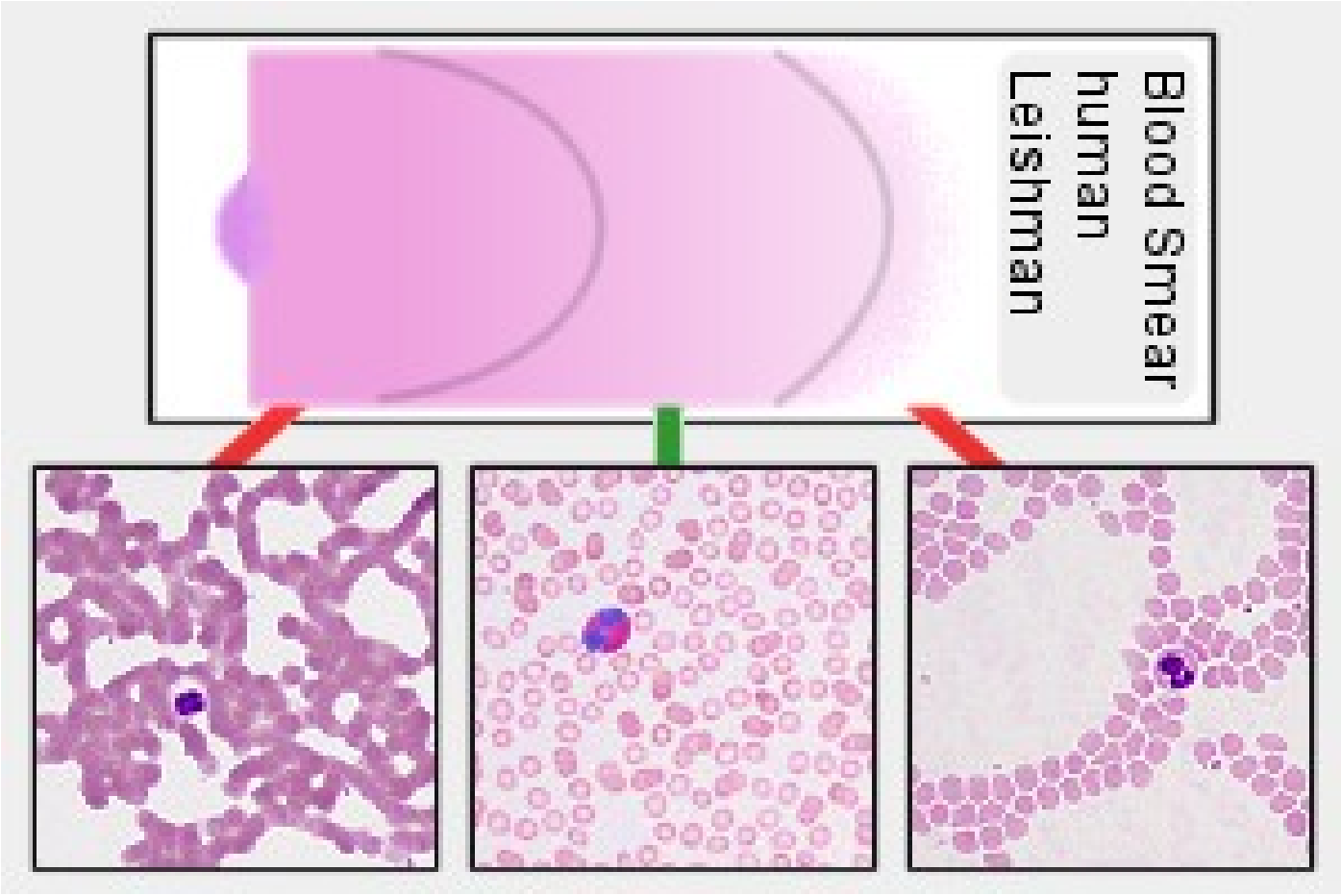
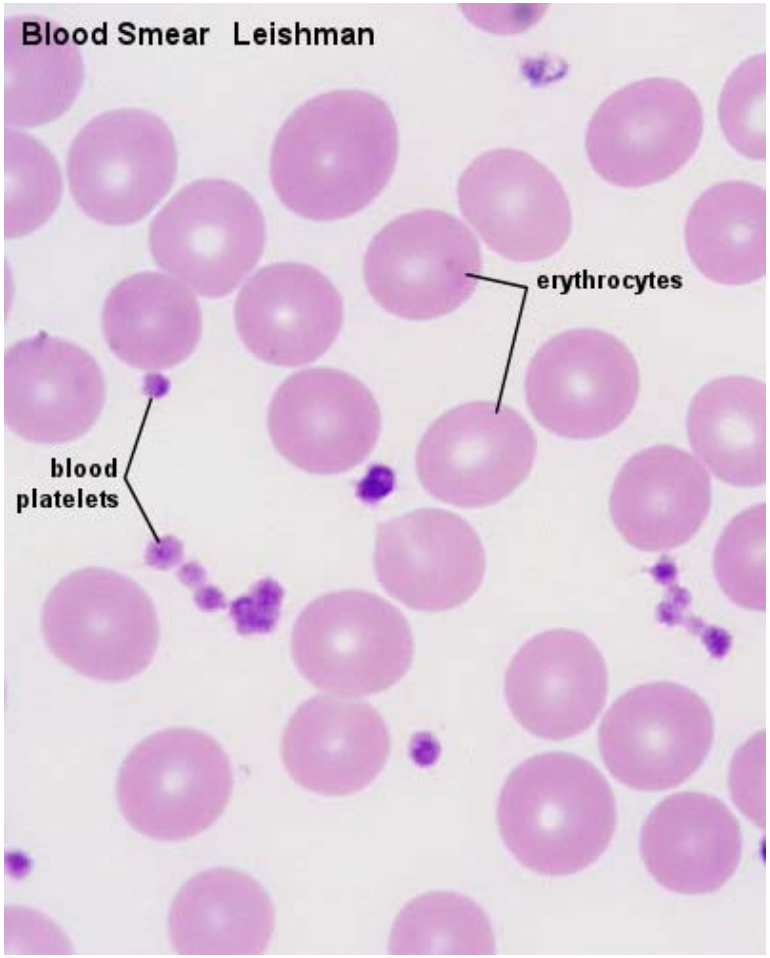


Figure 17.3

PBS under low power



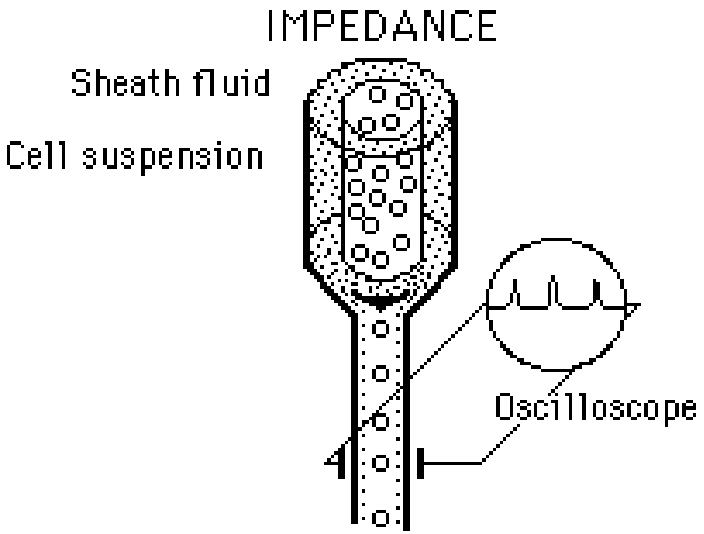
PBS under high power



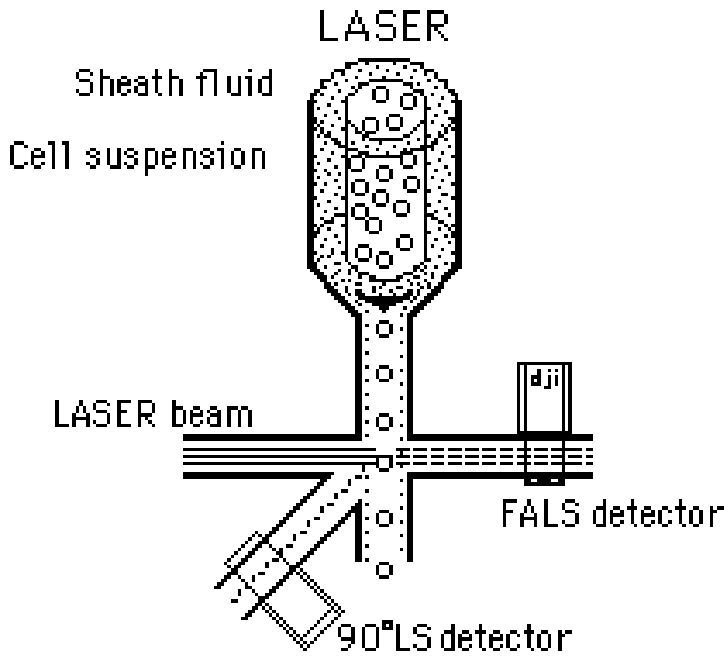
Mature Erythrocyte

Shape	Biconcave disc ?
Mean diameter	7.5µ(7-8 microns)
Thickness at periphery Thickness at center	2.5 µm 1 µm
Number: Males Females Children	5.2million ± 3lakh 4.7 million±3 lakh 4.4 million
Hemoglobin(g/dl of whole blood) At end of 3 m 10.5, Children upto 1 yr 12	Males 14- 18, Females 12-15.5 At birth 23
MCV	90±9fl
MCH	32±2 pg
MCHC (g/100ml of packed cells)	32- 34

Principle of automated cell counting



Current is measured across an aperature through which cells can pass, increasing the electrical resistance. Cells are counted by the pulse and cell volume inferred from the pulse amplitude.



As cells pass through an aperature a LASER beam strikes the cell. The reflected light is measured, providing a count and information about the cell size and internal composition.

RBC indices

- MCV gives you the average volume of erythrocytes.
- MCH gives you the average weight of hemoglobin per eyrthrocyte.
- MCHC gives you the average hemoglobin concentration per erythrocyte.

RBC indices

MCV: Average volume of the RBC

$$MCV = \frac{PCV \text{ in } \%}{RBC \text{ count in millions/mm3}} \times 10 \text{ cubic microns}$$

MCH: Average Hb concentration of a RBC

$$MCH = \frac{Hb(gm/dl)}{RBC \text{ count in million/mm3}} \times 10 \text{ picogram}$$

MCHC: Ave. Hb concentration per RBC

$$MCHC = \frac{Hb(gm/dl)}{PCV(\%)} \times 100$$

Biconcave disc shape of RBC

- Increased ratio of surface area : volume(40% more membrane)

  Facilitates gas transport
- More deformable

Maintenance of biconcave shape

- Elastic forces within the membrane
- Surface tension
- Electrical forces on the membrane surface due to albumin adsorption
- Osmotic /hydrostatic forces

Mature erythrocyte

Lacks: Ribosomes,mitochondria and nucleus thus

- Unable to synthesize new protein
- Unable to carry out oxidative reactions a/w mitochondria
- Unable to undergo mitosis

RBC metabolism

Require energy to

- Maintain shape & flexibility of cell membrane
- Maintain iron in Fe^{++} form
- Preserve the milieu of RBC (high K^{+} , low Na^{+} & Ca^{++})

Thus must have constant access to glucose

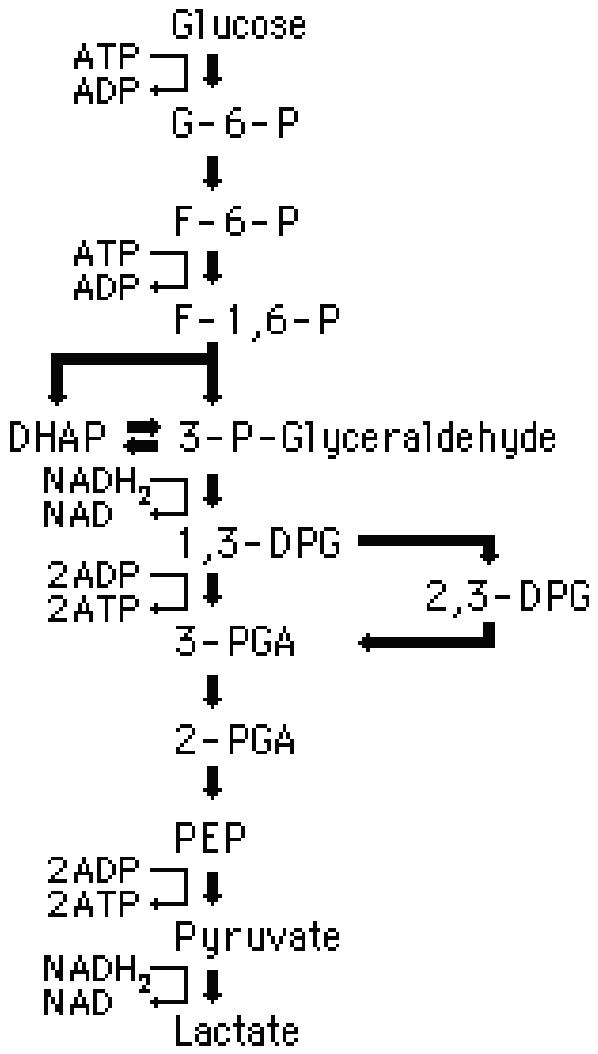
Glucose enters the RBC via facilitated diffusion

Mature RBCs do not have a Citric aid cycle for glucose utilization

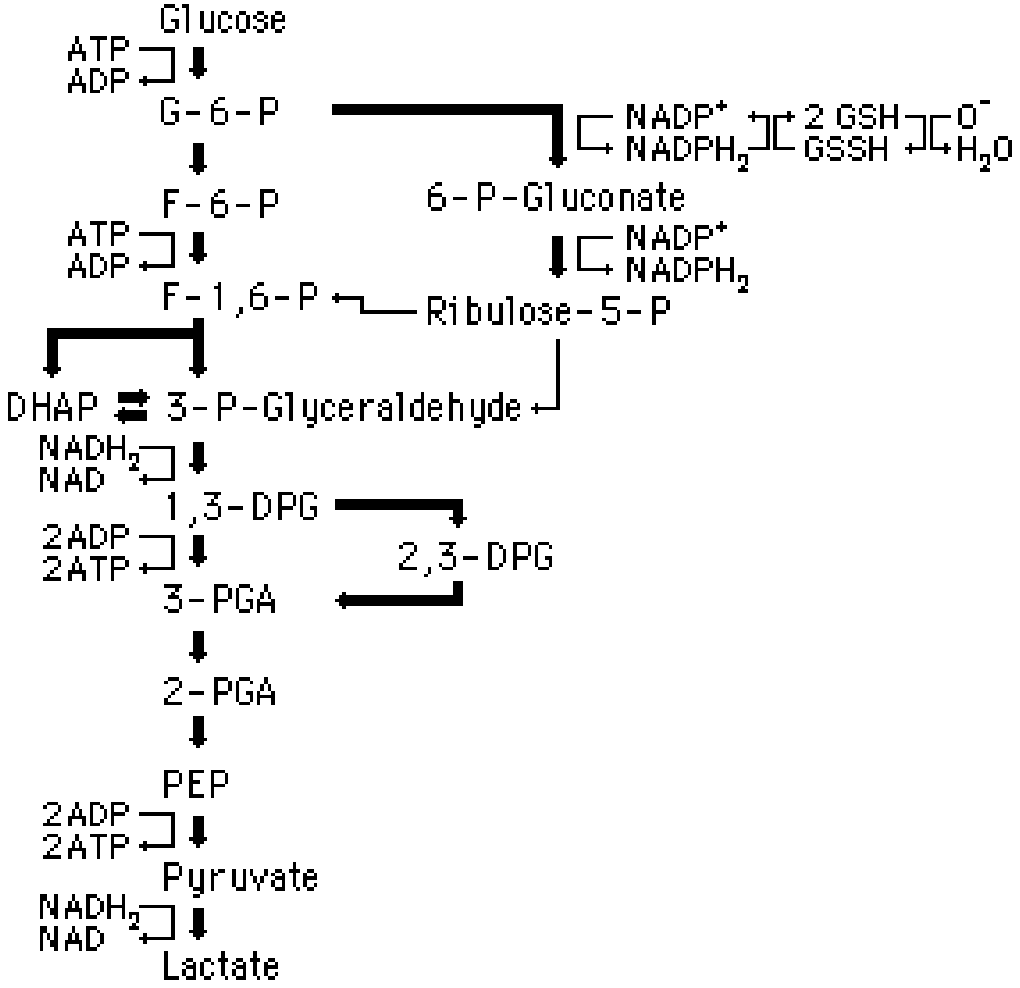
Less efficient pathways

- Anaerobic glycolysis (EMP)(95%)
- Pentose phosphate pathway(HMP shunt/ phosphogluconate pathway)

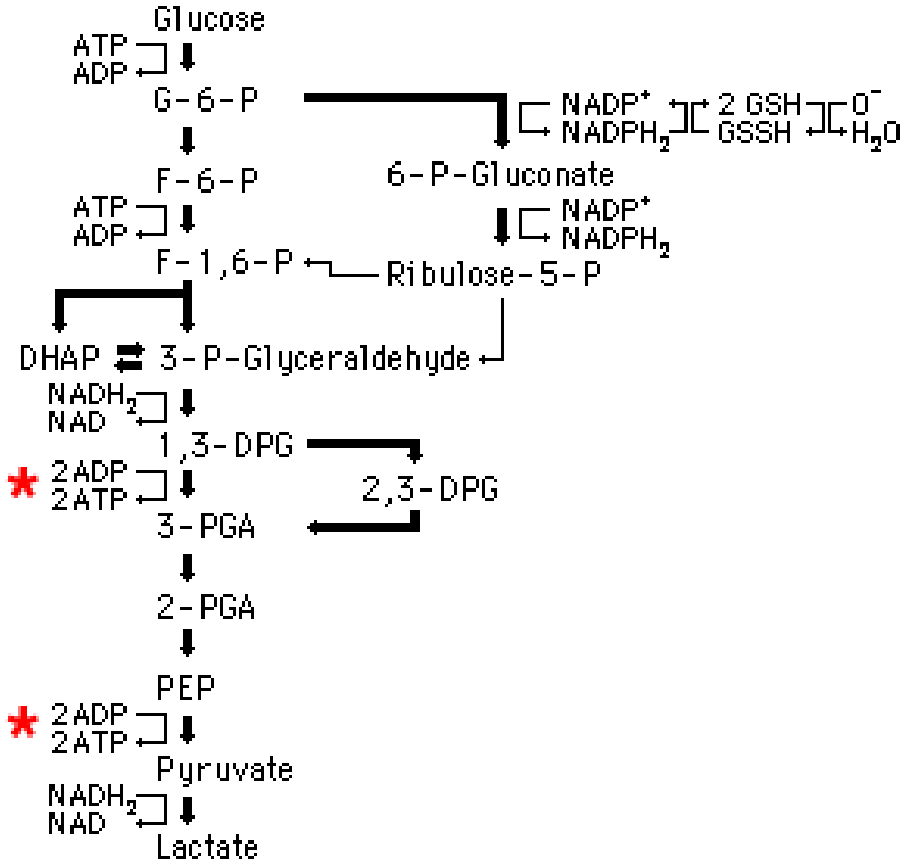
Anaerobic glycolysis(EMP)



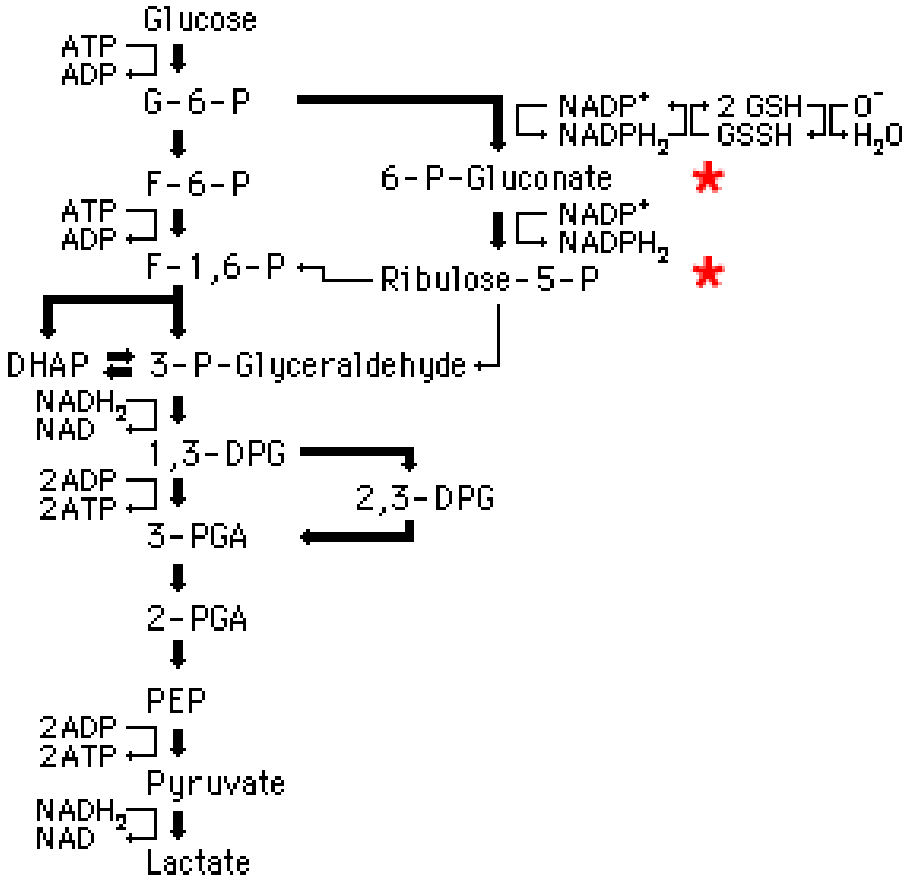
HMP shunt


Glucose
ATP
ADP
G-6-P
NADP+
NADPH2
2 GSH
GSSH
O-
H2O
6-P-Gluconate
F-6-P
ATP
ADP
NADP+
NADPH2
F-1,6-P
Ribulose-5-P
DHAP
3-P-Glyceraldehyde
NADH2
NAD
1,3-DPG
2ADP
2ATP
2,3-DPG
3-PGA
2-PGA
PEP
2ADP
2ATP
Pyruvate
NADH2
NAD
Lactate

ATP production in anaerobic glycolysis



NADPH synthesis

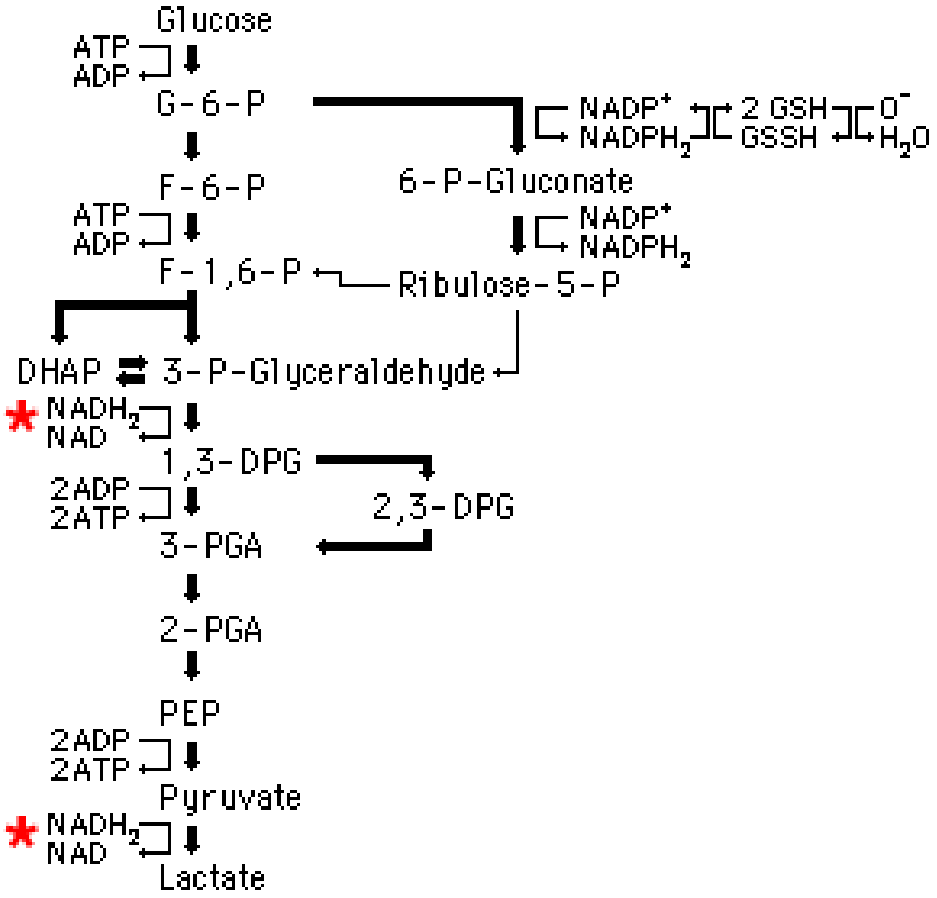


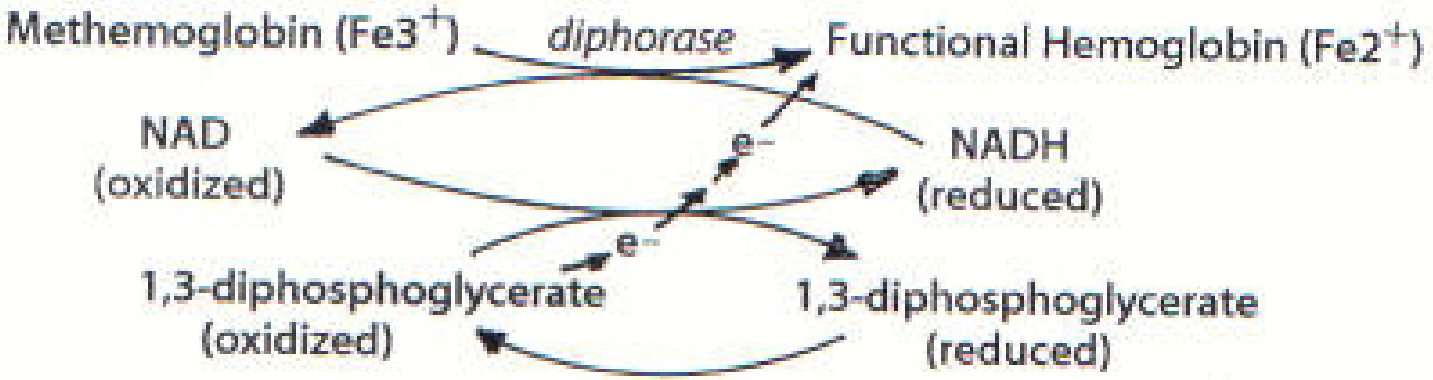
- Normally H_2O_2 is disposed off by catalase & glutathione peroxidase. The latter leads to an increase in production of GSSG(oxidized glutathione)
- Reduced glutathione(GSH) is regenerated from GSSG by action of glutathione reductase which depends on the availability of NADPH
- NADPH synthesis protect sulfhydryl groups in erythrocyte membranes and hemoglobin

Heinz bodies

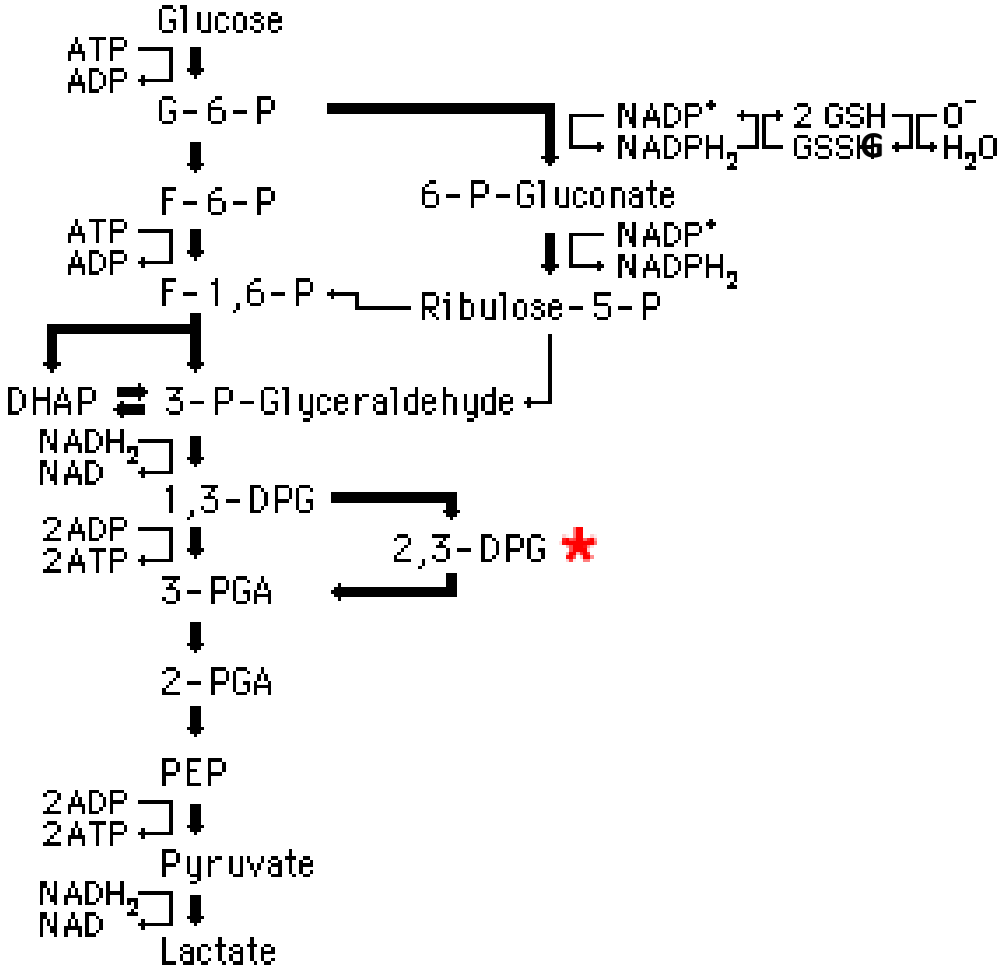
- Appear inside the RBC when it has been subjected to oxidative stress as a result of oxidation and subsequent precipitation of –SH groups of hemoglobin
- They stain purple with cresyl violet

Meth hemoglobin reduction

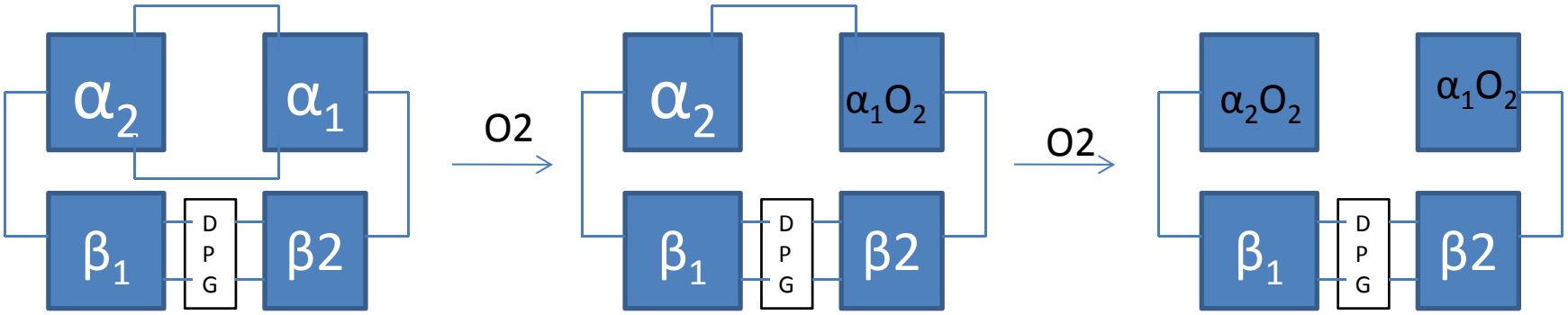



Methemoglobin ($Fe3^+$)
diphorase
Functional Hemoglobin ($Fe2^+$)
NAD
(oxidized)
e^-
NADH
(reduced)
e^-
1,3-diphosphoglycerate
(oxidized)
1,3-diphosphoglycerate
(reduced)

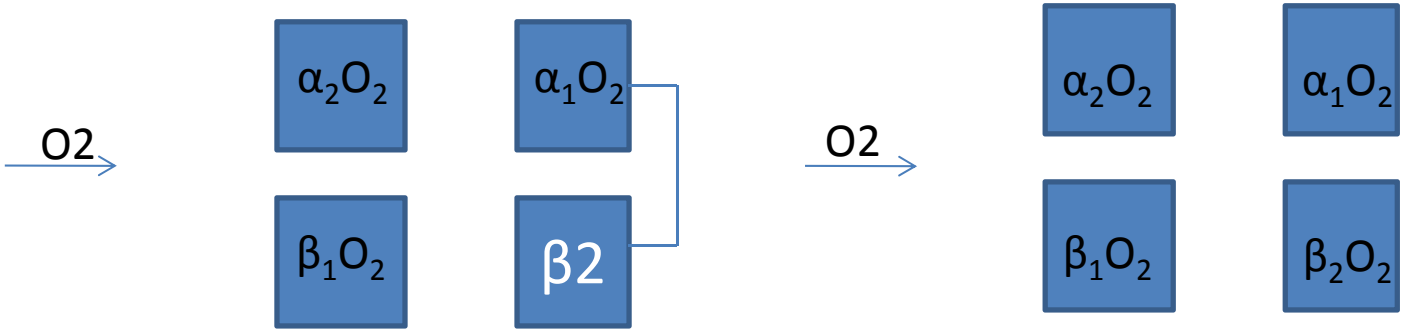
2, 3 DPG generation



Role of 2,3 DPG

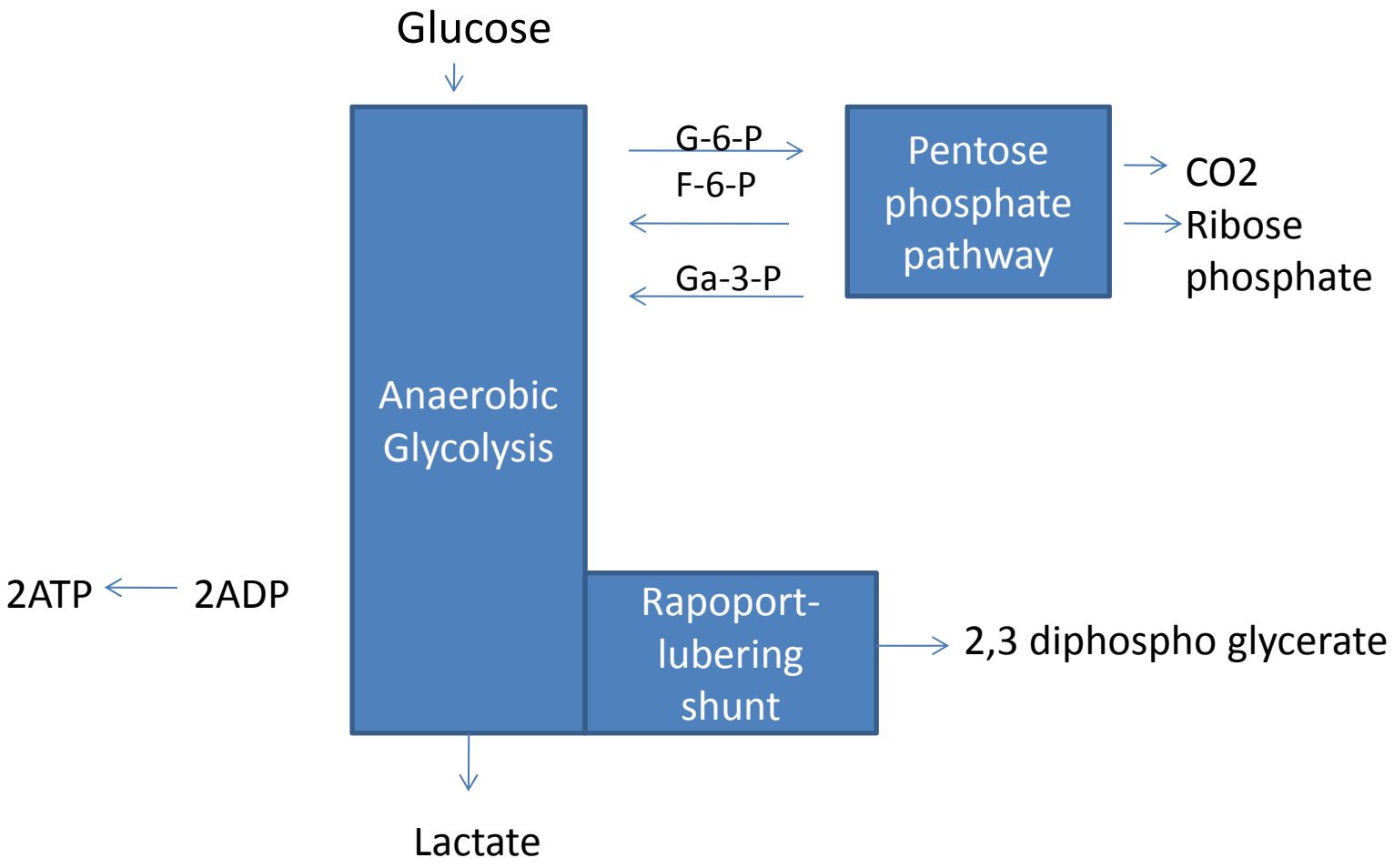


T state
Taut /Tense



R state
Relaxed

Energy metabolism in RBC



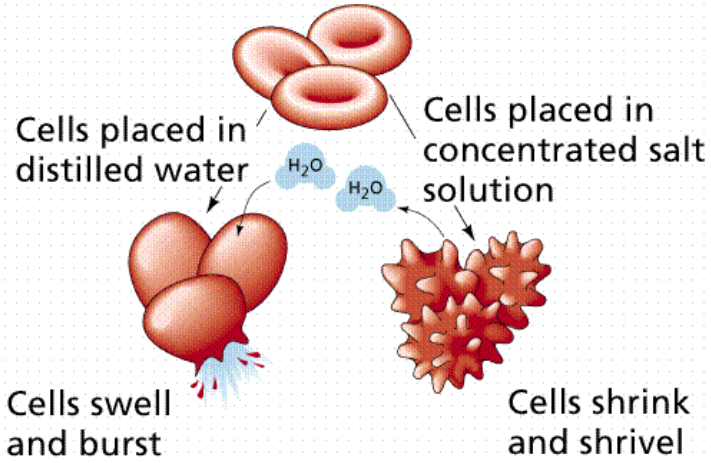
Products of metabolism in RBC

- NADH
- ATP
- 2, 3 DPG
- NADPH,major reducing agent in the RBC
- Conversion of hexoses to pentoses

Erythrocyte membrane and fragility

Broken by certain physical stimuli

- Mechanical fragility:RBCs shaken with glass bead X 1hr, 2-5% lysis
- Autohemolysis: blood kept at 370C for 24 hrs
  < 0.5% hemolysis
- Osmotic fragility: RBCs in physiological saline remain intact for hours



RBC membrane

1. *Peripheral proteins* -- spectrin, ankyrin, (band 4.1), actin. Comprise peripheral cytoskeleton, which supports membrane. All cells are thought to have a similar structure under the plasma membrane.

RBC membrane…

2. Intrinsic proteins

Examples

(1). Multipass (band 3/anion exchanger) -- Catalyzes reversible exchange of the anions HCO_3^- (bicarb) and Cl^- between RBC and plasma. Exchange allows max. transport of CO_2 in blood (as bicarb in solution

(a) Basic point: Bicarb is much more soluble in plasma than CO_2, so lots of bicarb (but not much CO_2) can be carried in the blood. Therefore need to covert CO_2 to bicarb when want to carry CO_2 in blood; need to do reverse to eliminate the CO_2 (in lungs).

(b) Conversion of CO_2 to bicarb (& vice versa) can only occur ***inside*** RBC, where the enzyme carbonic anhydrase is. Carbonic anhydrase catalyzes:

$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$

RBC membrane…

(c). Gases can pass through membranes by diffusion -- CO_2 can exit or enter RBC as needed. However bicarb cannot pass through membranes. You need the anion exchanger to get bicarb in and out of RBC.

(d). Where CO_2 is high, as in tissues, CO_2 diffuses into RBC and is converted to bicarb inside the RBC. (Reaction above goes to right.) Then bicarb leaves RBC in exchange for chloride using the anion exchanger.

(e). In lungs, the process is reversed -- bicarb reenters the RBC in exchange for chloride using the anion exchanger. The bicarb is converted back to CO_2 inside the RBC (reaction above goes to left). Then the CO_2 diffuses out of the cells and is exhaled.

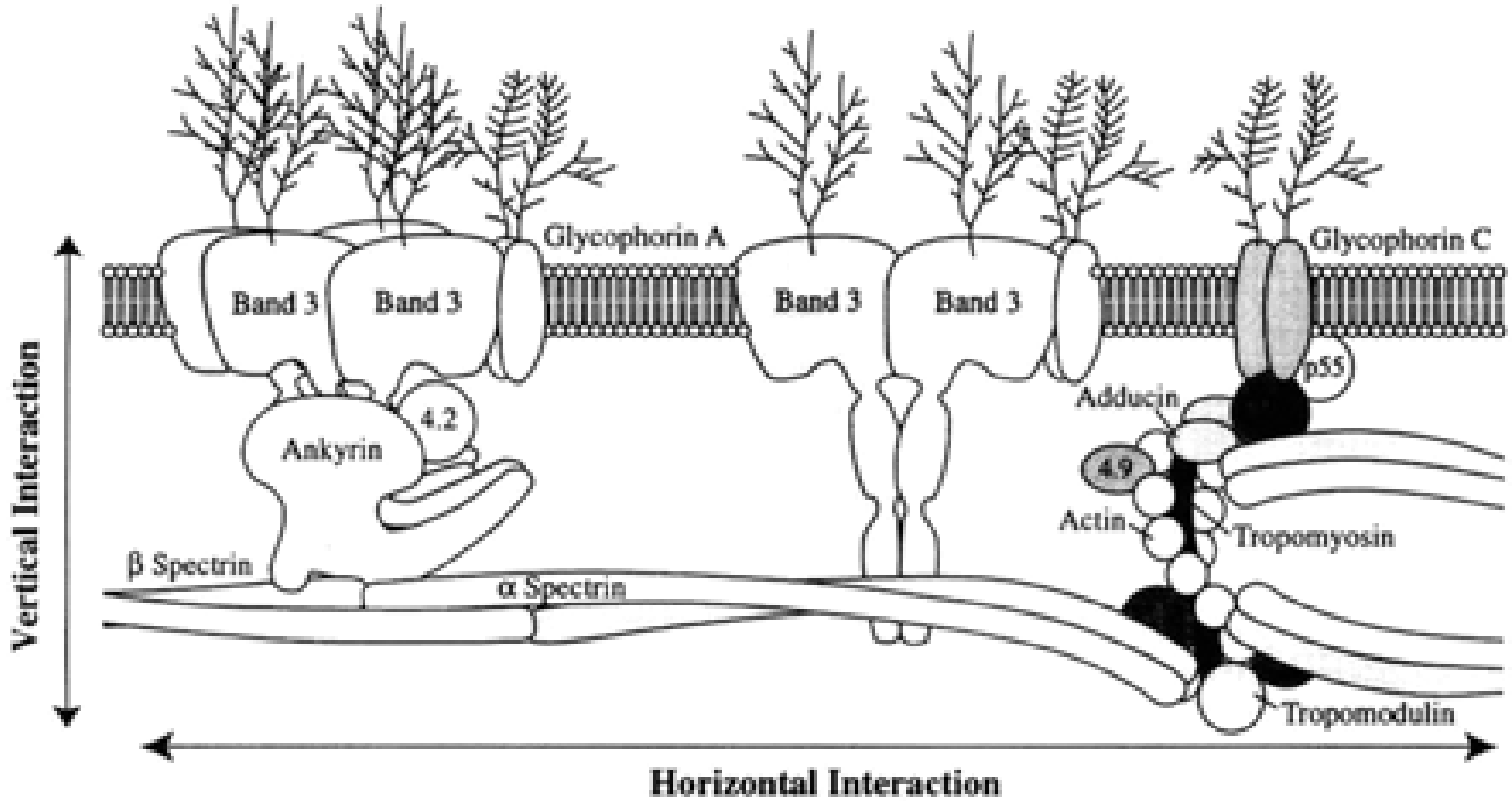
(2). Single pass (glycophorin) -- function of protein not known.

(a). Large amount of (-) charged modified carbohydrate -- sialic acid -- may cause RBC to repel each other and prevent clumping of RBC.

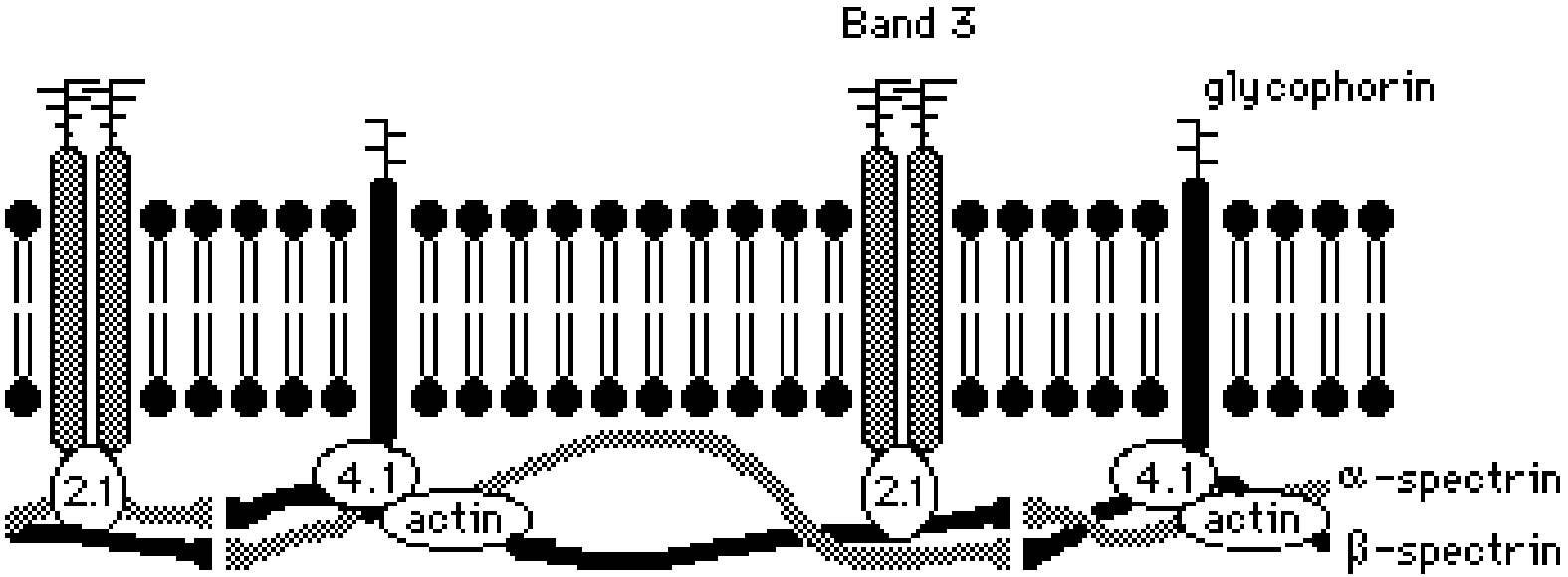
(b). Loss of terminal sugars may occur with age and trigger destruction of "old" RBC.

(c). Glycophorins make up a gene family; variations in glycophorin A are responsible for MN blood type differences. Variations in glycophorin C are correlated with resistance to malaria.

RBC membrane structure



RBC membrane structure



Protein 4.1 binds spectrin to glycophorin C

Protein 2.1(Ankyrin) binds spectrin to anion exchanger band 3

Q

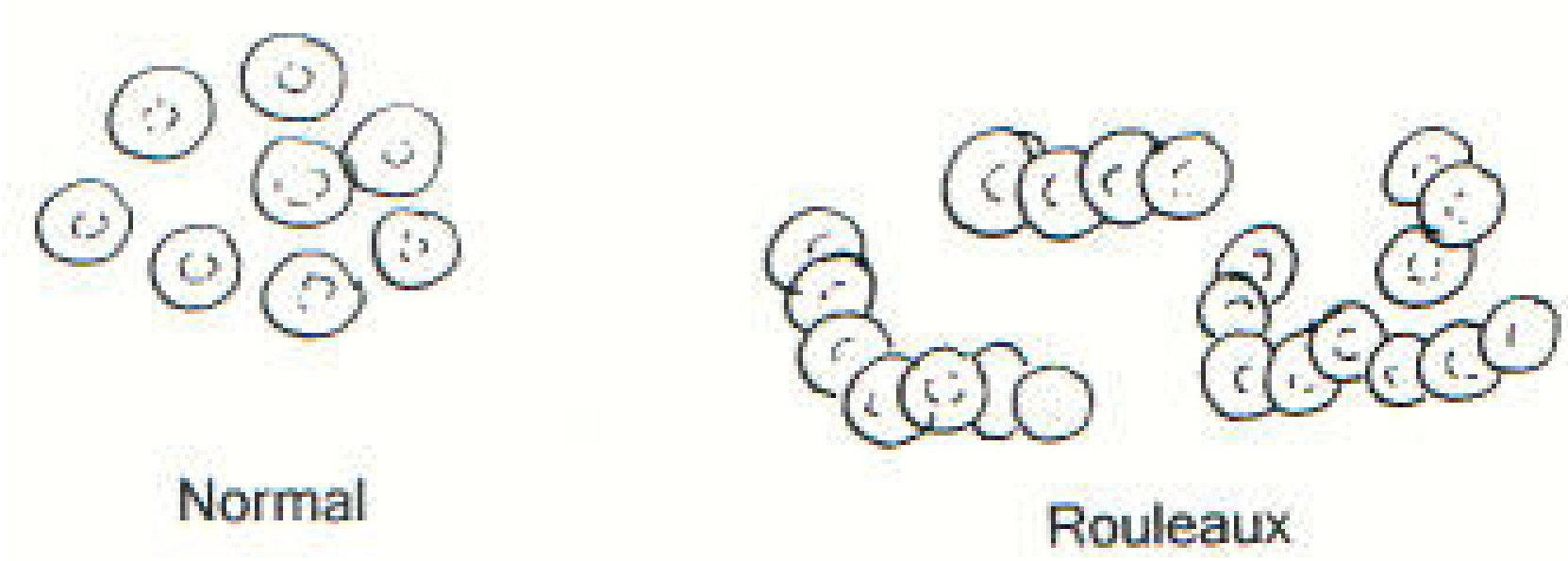
- Your patient is a 44 year -old woman complaining of "exhaustion".
- hematocrit is 0.15
- RBC count 1.0 million
- reticulocyte count 2%.

What do these findings indicate to you

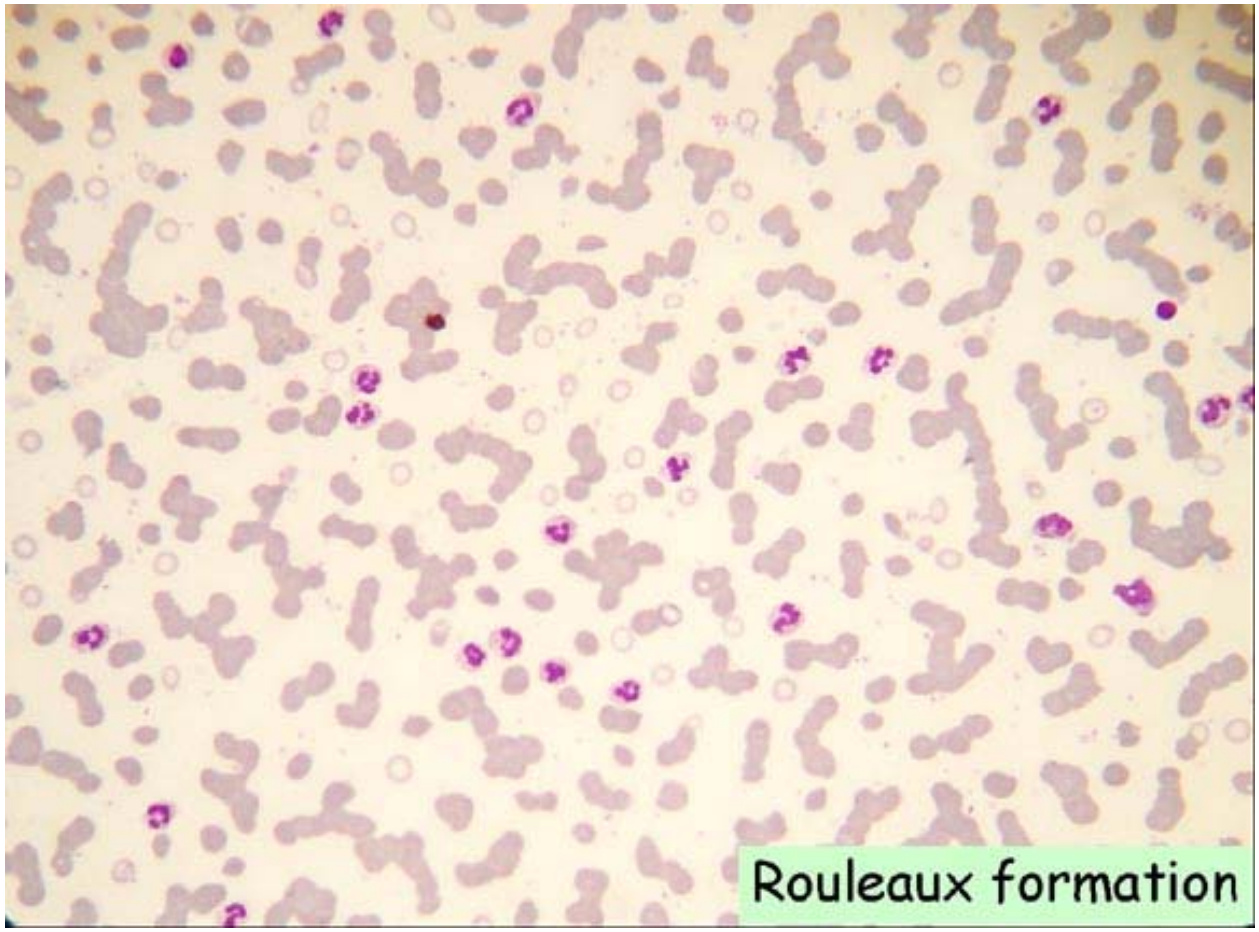
- ➢ Patient is recovering from anemia
- ➢ Patient is going into marrow failure

- When expressed as a % of total RBCs the reticulocyte count may overstate the actual number of reticulocytes. Therefore:
- In this case:
- Thus the retic count is not increased (normal being 0.5-1.5%), but is in fact relatively low in an anemic patient indicating no marrow response and suggesting marrow failure.
- The use of the absolute rectic count avoids this problem. In this case:
- (0.02 x 1,100,000) = 22,000 retics, which is abnormally low for someone with such a low Hct

Rouleaux formation



Increased amount of fibrinogen in the blood can cause rouleaux formation


Rouleaux formation

Anisocytosis

Variations in erythrocyte size

- Microcytic : MCV = <80 fL & size = <6 µM

  Eg.iron-deficiency anemias.
- Normocytic : MCV = 80 - 100 fL & size = 6 - 9 µM
- Macrocytic: MCV = >100 fL & size = >9 µM

  Eg. hepatic diseases & vitamin B12 and folic acid deficiency anemias

Poikilocytosis

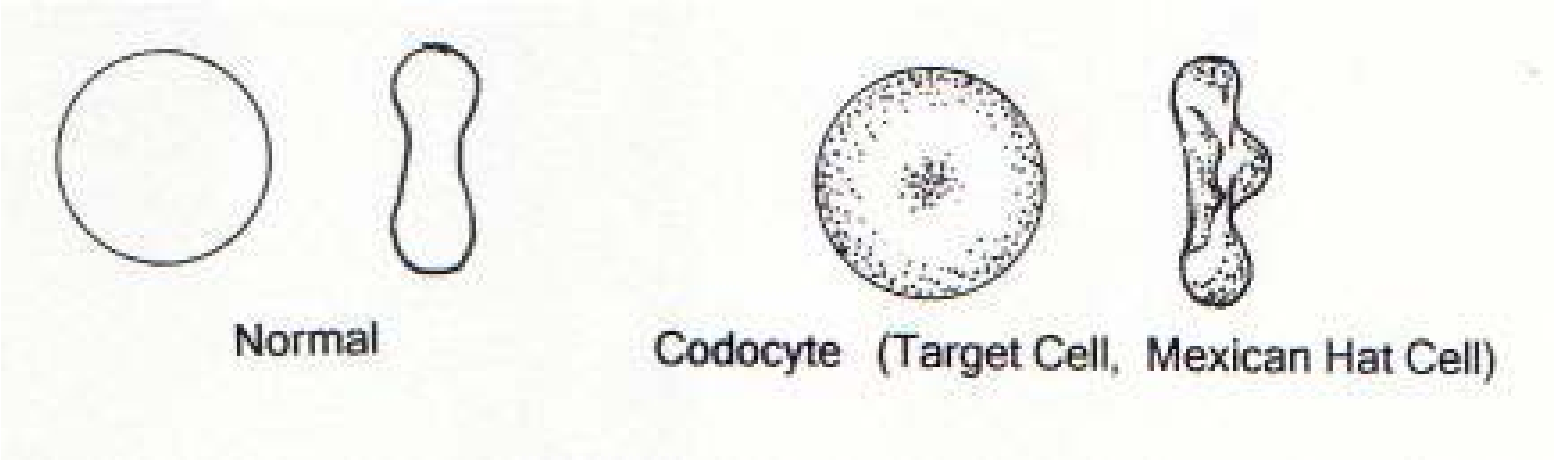
Variation in the shape of erythrocytes

Due to chemical or physical alteration in the red blood cell membrane or the actual contents of the cell

Spherocyte

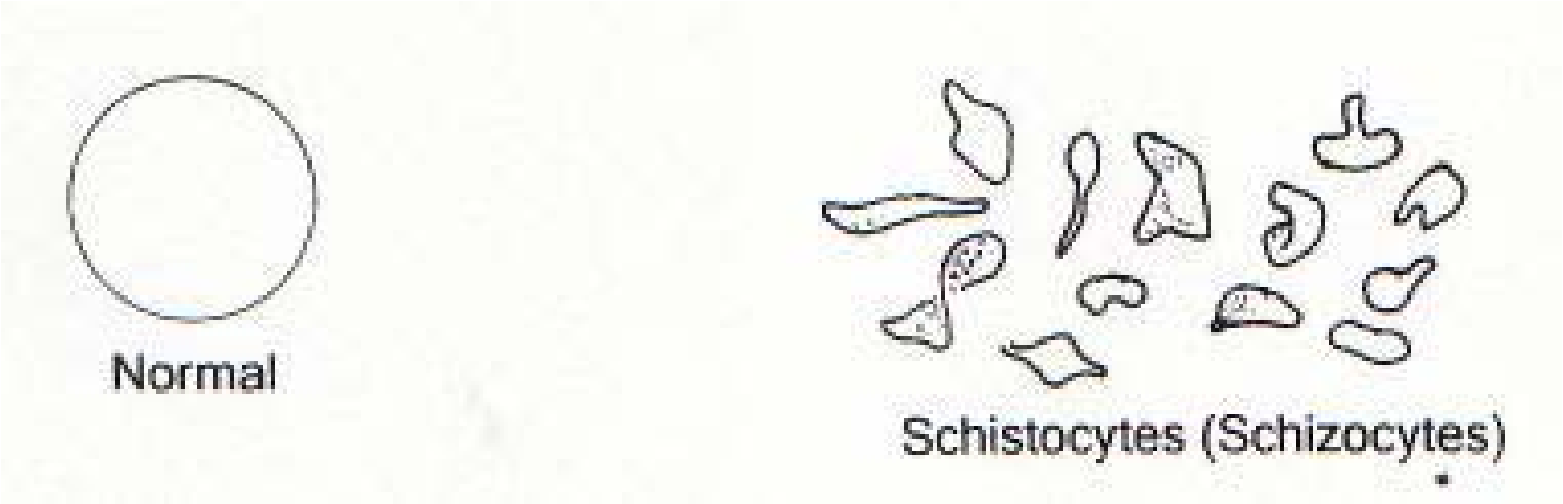
Observed in immune induced hemolysis, post blood transfusions, and congenital anemia

Target cell



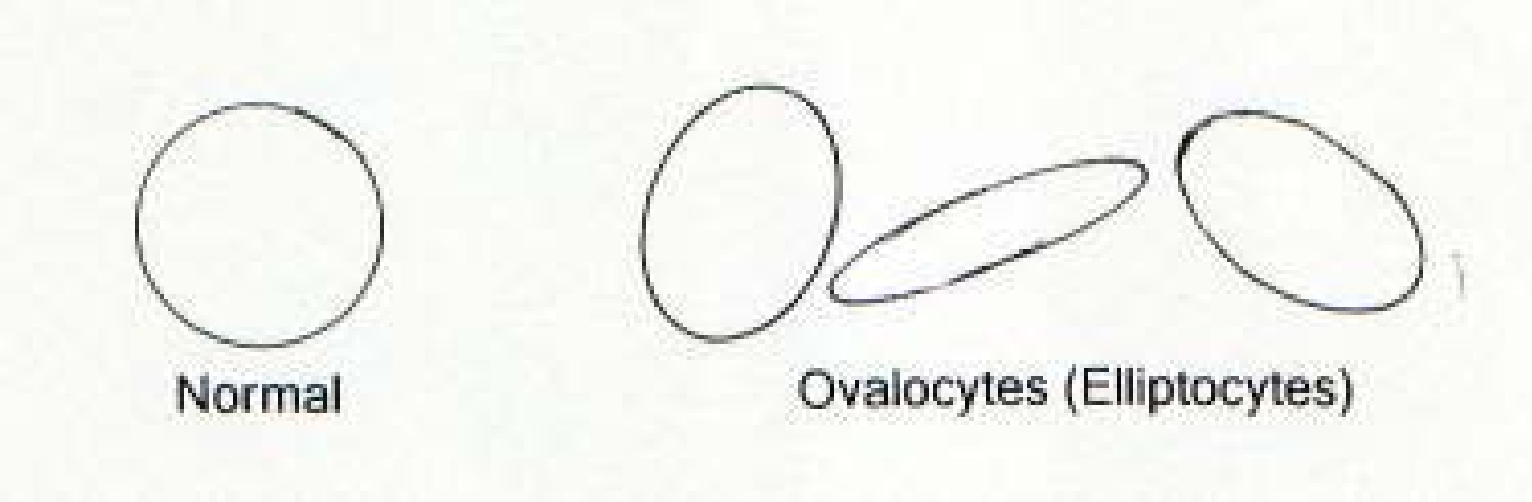
Observed in hemoglobinopathy, hepatic diseases, iron deficiency anemia, hemolytic anemia, and splenectomy.

Schistiocytes



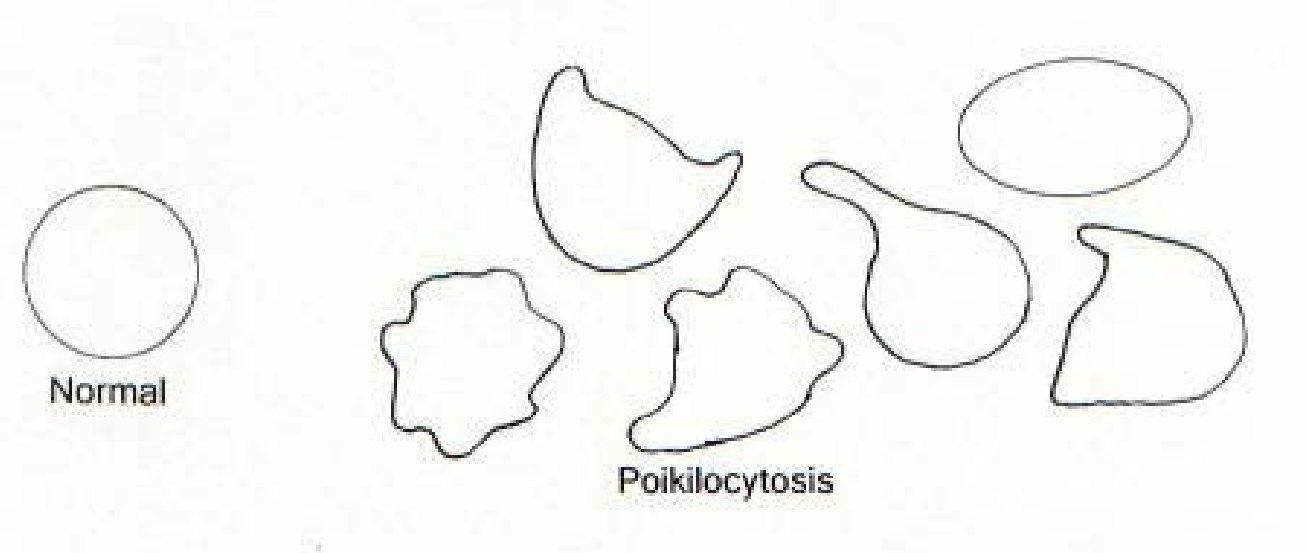
Uremia, microangiopathic hemolytic anemias, hemolytic anemias cause by physical agents, and disseminated intravascular coagulation (DIC)

Ovalocytes



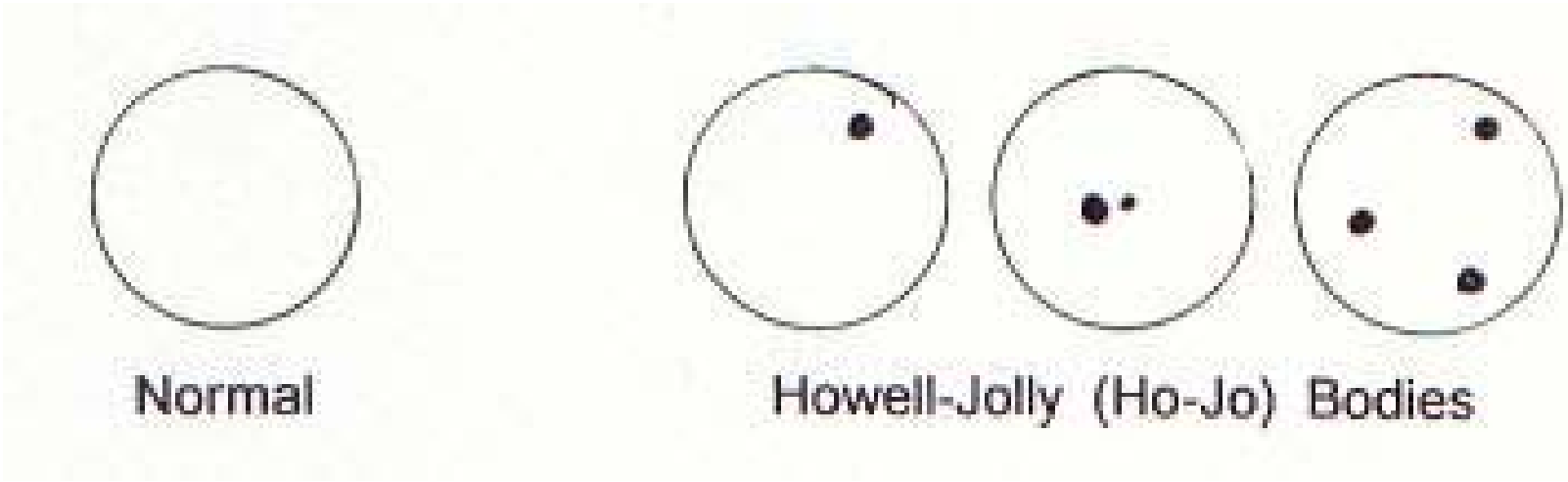
Hereditary defect present in the RBC cytoskeletal proteins (the spectrin chain), iron deficiency anemia, leukemia associated anemias, thalassemia, and dyserythropoiesis

Poikilocytosis



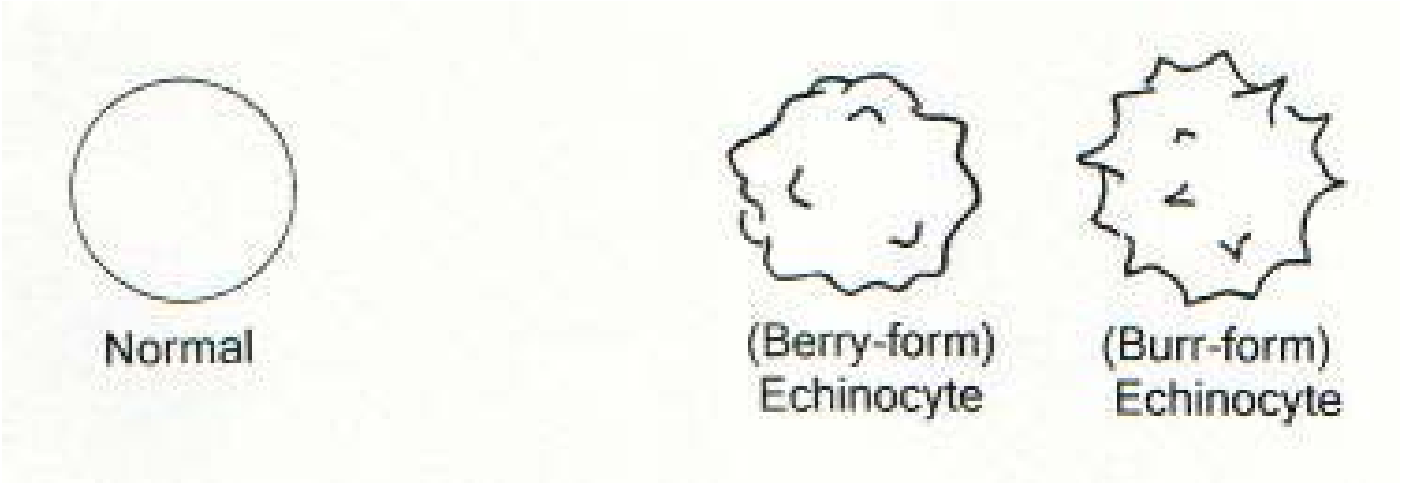
Indicator of abnormal erythropoiesis due to bone marrow effects and/or abnormal RBC destruction

Howell-Jolly bodies



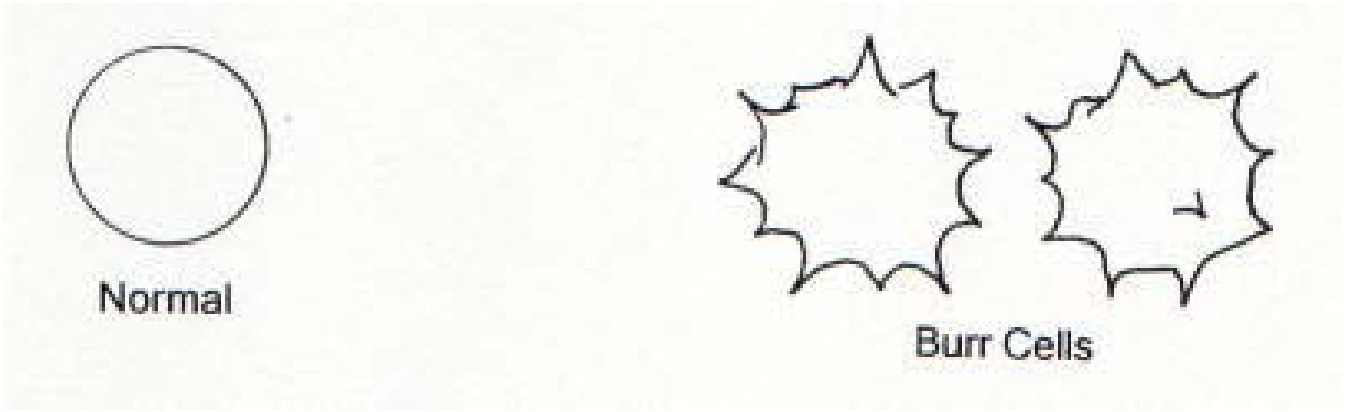
Observed in hemolytic anemias, pernicious anemia, post-operative conditions, splenectomy, or splenic atrophy

Echinocyte



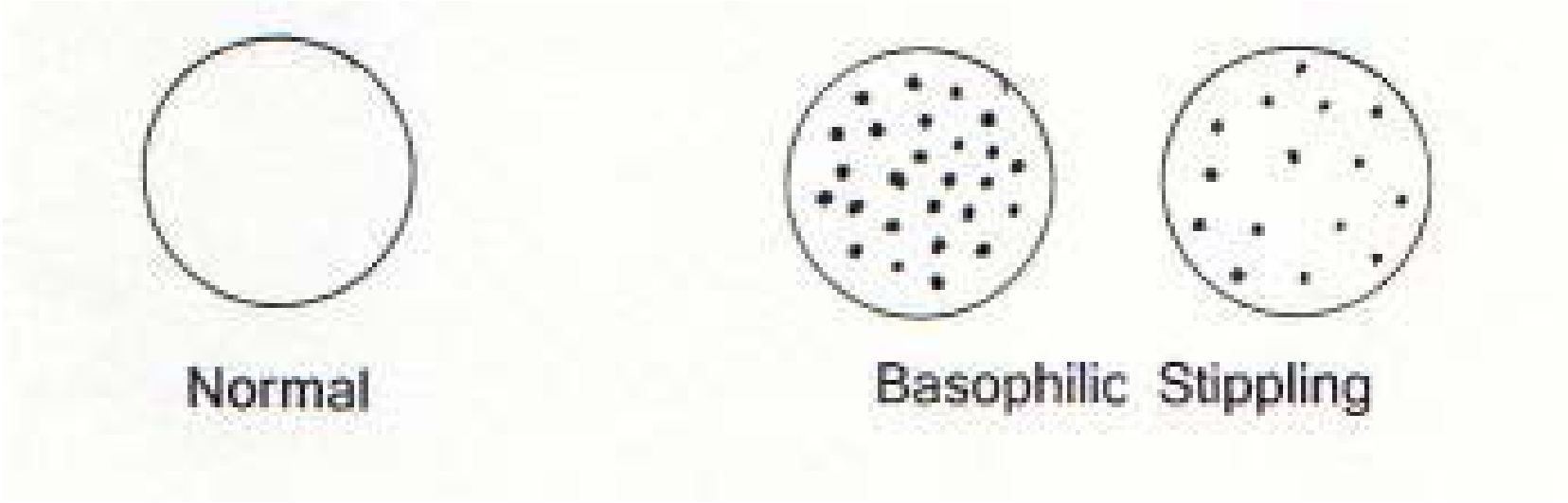
Observed in uremia, acute blood loss, stomach cancer, and pyruvate kinase deficiency

Burr cell



Observed in uremia, acute blood loss, stomach cancer, and pyruvate kinase deficiency

Basophilic stippling



Observed in lead poisoning, alcoholism megaloblastic anemias

Erythrocytes hemoglobinization

- Normochromic RBC normal amount of hemoglobin which stains uniformly

  MCH = 27 to 32 pg & MCHC = 31 to 37%.
- Hypochromasia / hypochromia

  MCH = <27 pg & MCHC = <31

  Eg.iron-deficiency anemia and thalassemia, any hemoglobinopathy

Hematocrit/ Packed cell volume

Percentage of the total volume of blood that is occupied by packed red blood cell

Normal values are as follows:

- Adult male = 42% to 53%
- Adult female = 36% to 46%
- Newborn = 50% to 62%
- One year = 31% to 39%

Hematocrit

- Increased

Polycythemia

Shock associated with surgery, burns, or traumas

Dehydration

- Decreased

Anemias

Pregnancy

receiving IV fluids

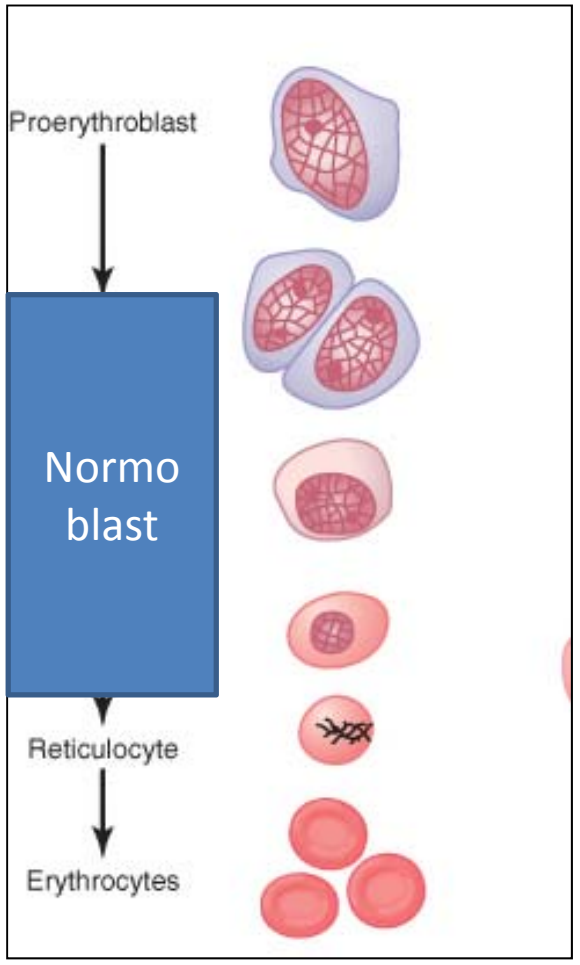
Cardiac decompensation (a failure to maintain a good blood circulation)

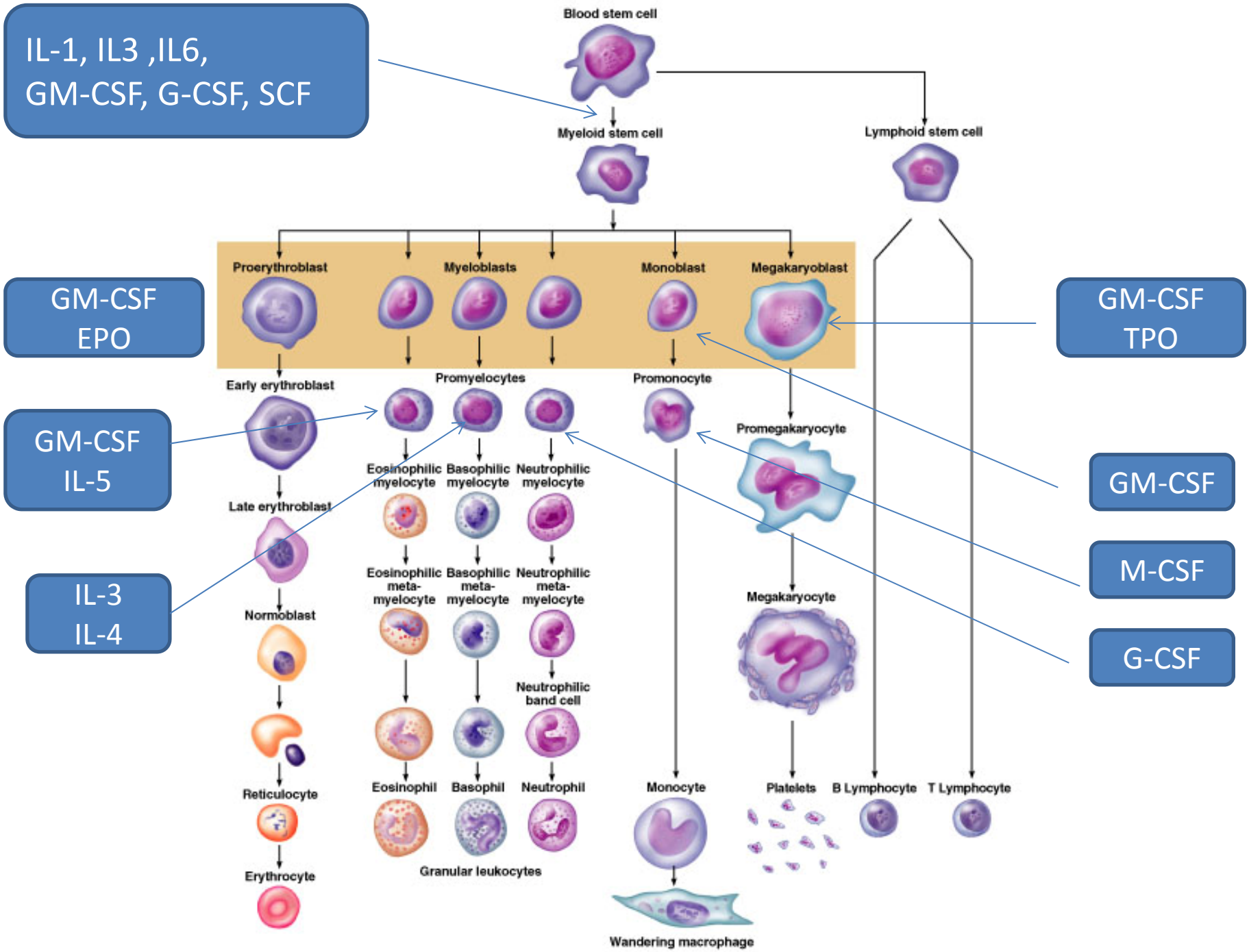
Factors required for normal erythropoiesis

- Dietary factors

Protein
Iron
Copper
Manganese
Vitamin C
Folic acid
Vitamin B12

- Intrinsic factors
- Hormones: Thyroid & corticoid




IL-1, IL3 ,IL6,
GM-CSF, G-CSF, SCF
Blood stem cell
Myeloid stem cell
Lymphoid stem cell
Proerythroblast
Myeloblasts
Monoblast
Megakaryoblast
GM-CSF
EPO
GM-CSF
TPO
Early erythroblast
Promyelocytes
Promonocyte
Promegakaryocyte
GM-CSF
IL-5
GM-CSF
Late erythroblast
Eosinophilic myelocyte
Basophilic myelocyte
Neutrophilic myelocyte
M-CSF
IL-3
IL-4
Eosinophilic meta-myelocyte
Basophilic meta-myelocyte
Neutrophilic meta-myelocyte
Megakaryocyte
G-CSF
Normoblast
Neutrophilic band cell
Reticulocyte
Eosinophil
Basophil
Neutrophil
Monocyte
Platelets
B Lymphocyte
T Lymphocyte
Erythrocyte
Granular leukocytes
Wandering macrophage

Dietary factors affecting erythropoiesis

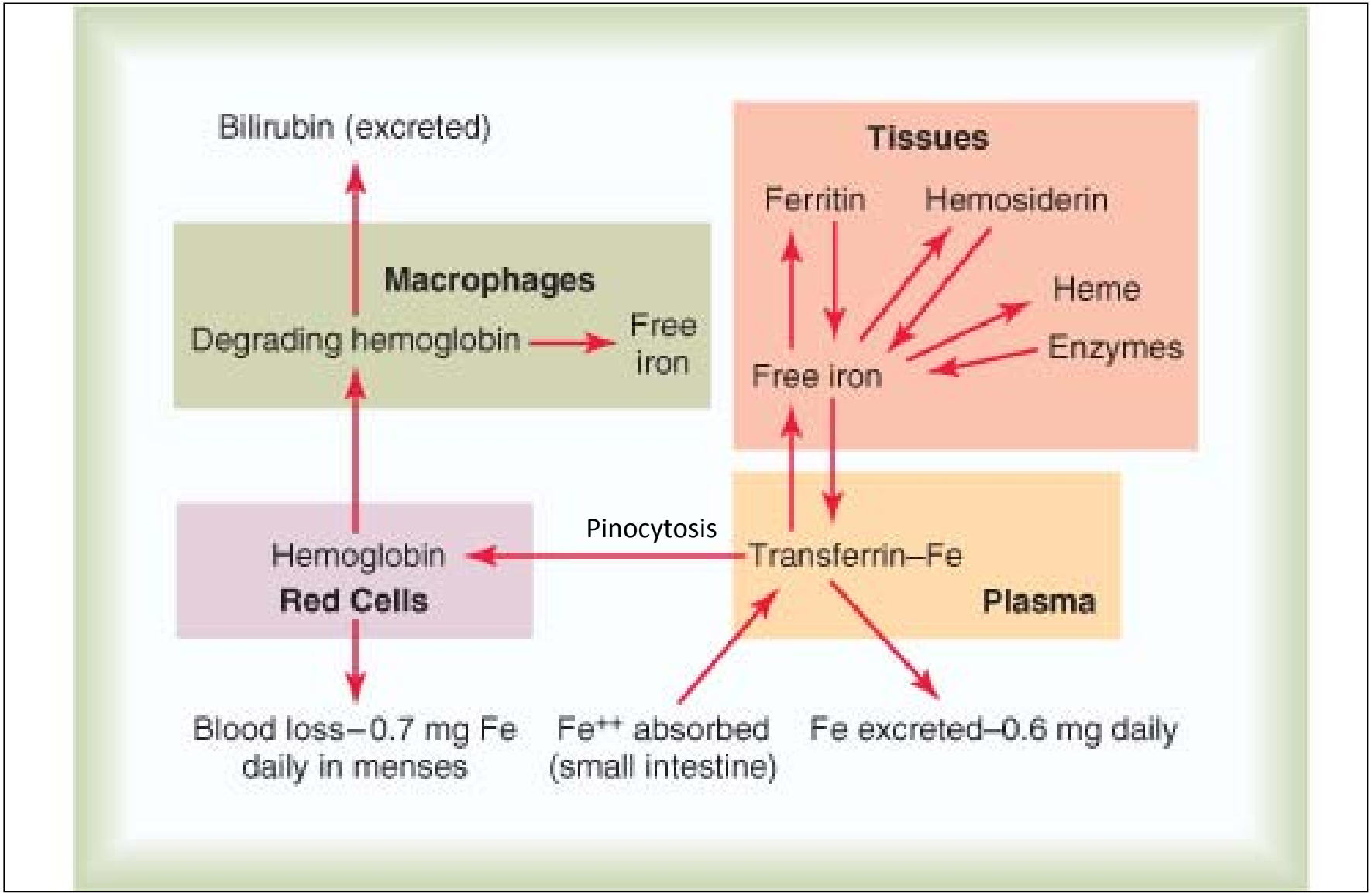
Protein: all 10 are important

Histidine, Valine, Leucine, Isoleucine, Lysine, Arginine, Methionine, Tryptophan, Phenylalanine, Threonine, Glycine

In PEM anemia results

Normocytic, normochromic, reticulocyte count normal, slightly hypocellular bone marrow

Iron


Bilirubin (excreted)
Tissues
Ferritin
Hemosiderin
Heme
Enzymes
Macrophages
Degrading hemoglobin
Free iron
Free iron
Pinocytosis
Hemoglobin
Red Cells
Transferrin–Fe
Plasma
Blood loss–0.7 mg Fe daily in menses
Fe++ absorbed (small intestine)
Fe excreted–0.6 mg daily

Iron metabolism

Why is iron required?

Hemoglobin: 65%

Myoglobin: 4%

Cytochromes

Cytochrome oxidases

Peroxidase

Catalase

1%

Transferrin: 0.1%

Ferritin(Liver &RES): 15-30%

Iron metabolism

Total body iron 4-5gm
Daily losses
Males: 1mg/day
Females: 2 mg/day
Absorption of iron: 3-6 % of ingested amount
Site of absorption: Duodenum
Inhibitors of absorption:
Phytic acid, phosphates, oxalates & carbonates
Promoters of absorption: Citric acid

Iron absorption from the gut

2 pathways for iron absorption

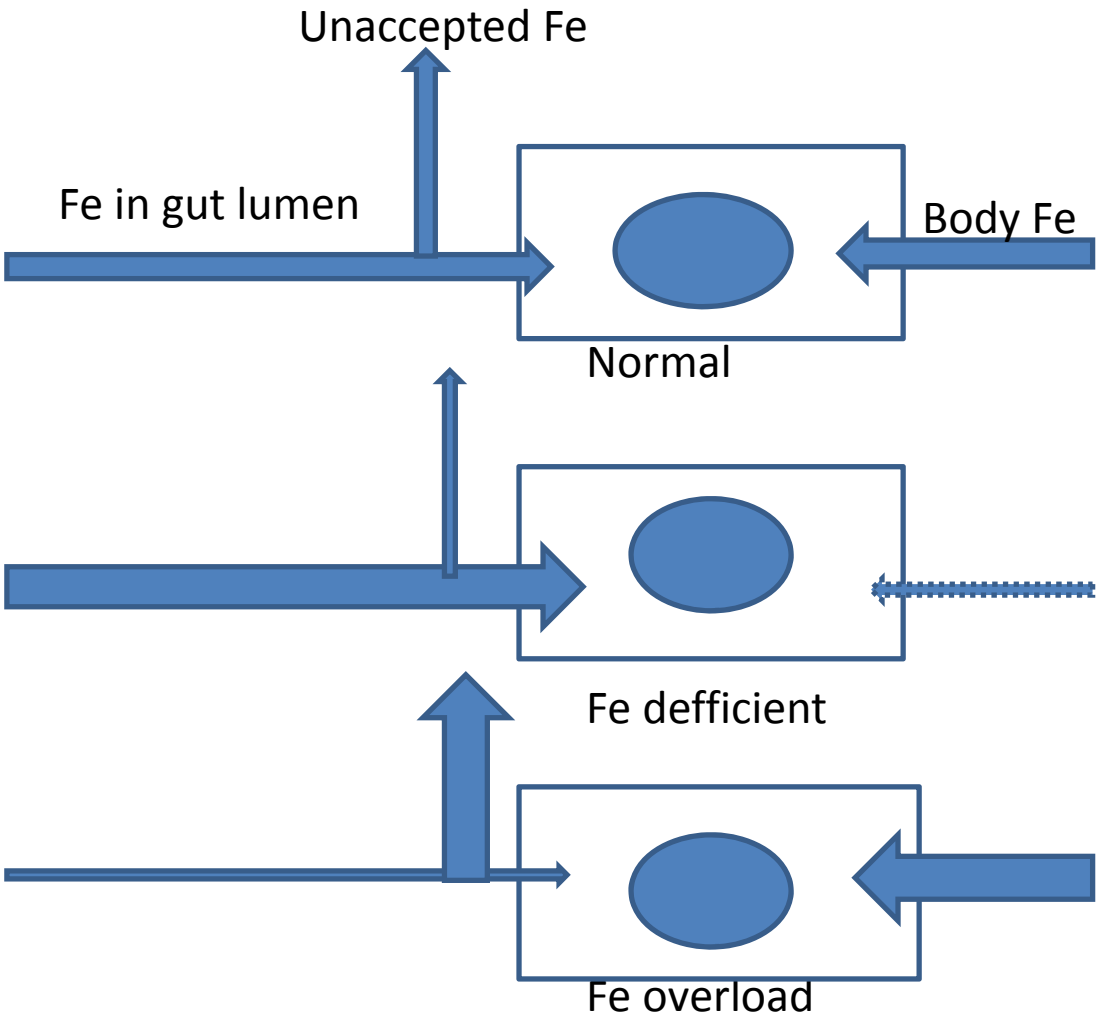
- Heme iron
- Non heme iron

2 factors determine absorptive rate

1. Amount of storage iron
2. Rate of erythropoiesis

Mucosal block theory

Modified mucosal block theory



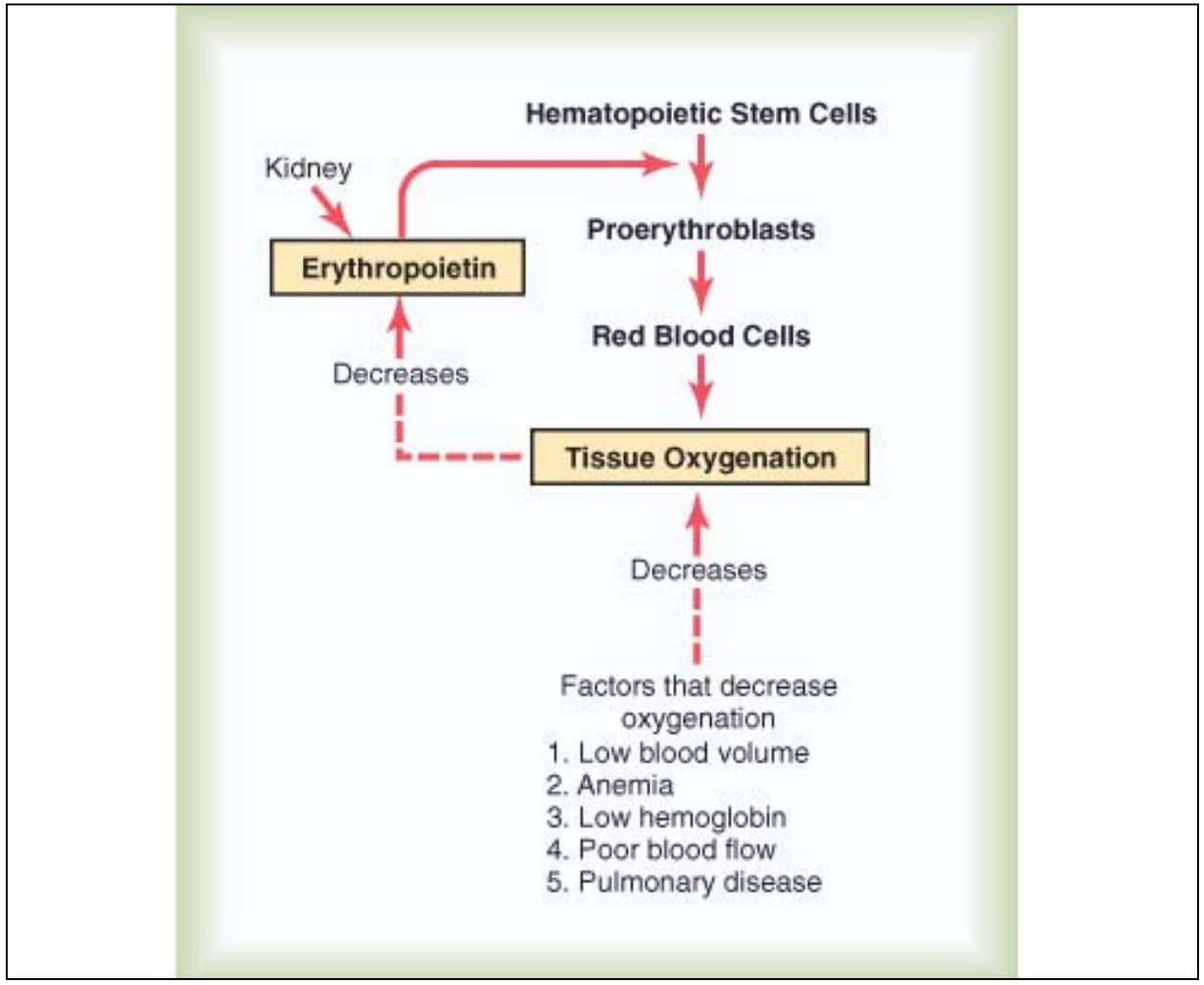
Copper: Promotes absorption, mobilization & utilization of iron

Vitamin C

Folic acid

Vitamin B12

Regulation of RBC production



Erythropoietin Mechanism

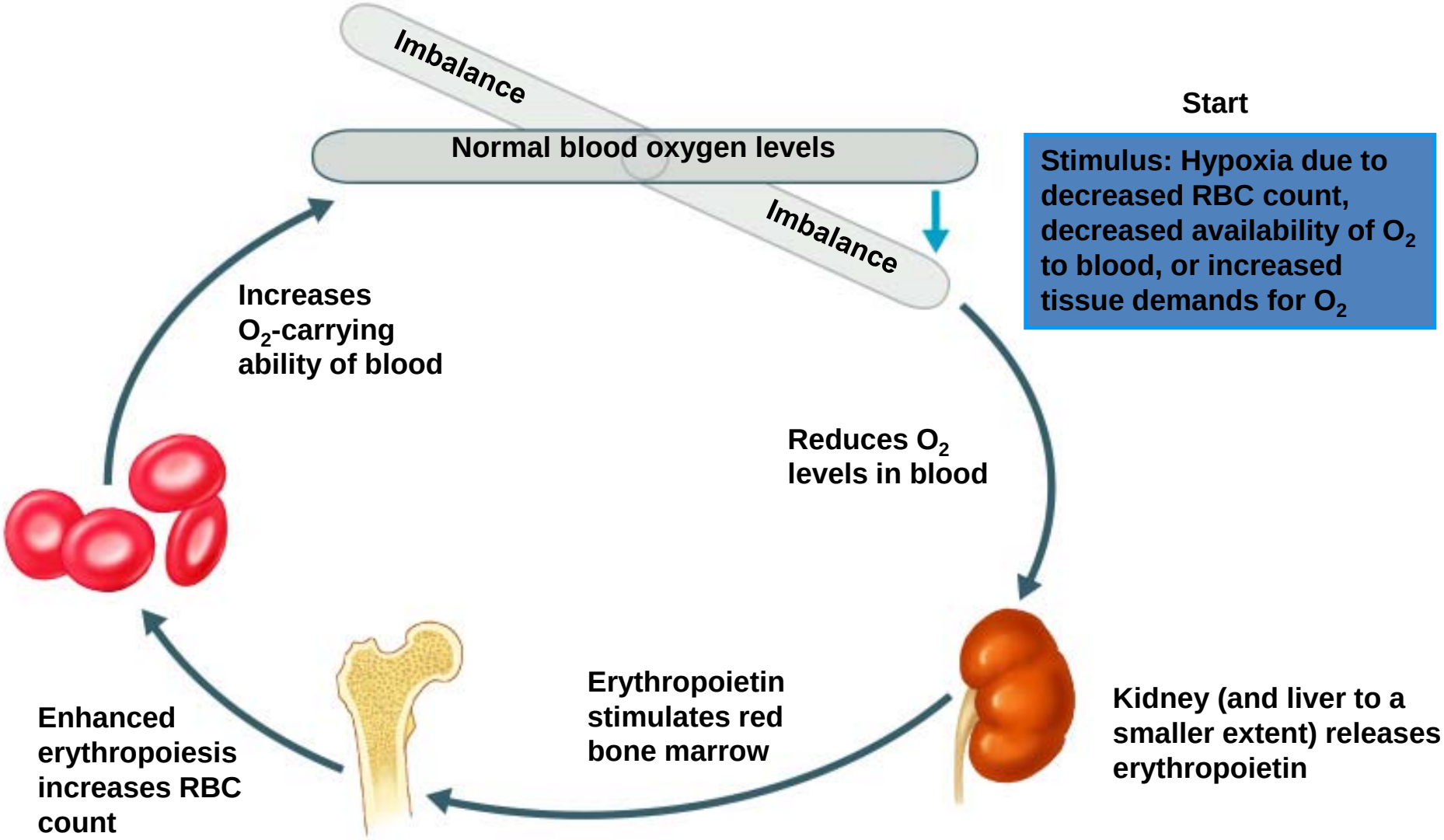


Figure 17.6

Mechanism of action of erythropoietin

Erythroid cell most sensitive to EPO is proerythroblast

EPO+ receptor

↓

↑ Ca^{++}

↑ Intracellular cAMP, cGMP

↑ Tyrosine specific protein kinase

↑ Phosphatidylinositol

↑ Protein kinase C

Erythropoietin

Site of synthesis

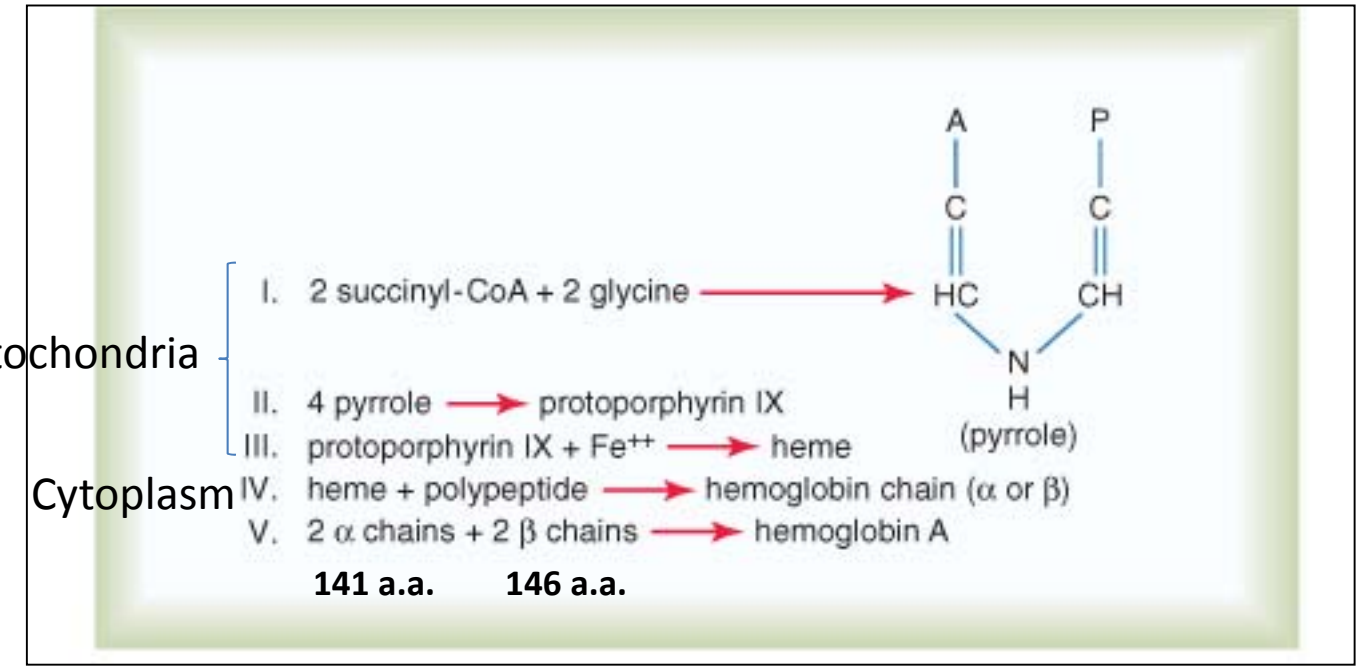
90% kidney, interstitial cells in the peritubular capillaries

10% liver, Perivenous hepatocytes

Factors influencing erythropoietin production

- Hypoxia:Hypobaric, bleeding, cardio respiratory disturbance, carboxyhemoglobin
- Vasoconstrictors: 5-HT, PG E1(By inducing renal hypoxia)
- Nucleotides: cAMP, NAD, NADPH
- Products of RBC destruction
- Hormones: Androgens, ACTH, TSH, GH, Prl, PTH

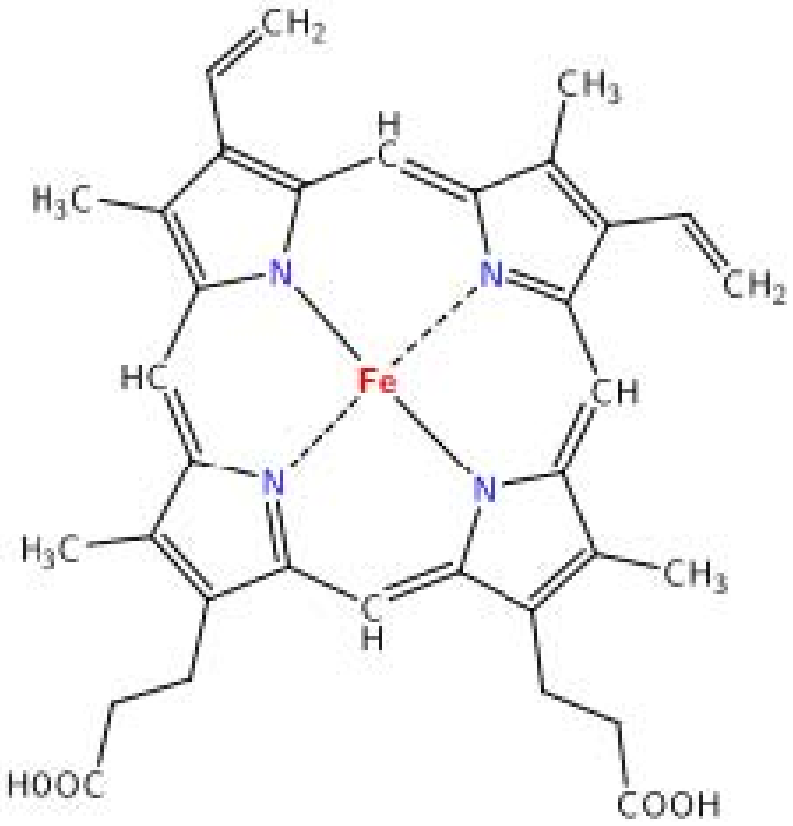
Formation of hemoglobin



Characteristics of oxygen combination with hemoglobin

- Oxygen is carried in molecular form
- Forms coordinate bond with iron atom

Structure of hemoglobin



Structure of Hemoglobin

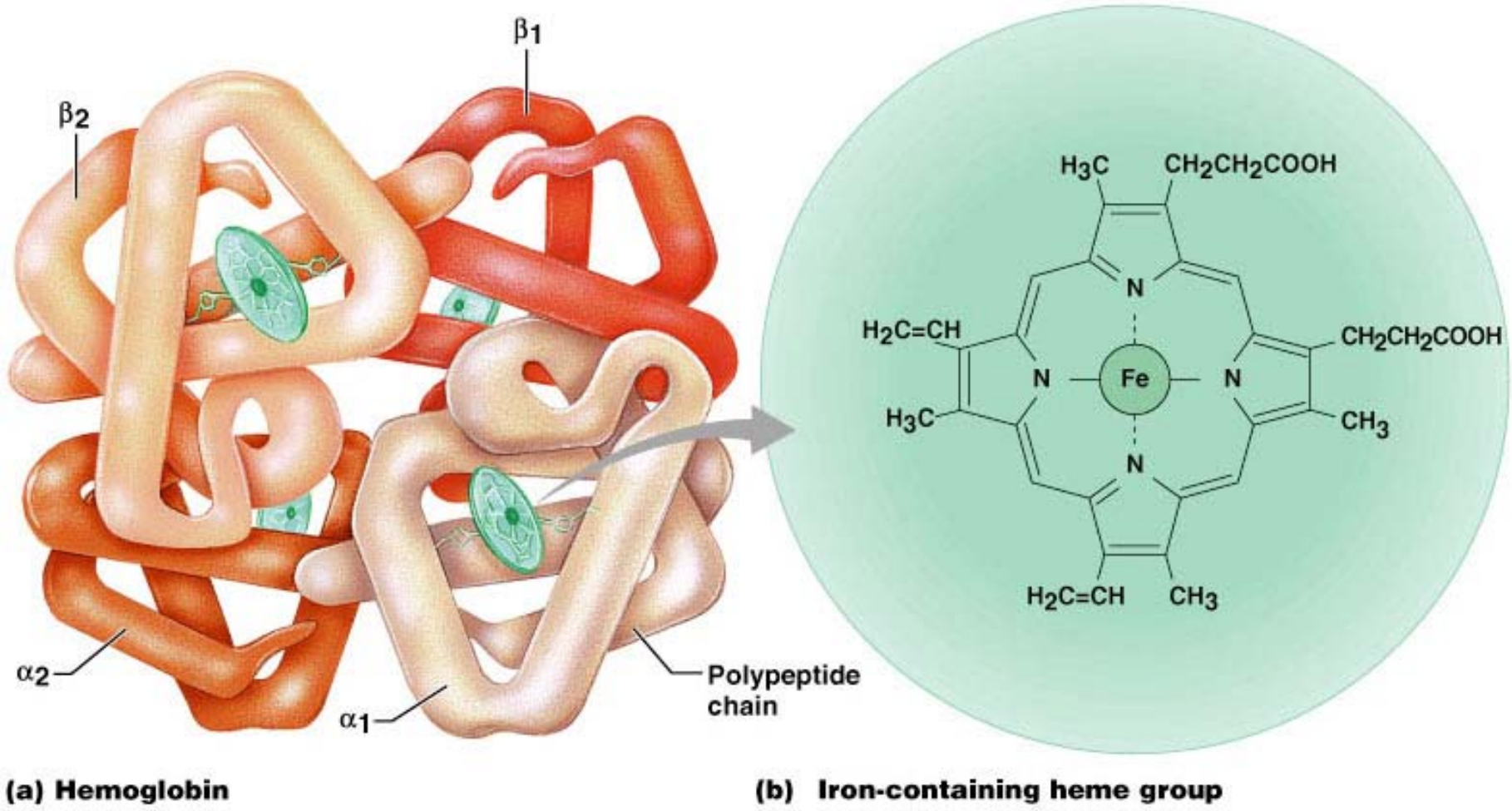
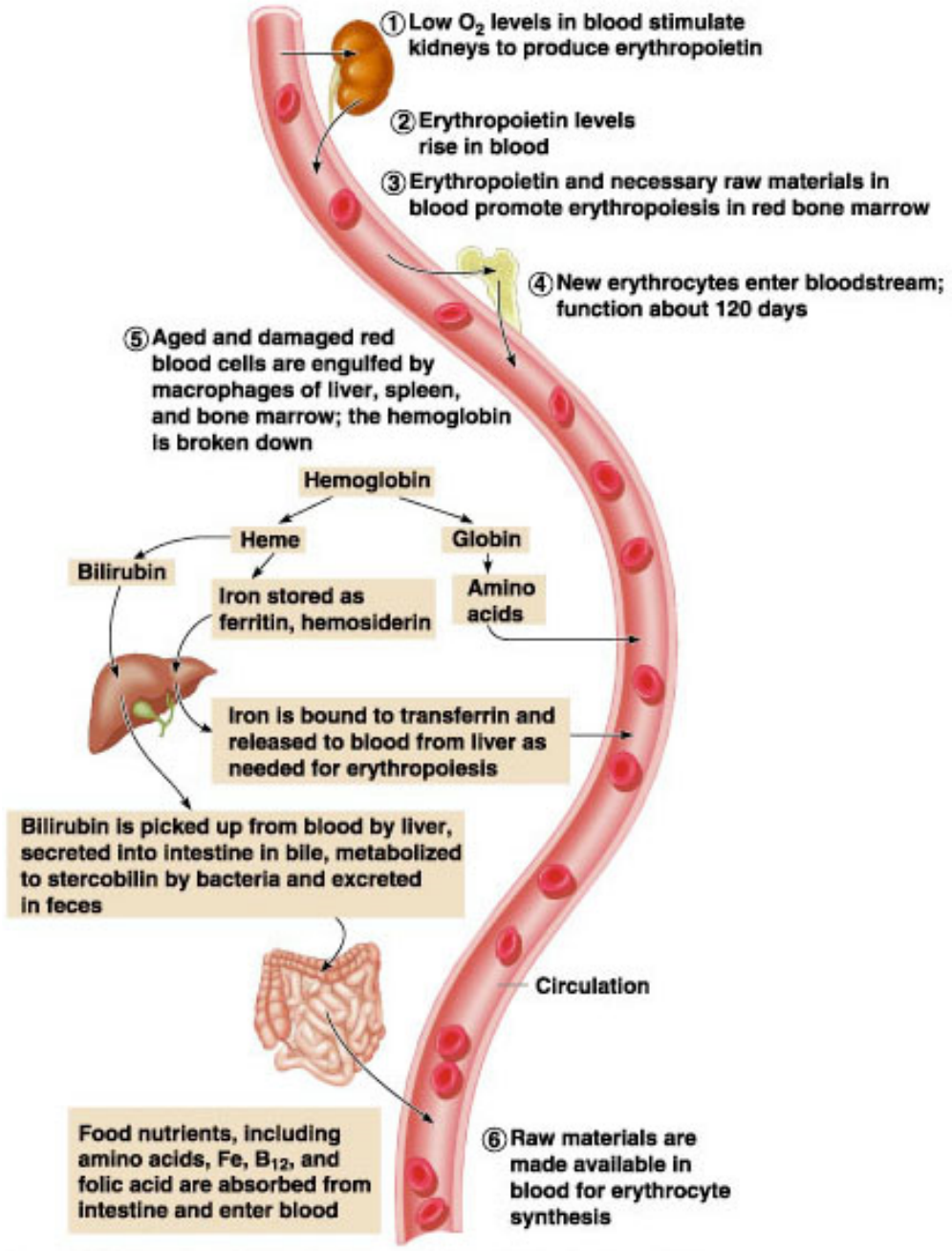
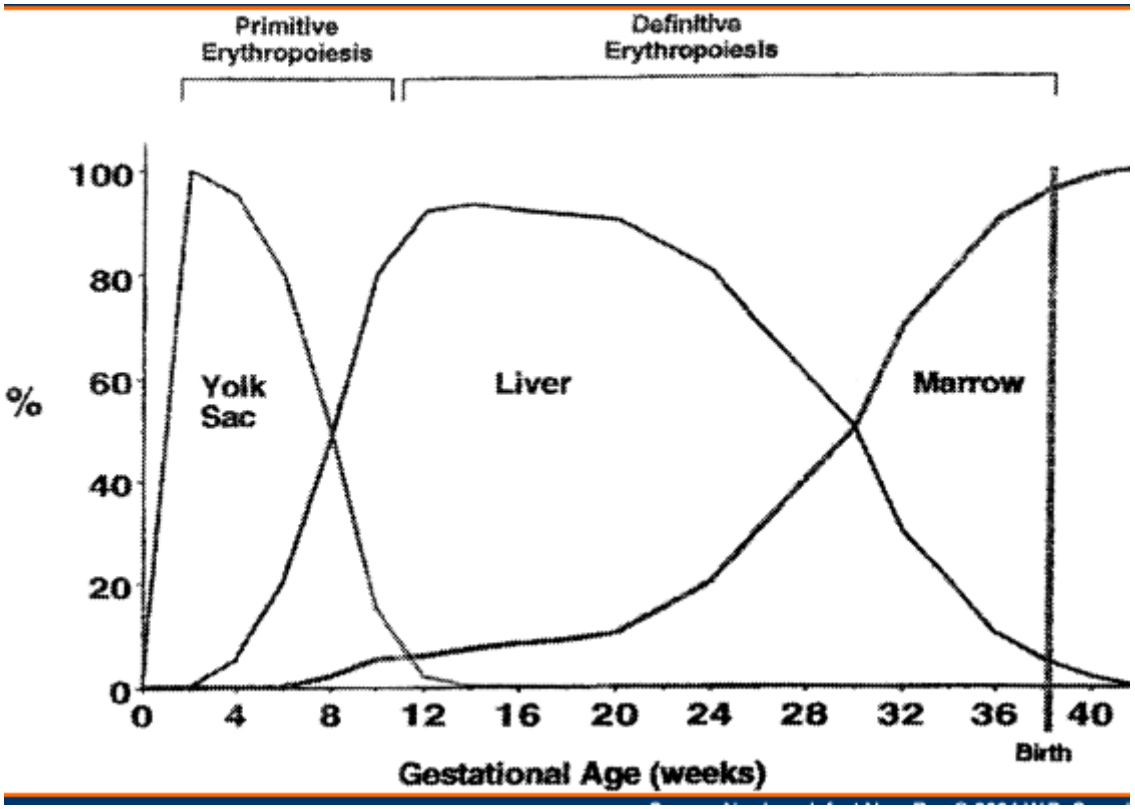


Figure 17.4

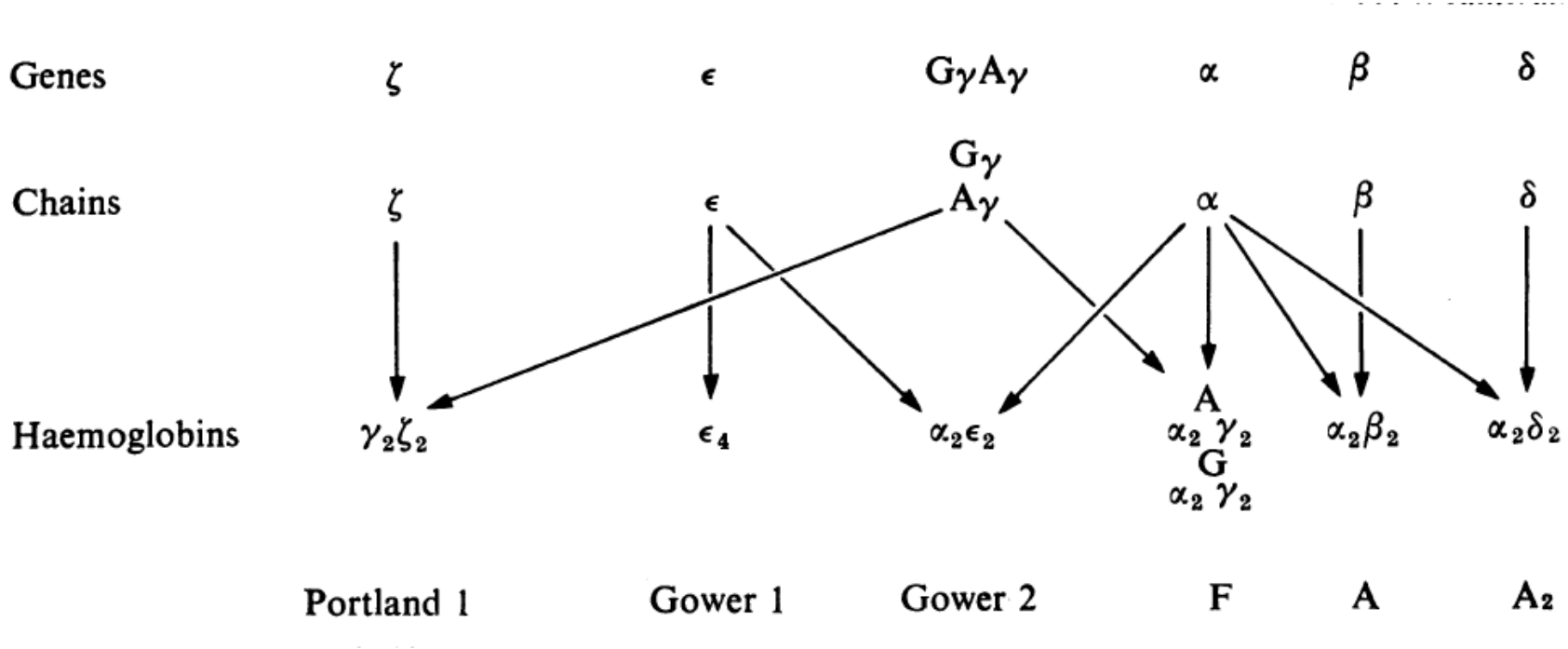
Life Cycle of Red Blood Cells



Sites of erythropoiesis



Genes regulating hemoglobin synthesis



Normal hemoglobin types

In the embryo

- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$)
- Hemoglobin Portland ($\zeta_2\gamma_2$)

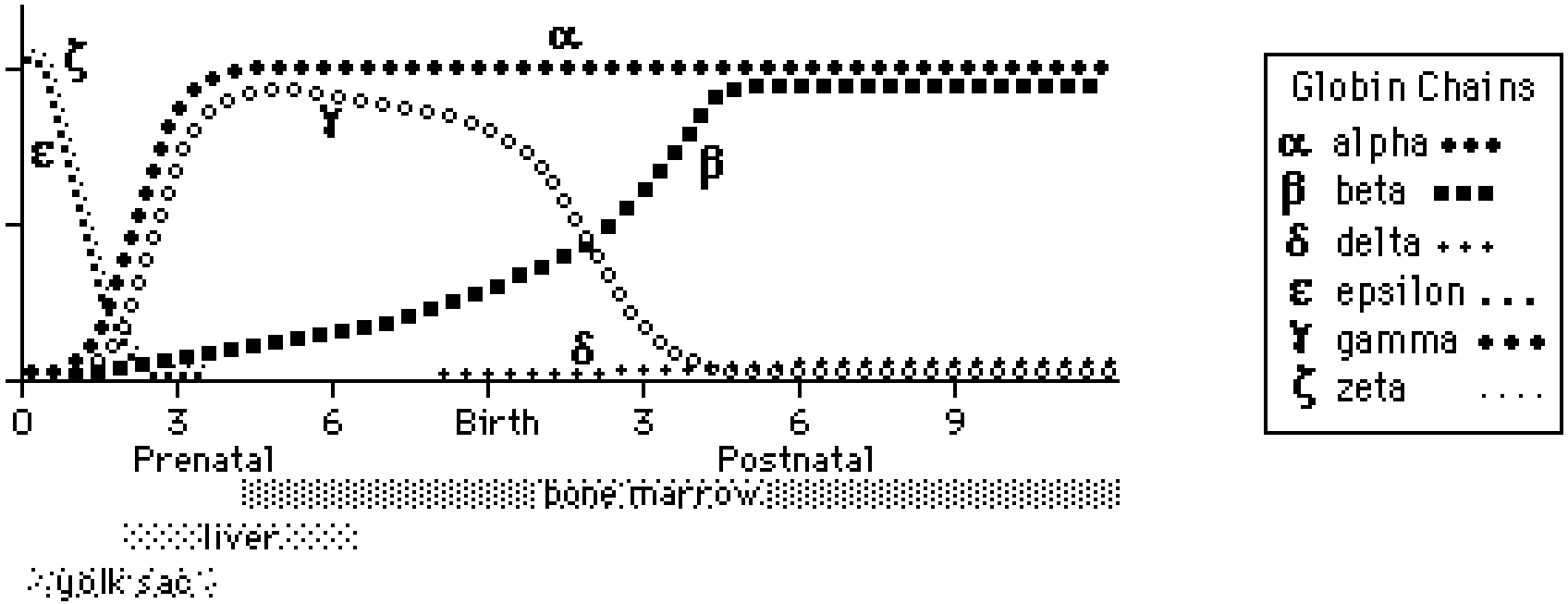
In the fetus

- Hemoglobin F ($\alpha_2\gamma_2$)

In adults:

- Hemoglobin A ($\alpha_2\beta_2$) The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells

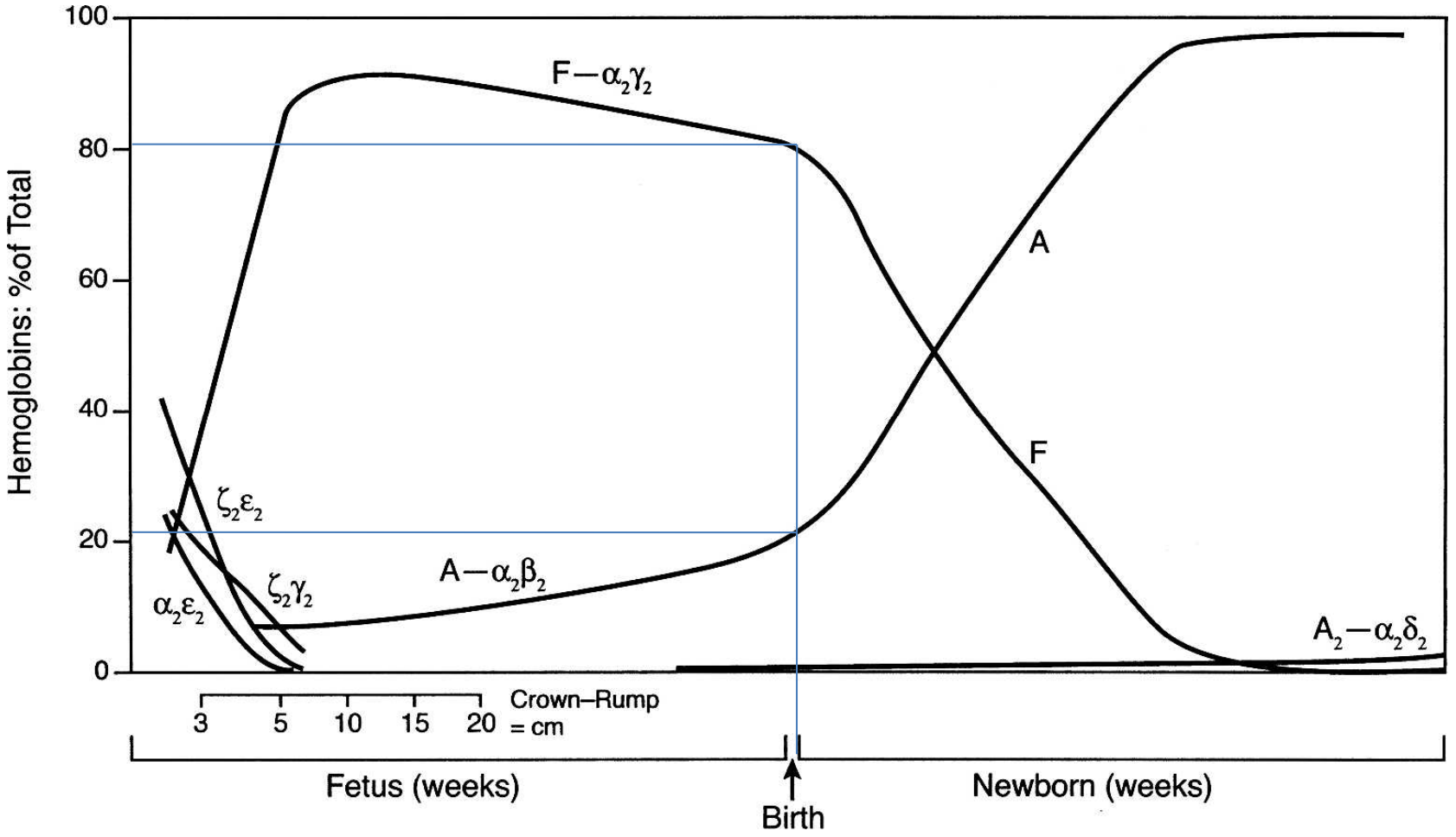
  Elevated in persons with sickle-cell disease.


ζ
ε
γ
α
β
δ
0
3
6
Birth
3
6
9
Prenatal
Postnatal
bone marrow
liver
yolk sac
Globin Chains
α alpha
β beta
δ delta
ε epsilon
γ gamma
ζ zeta

Time course of appearance of different hemoglobins

- Gower 1 ($\zeta_2\varepsilon_2$): First 3 months of embryo
- Gower 2 ($\alpha_2\varepsilon_2$): Most important embryonic hemoglobin, first 3 months
- Hemoglobin Portland ($\zeta_2\gamma_2$)
- Hemoglobin F ($\alpha_2\gamma_2$): Appears in 5th week of IUL peaks at 7th month(95%), at birth (80%), by 6 months totally replaced
- Hemoglobin A_1 ($\alpha_2\beta_2$): Appears in 5th month of IUL
- Hemoglobin A_2 ($\alpha_2\delta_2$): makes up 3 % of adult hb

Developmental profile of hemoglobins



Variant forms of hemoglobin which cause disease

- Hemoglobin H (β_4) - tetramer of β chains, which may be present in variants of α thalassemia
- Hemoglobin S ($\alpha_2\beta^S_2$) - β-chain gene, causing a change in the properties of hemoglobin which results in sickling of red blood cells.
- Hemoglobin C($\alpha_2\beta^C_2$) - Variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin AS - A heterozygous form causing Sickle cell trait with one adult gene and one sickle cell disease gene
- Hemoglobin SC disease - Another heterozygous form with one sickle gene and another encoding Hemoglobin C.

Hemoglobin types

- HbA IC: Glycated hemoglobin(Glucose attached to terminal valine in each beta chain
- Meth Hb: Fe^{++} changed to Fe^{+++}
- Carboxy hemoglobin

Differences between adult and fetal hemoglobin

Age related changes in RBC

[1] Increased membrane bound IgG
[2] Increased cell density
[3] Increased intracellular sodium
[4] Decrease enzyme activity
[5] Decrease hb affinity for oxygen
[6] Decreased cell cholesterol
[7] Changes in MCHC and MCV
[8] Cell becomes more spherical
[9] Increased intracellular viscosity
10] Increased methemoglobin
[11]Decrease intracellular potassium
[13]Decrease in sialic acid

Mechanism of red cell destruction

Averege life span 120 days

4 major mechanisms of destruction

1. Osmotic lysis
2. Erythrophagocytosis
3. Complement induced cytolysis
4. Fragmentation

Heme oxygenase system responsible for hemoglobin degradation is located in the phagocytic cells of Liver, Spleen& Bone marrow

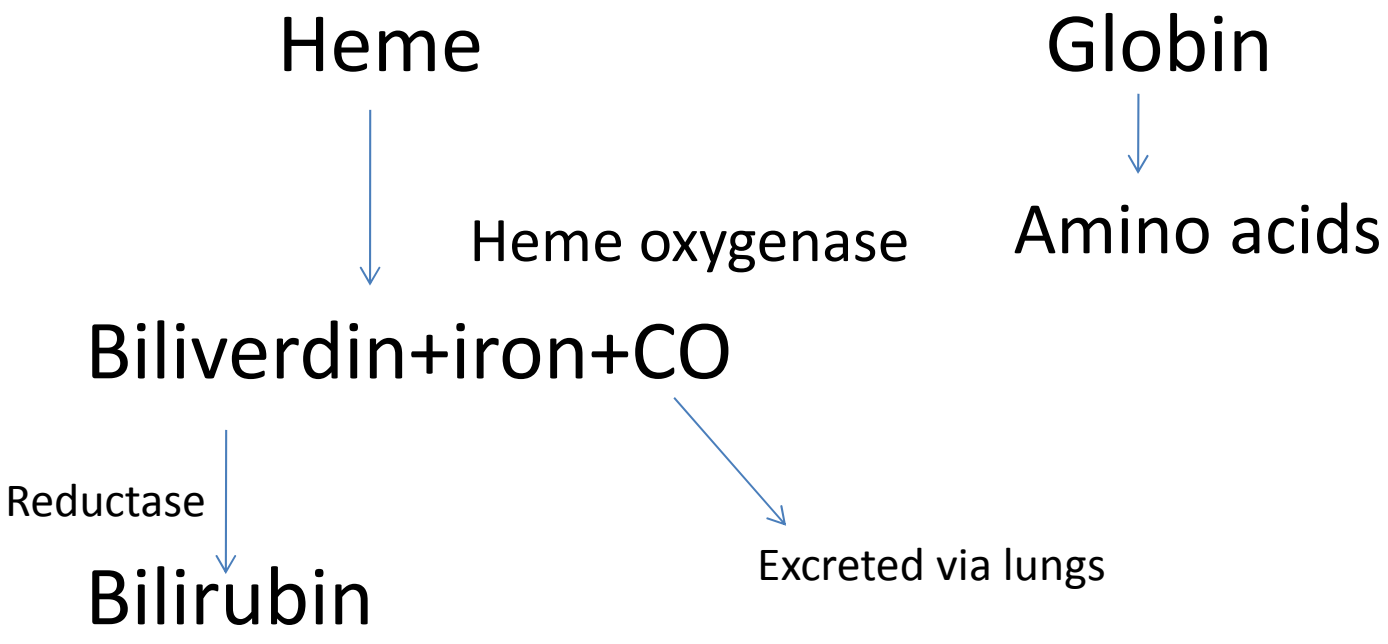
Sites of erythrocyte destruction

- Extravascular hemolysis(80-90 %)

  Spleen, Liver, Macrophages, Lymph node and Bone marrow
- Intravascular hemolysis

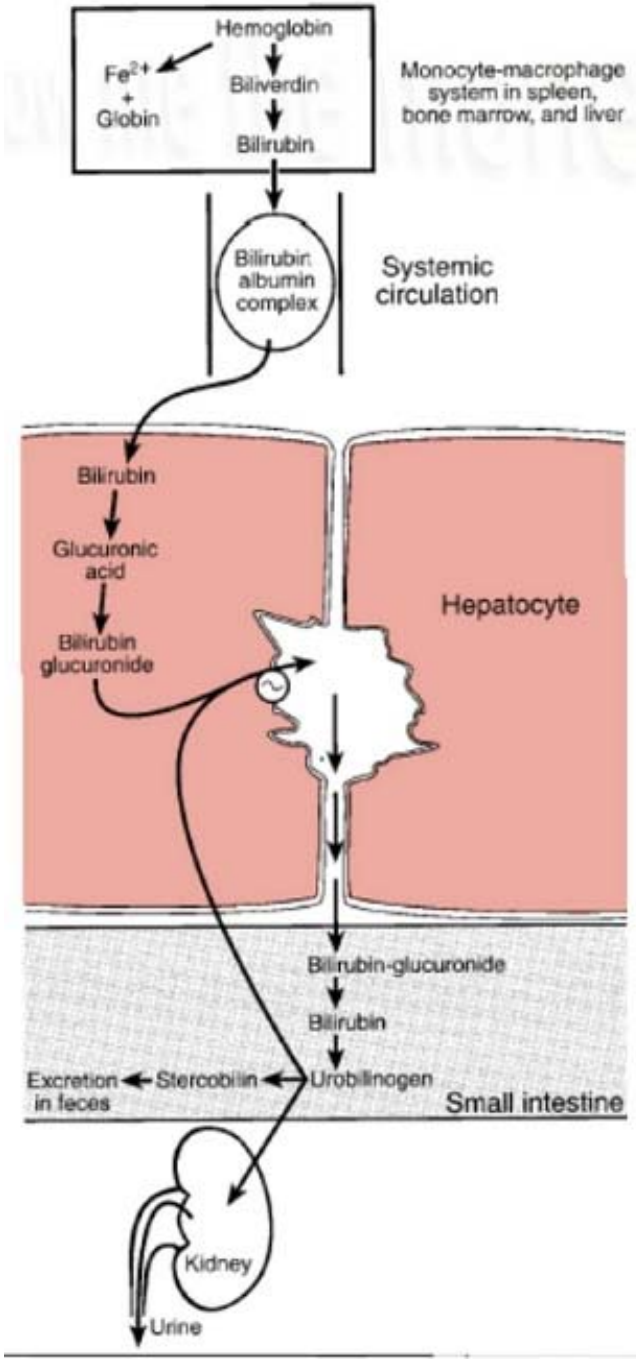
  Hemoglobin is discharged directly into the circulation & is removed by several mechanisms

Hemoglobin catabolism



(Plasma levels: <1mg/dl)

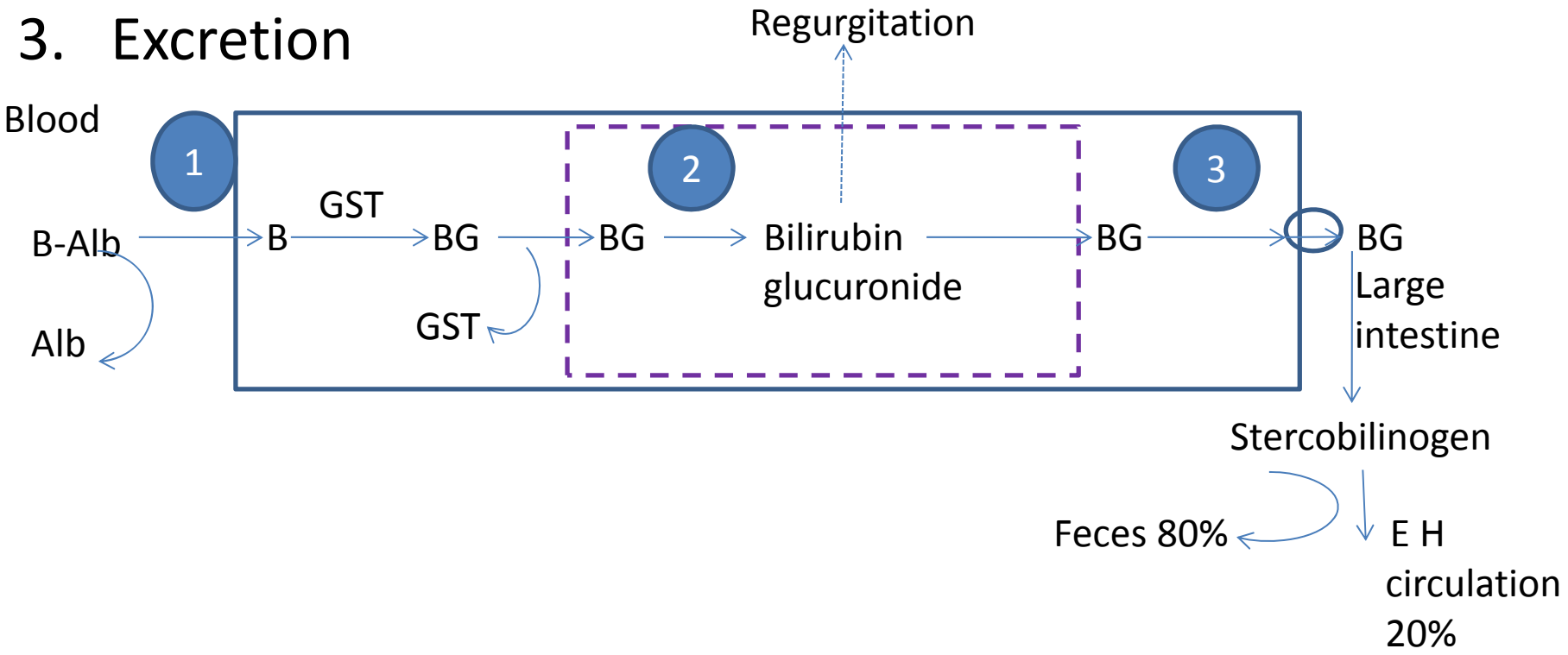
Conjugated/Unconjugated


Hemoglobin
Fe^{2+}
+
Globin
Biliverdin
Bilirubin
Monocyte-macrophage system in spleen, bone marrow, and liver
Bilirubin albumin complex
Systemic circulation
Bilirubin
Glucuronic acid
Bilirubin glucuronide
Hepatocyte
Bilirubin-glucuronide
Bilirubin
Excretion in feces
Stercobilin
Urobilinogen
Small intestine
Kidney
Urine

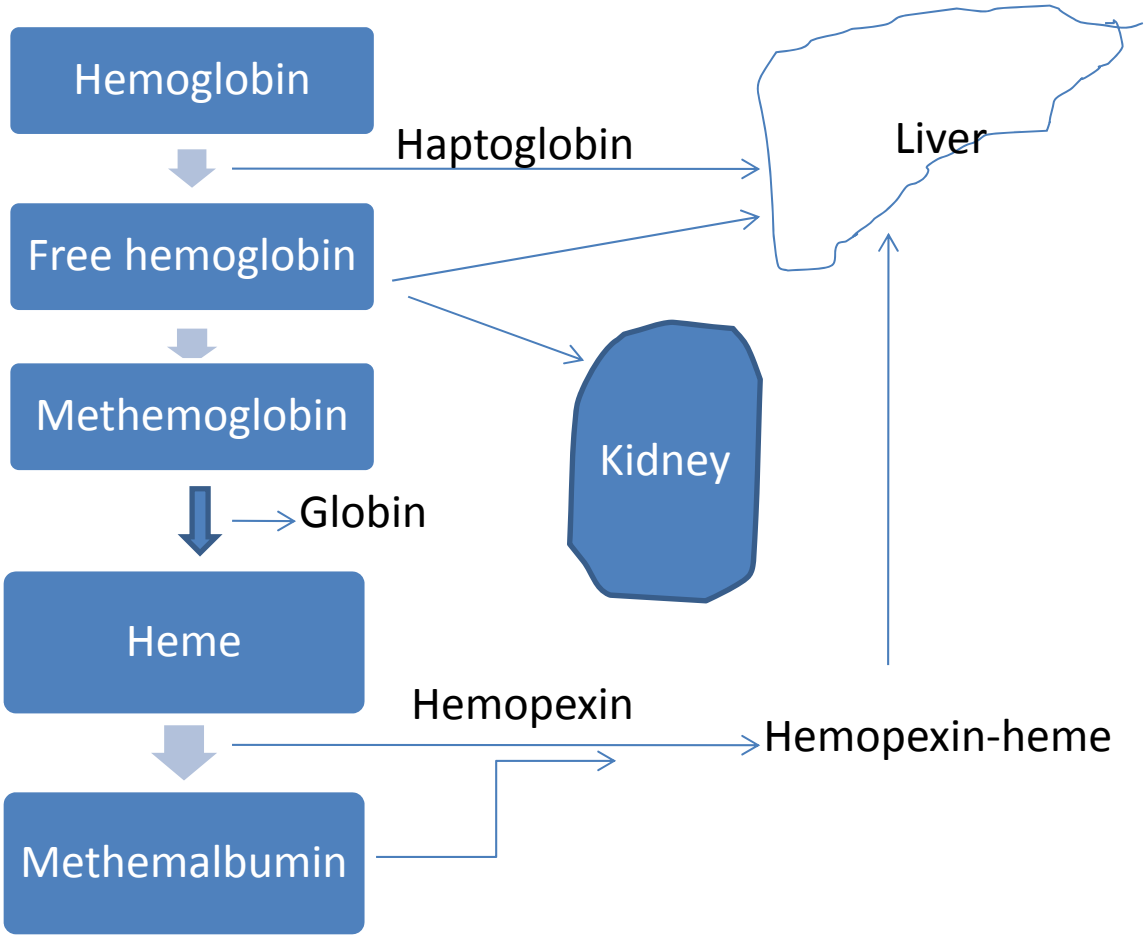
Hepatic handling of bilirubin

3 steps

1. Uptake
2. Conjugation
3. Excretion



Hemoglobin catabolism: Intravascular



Jaundice

Definition : Yellowish discolouration of skin and eyes due to an elevation in the concentration of bilirubin in blood

Clinically detected only when bilirubin > 2.5 mg/dL

First site where it is detected : sclera

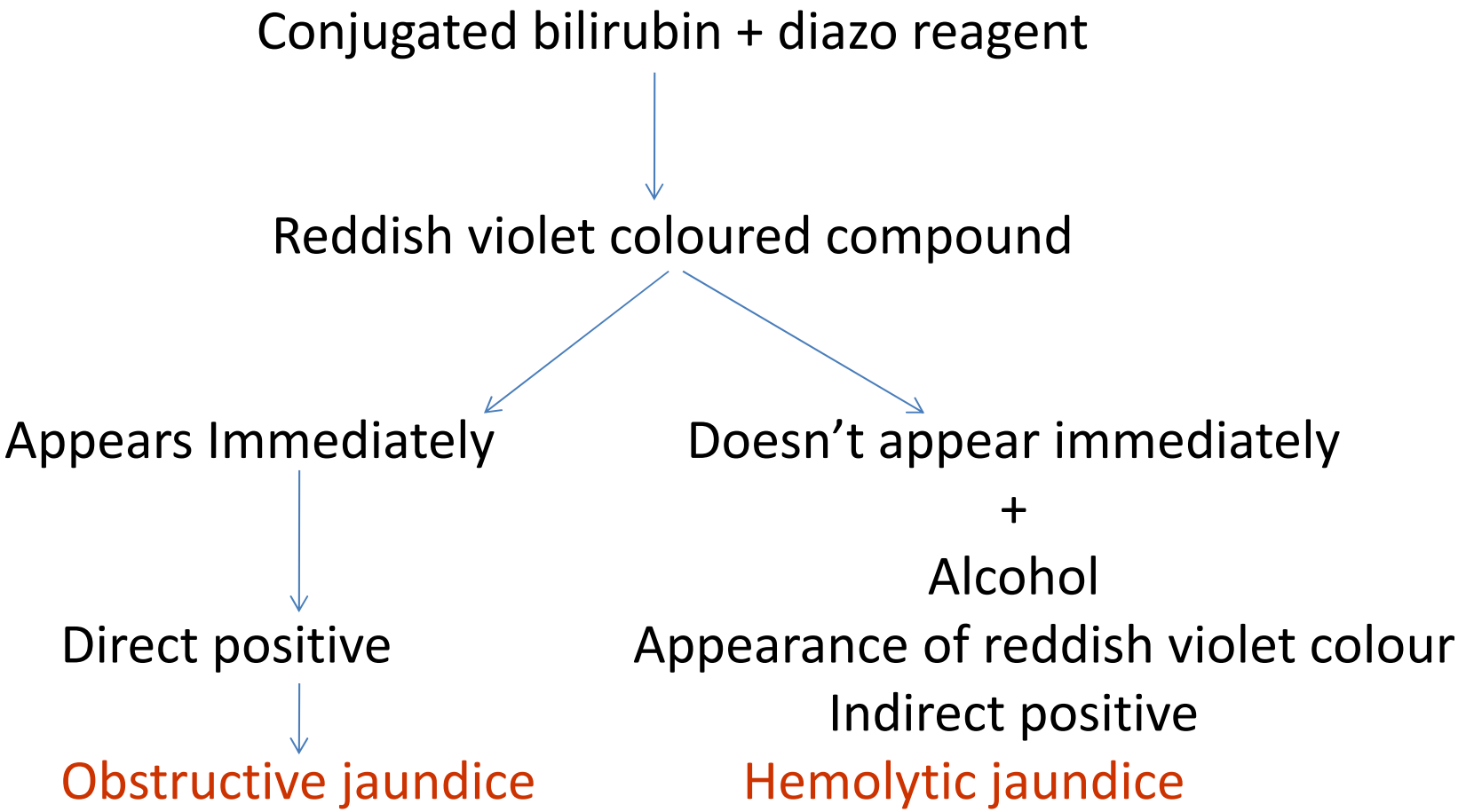
Types:

- Hemolytic
- Hepatic
- Obstructive

Types of jaundice

	Jaundice		
	Hemolytic	Hepatic	Obstructive
Fecal stercobilinogen	Increased	Decreased	Absent
Urinary urobilinogen	Increased	Decreased	Absent
Urinary bilirubin	Absent	Present	Present
Liver function test	Normal	Impaired	May be impaired

Van den Berg test: Principle



	Jaundice		
	Hemolytic	Hepatic	Obstructive
Fecal stercobilinogen	Increased	Decreased	Absent
Urinary urobilinogen	Increased	Decreased	Absent
Urinary bilirubin	Absent	Present	Present
Liver function test	Normal	Impaired	May be impaired
Van den Bergh test	Indirect	Biphasic	Direct

Physiological jaundice

Neonatal jaundice

More common in premature & LBW babies

Appears on 2-3rd day of life

Disappears within a week

Rarely exceeds 12 mg/dL

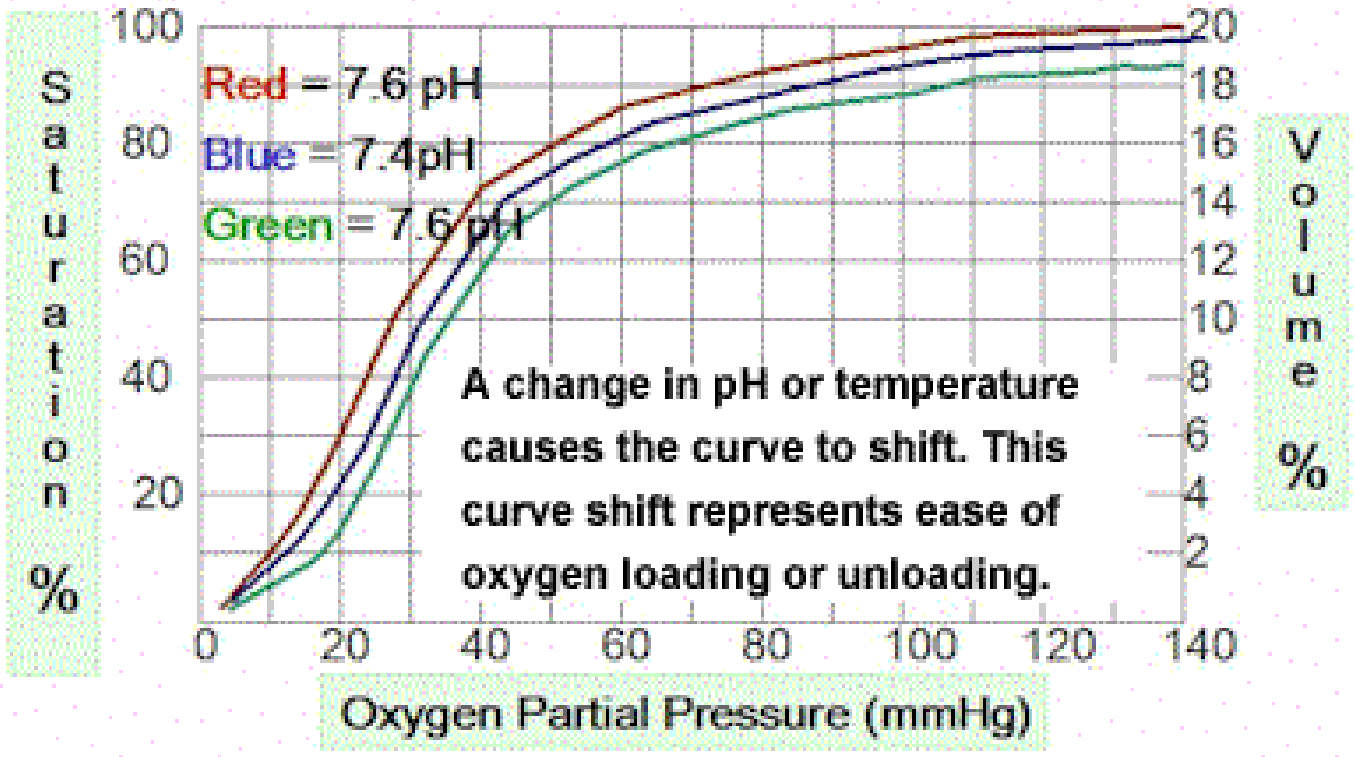
Cause: immaturity of Liver function

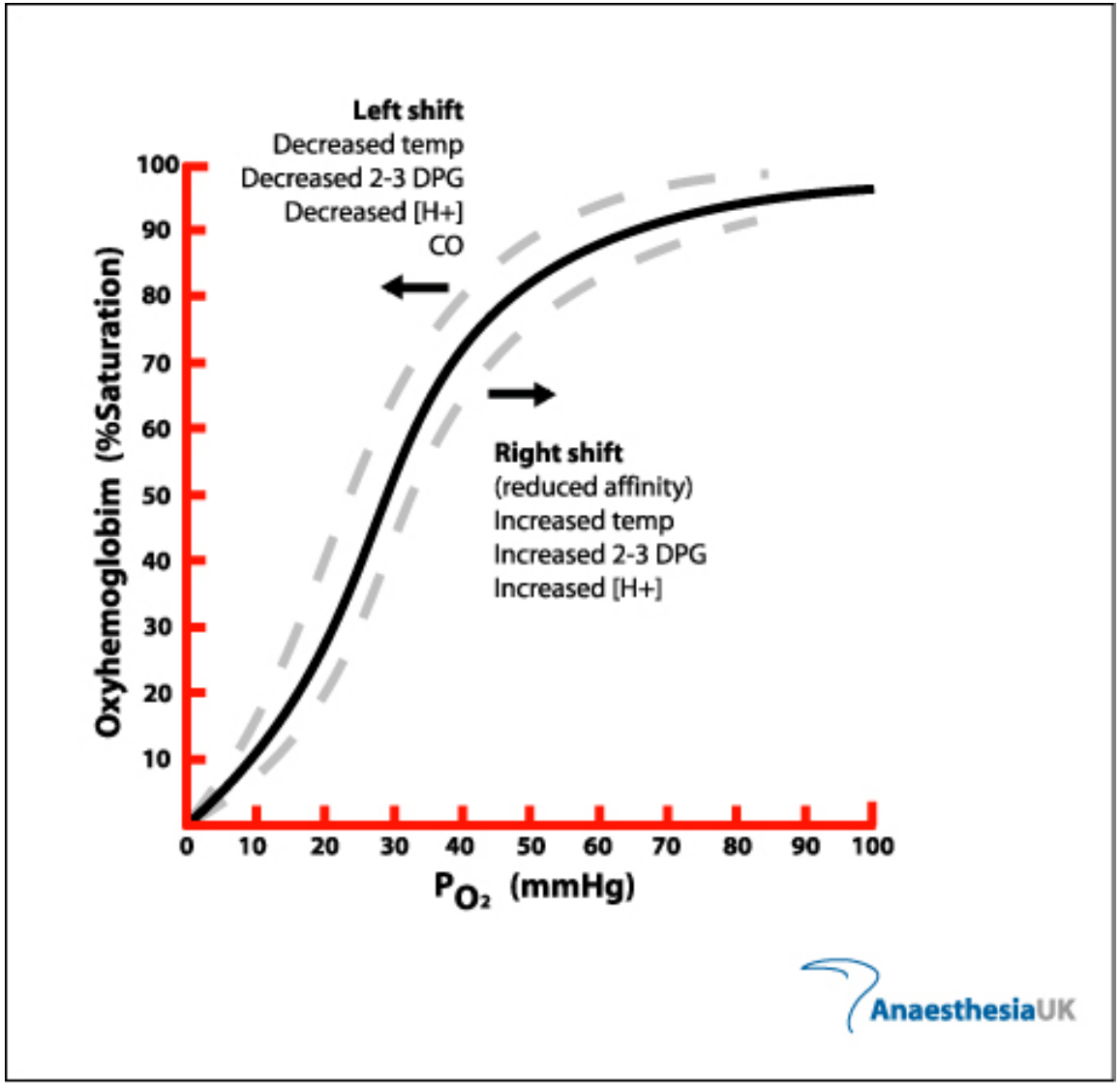
In utero the bilirubin formed is excreted mainly by the placenta

Oxygen transport by blood

- Dissolved oxygen is consumed first by cells in organs and tissues.
- Heme-bound oxygen, which begins a sequential unloading of its four oxygen molecules.
- During oxygen unloading, the hemoglobin tetramer undergoes intramolecular conformational changes called cooperativity.
- Once the first oxygen has been unloaded, the unloading of the second oxygen is facilitated. The second oxygen can dissociate after a much smaller change in oxygen pressure than was needed to unload the first. Another conformational change facilitates dissociation of the third oxygen.
- Cooperativity is an important phenomenon that permits the loading and unloading of large amounts of oxygen at physiologically relevant oxygen pressures.

Oxyhemoglobin Dissociation Curve




Left shift
Decreased temp
Decreased 2-3 DPG
Decreased [H+]
CO
Right shift
(reduced affinity)
Increased temp
Increased 2-3 DPG
Increased [H+]
Oxyhemoglobim (%Saturation)
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
P_{O_2} (mmHg)
AnaesthesiaUK

Physiological significance of the shape of the oxygen dissociation curve

Flat upper part

The flat upper part acts as a buffer in the sense that the pO2 can drop to about 80 mmHg and yet the haemoglobin will still remain highly saturated (96%) with oxygen. This keeps the arterial oxygen concentration high despite impairment in saturation in the lung.

Steep lower part

If the tissues require more oxygen, substantial amounts of oxygen can be removed from haemoglobin without much further drop in pO2. The pressure gradient for diffusion of oxygen from capillary to cell tends to be relatively well maintained despite the much increased oxygen extraction

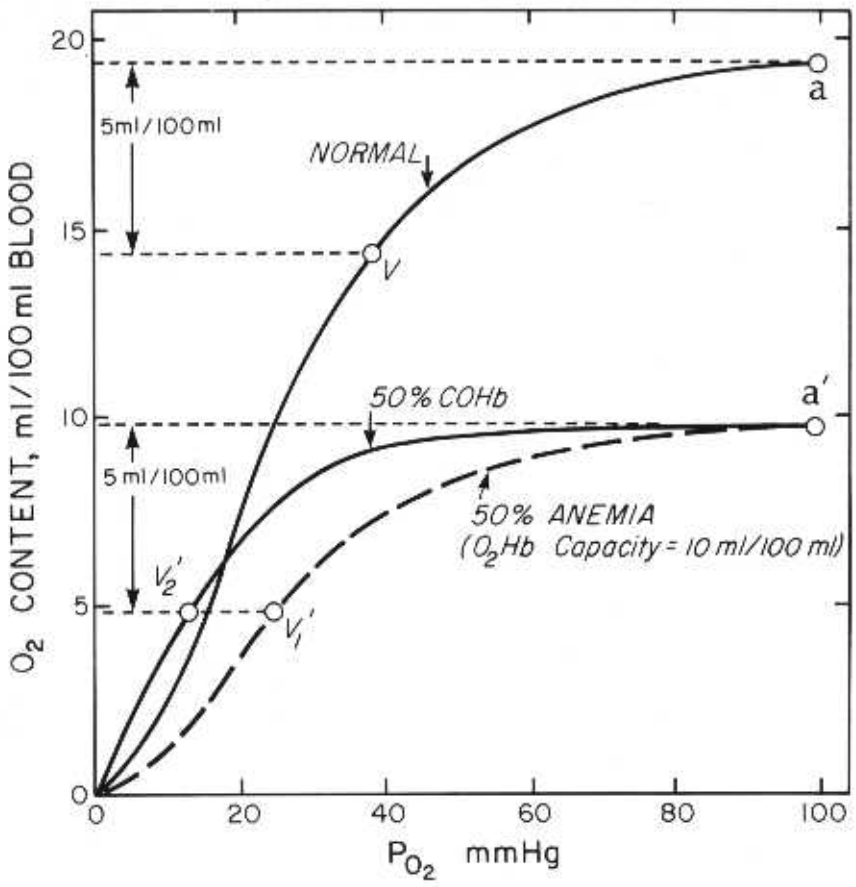
Physiological significance of the shape of the oxygen dissociation curve

Summary, the shape of the ODC provides this double buffering effect because:

- The flat upper part tends to ‘buffer’ haemoglobin saturation against a substantial drop in pO2. This is useful in the lungs to maintain the arterial haemoglobin saturation.
- The steep lower part has 2 advantages: Large O2 unloading & a maintained O2 diffusion gradient (ie the pO2 gradient from capillary to cell).

- Haemoglobin binds with CO , 240 times more readily than with oxygen.
- The presence of carbon monoxide on one of the 4 haem sites causes the oxygen on the other haem sites to bind with greater affinity.
- This makes it difficult for the haemoglobin to release oxygen to the tissues and has the effect of shifting the curve to the left
- With an increased level of carbon monoxide, a person can suffer from severe hypoxaemia while maintaining a normal pO_2.

Oxyhemoglobin dissociation curve



- When fully saturated with oxygen 1 gm of hb carries 1.34 ml of O2
- 100 ml of arterial blood contains 20 ml O2 (hb = 15 gm%)
- 100 ml of venous blood contains 15 ml O2
- 5 ml or 25% O2 extracted by tissues
- If hb is only 7.5 gm % the O2 it contains is 10 ml

Anemia

Definition

Deficiency of hemoglobin in blood as a result of

- too few RBCs(< 4million/cumm)
- too little hemoglobin
- WHO, 1992
- Hb < 7.0 g % severe anemia,
- 7.0 – 9.9 g % moderate anemia
- 10.0 – 10.9 g% mild anemia in pregnant women and 10.0 –11.9g% for non-pregnant women

Manifestations of anemia

- Reduction in oxygen carrying capacity of blood
- Degree of change in the total blood volume
- Rate of development of the above two factors
- Associated manifestations of the underlying disorder
- Capacity of the CVS and respiratory system to compensate

Mechanisms for compensation of the loss of oxygen carrying capacity

- Increase in 2,3 DPG
- Redistribution of blood flow
- Increased cardiac output

- Insidious onset

  Physiological adjustments in CVS

  Changes in oxygen hemoglobin dissociation curve
- Acute onset

  Symptoms related to acute hemorrhage

Physical signs of chronic anemia

Cardiac signs

In severe anaemia

- Hyperdynamic circulation: a fast heart rate (tachycardia), flow murmurs, and cardiac enlargement. There may be signs of heart failure
- Atrial fibrillation

Skin signs

- Pallor in the mucous membrane of the mouth,
- conjunctiva, lips & nail bed
- Skin may be pale in the absence of anemia or it may fail to appear pallid in the presence of anemia
- Loss of normal skin elasticity & tone
- Thinning, loss of lusture & early greying
- Nails lose lusture, become brittle, cholioneychia
- Chronic leg ulcers
- Glossitis
- Fissures at the angles of mouth
- Jaundice in haemolytic anaemia
- Bone deformities (found in thalassaemia major) or

Neuromuscular signs

Severe anemia

Headache, vertigo, tinnitus, fainting, scotomas, lack of mental concentration, drowsiness, restlessness, muscular weakness

Paresthesia

GIT signs

Glosssitis & atrophy of the papilla of the tongue

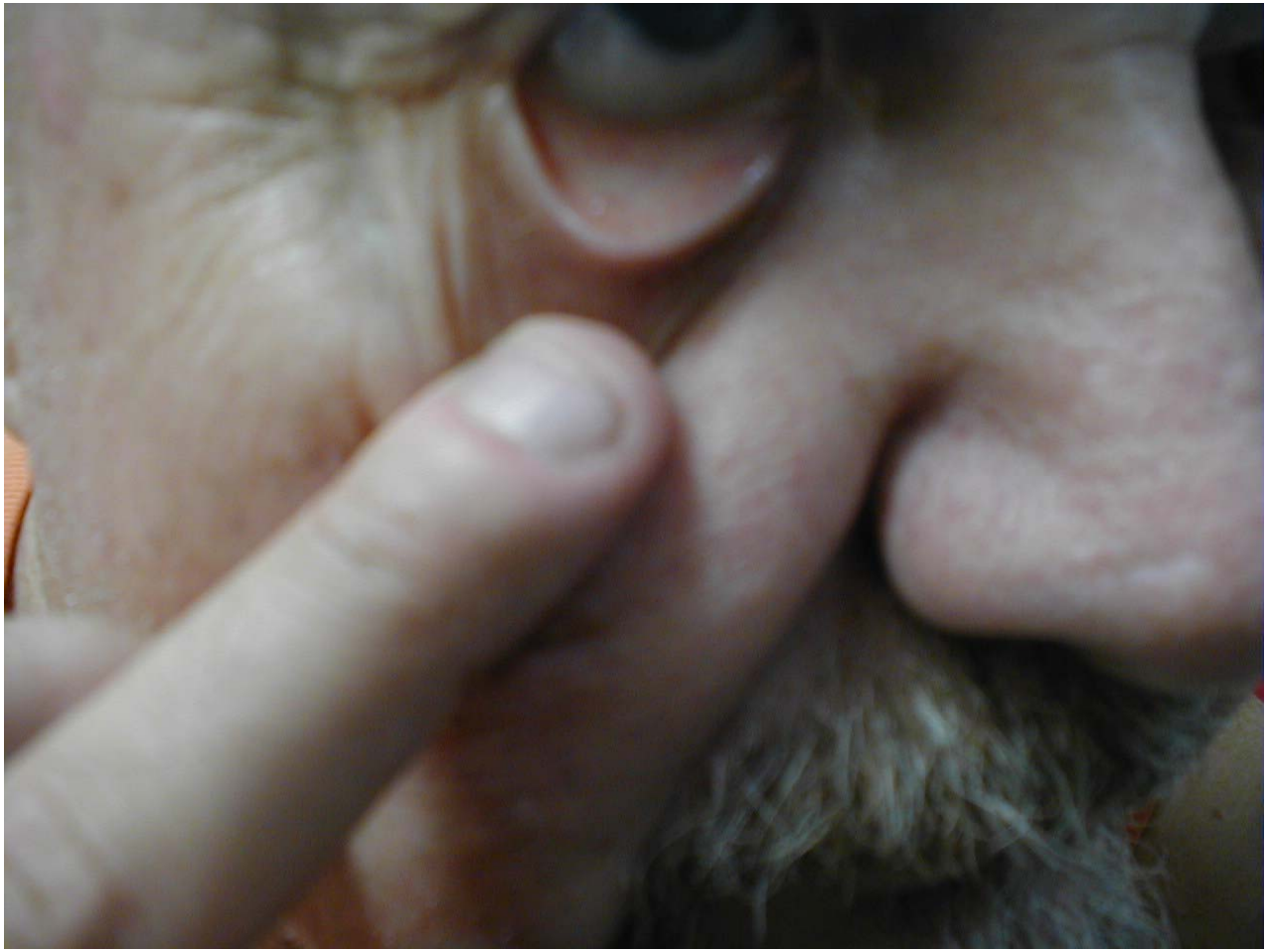
Painful ulcerative & necrotic lesions

Dysphagia

Genitourinary signs

Slight proteinuria

Microscopic hematuria



Classification of anemia: Based on underlying mechanism

- **Blood loss**
  - Acute: Trauma
  - Chronic: Lesions of GIT,hook worm infestation, gynaecological
- **Hemolytic anemia**
  - Intracorpuscular defect: Membrane defect, Enzyme defect, Hb defect
  - Extracorpuscular defect: Antibody mediated, mechanical trauma, infections
- **Impaired production**
  - Disturbance of proliferation & differentiation
  - Disturbance of proliferation & maturation

Signs

- Pallor (pale skin, mucosal linings and nail beds)
- Koilonychia(in iron deficiency),
- Leg ulcers (seen in sickle cell disease).

Morphological classification of anemia

	Normochromic	Hypochromic
Normocytic		
Macrocytic MCV > 100		
Microcytic MCV < 80		

Signs & symptoms of blood loss anemia

Acute blood loss

- >30% loss sudenly: Postural hypotension, increase heart rate
- > 40% blood loss: Hypovolumic shock, confusion, air hunger, diaphoresis, decrease hemoglobin, increase heart rate, CNS hypoxia leading to headache, dimness of vision & faintness

Chronic blood loss

- Forceful apical pulse
- Strong peripheral pulse
- Wide pulse pressure
- Mid/holosystolic murmur
- Pallor: Skin & mucous membranes

Hemolytic anemia

Intracorpuscular defect

- Membrane defect: Spherocytosis
- Enzyme defect: Pyruvate kinase def., hexokinase def.
- Hb defect: Thalassemia, sickle cell anemia

Extracorpuscular defect

- Antibody mediated: transfusion mediated, erythroblastosis
- Mechanical trauma
- Infections : Malaria

Hemolytic anemia

Clinical features

- Hemoglobinemia
- Hemoglobinuria
- Methhemoglobinemia
- Jaundice: Unconjugated hyperbilirubinemia
- Hemosiderinuria

Specific types of hemolytic anemias

Spherocytosis

Thalassemia

Sickle cell anemia

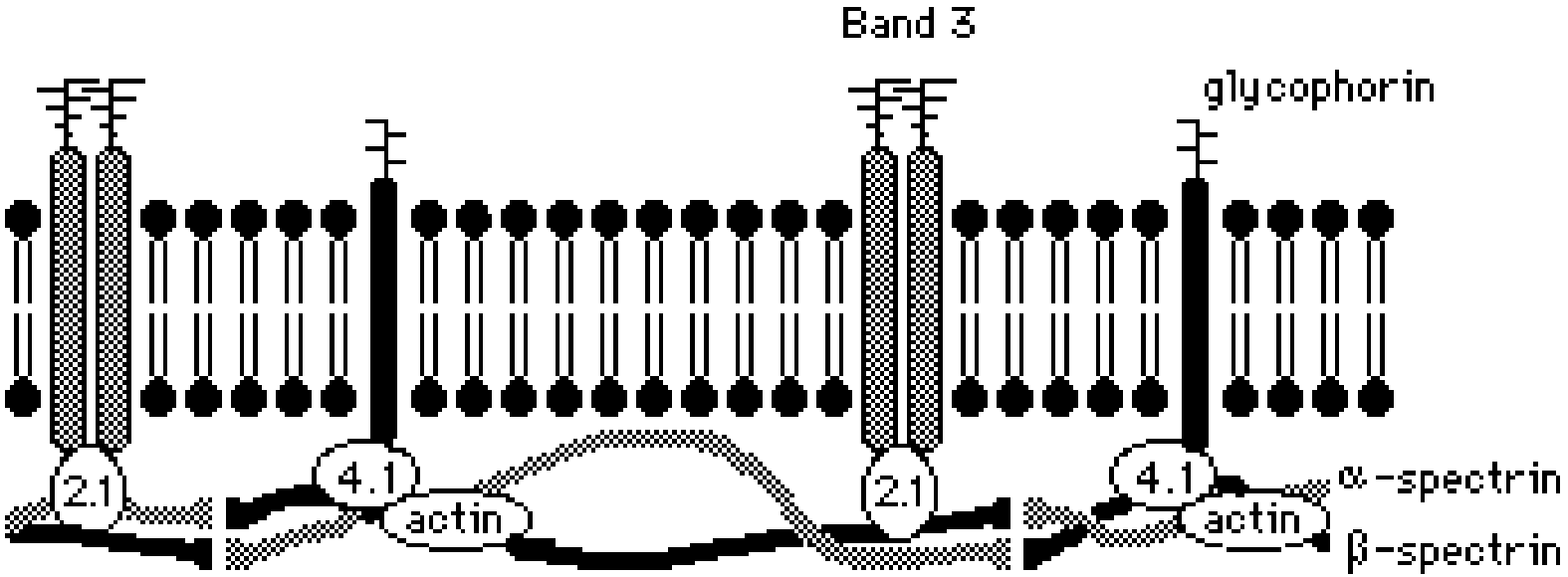
Extracorpuscular defect

Erythroblastosis

Mechanical trauma

Malaria

RBC membrane structure



Protein 4.1 binds spectrin to glycophorin C

Protein 2.2(Ankyrin) binds spectrin to anion exchanger band 3

Spherocytosis

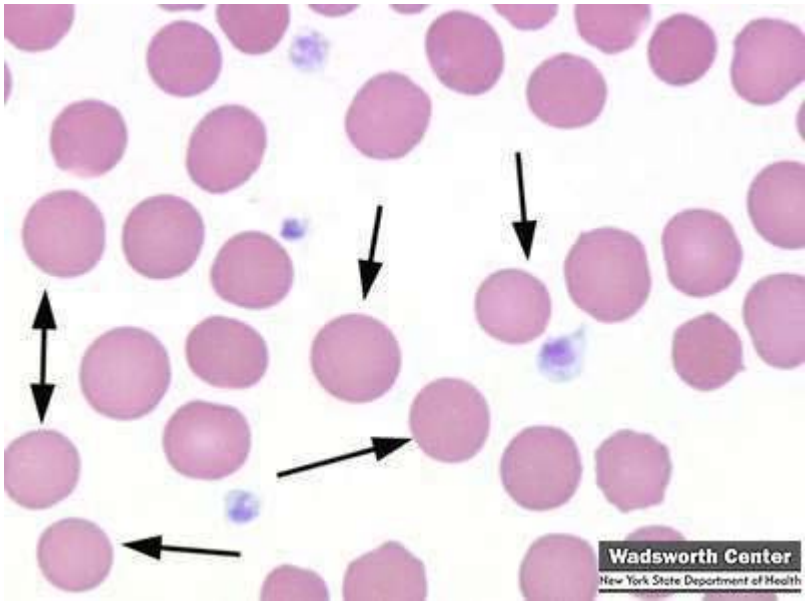
- Deficiency of spectrin due to a primary defect in
- Ankyrin gene
- Protein 3

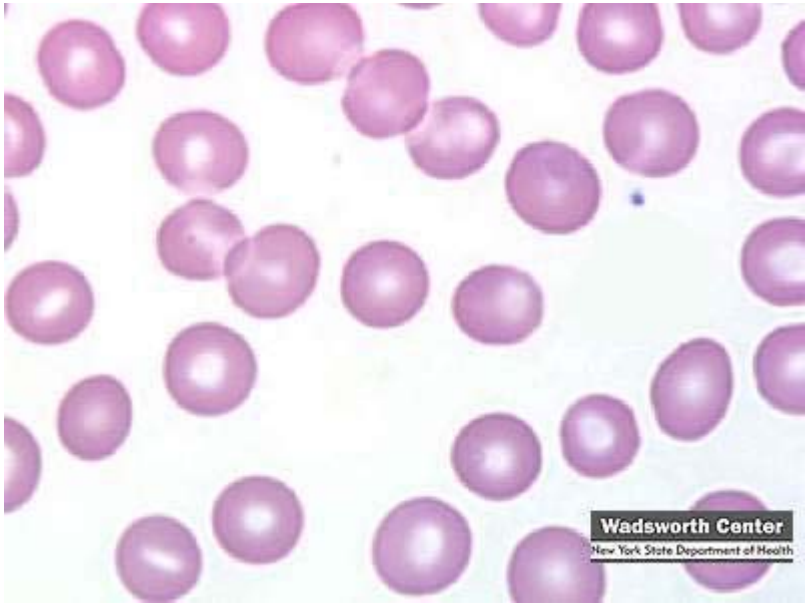
Effect: reduced membrane stability/ plasticity

Premature lysis of the cells in spleen

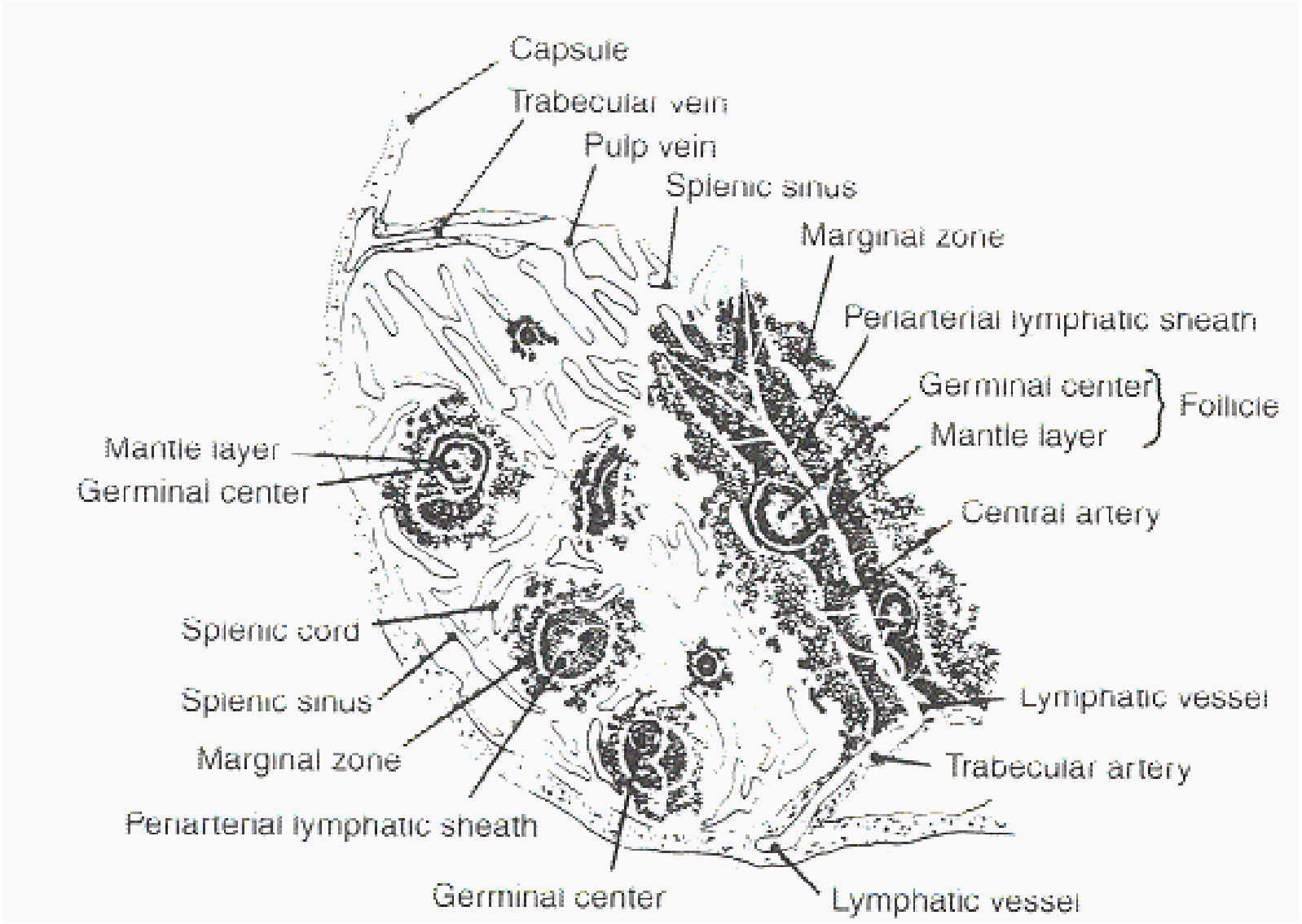
C/F: anemia, spleenomegaly & jaundice

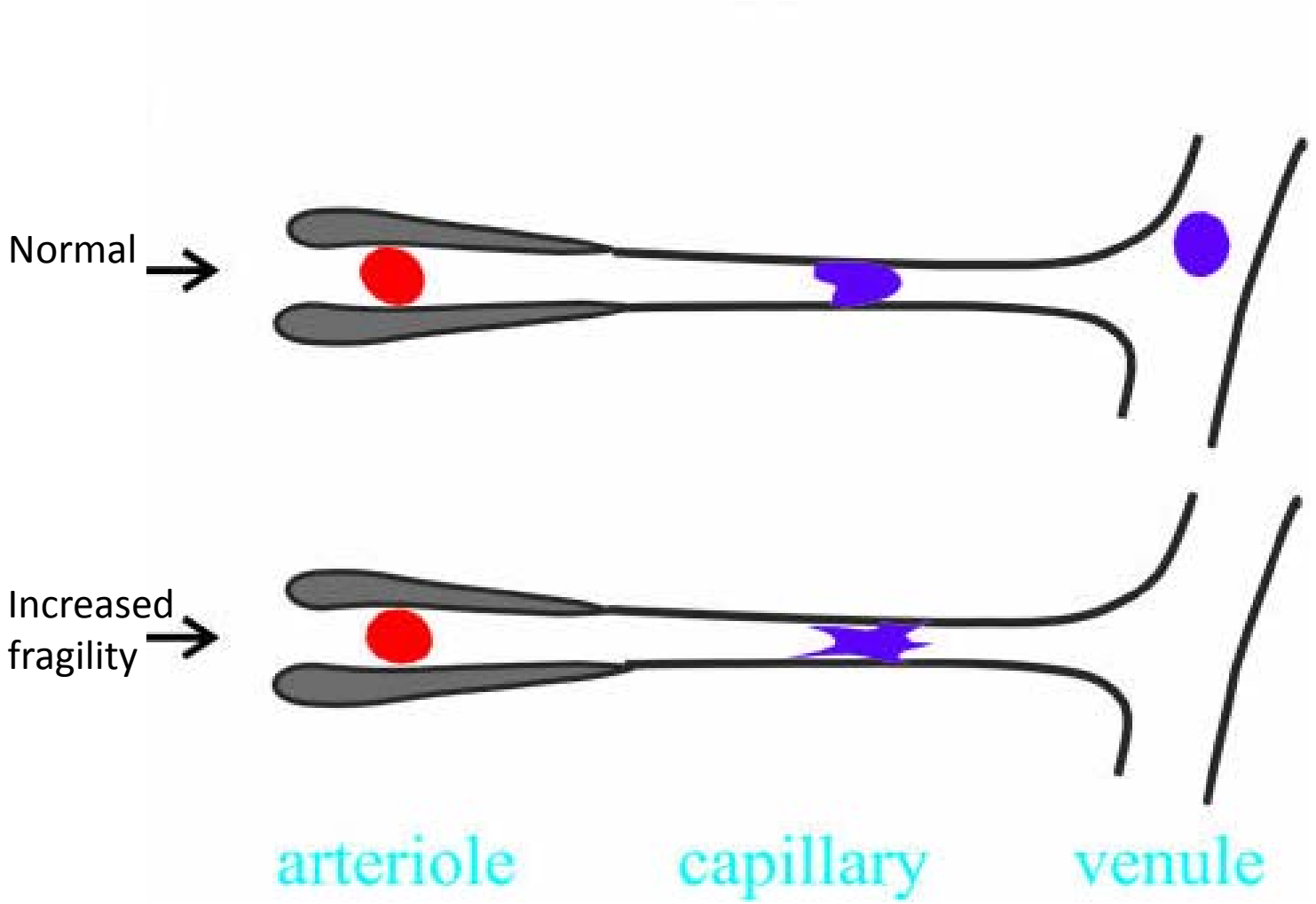
Spherocytosis



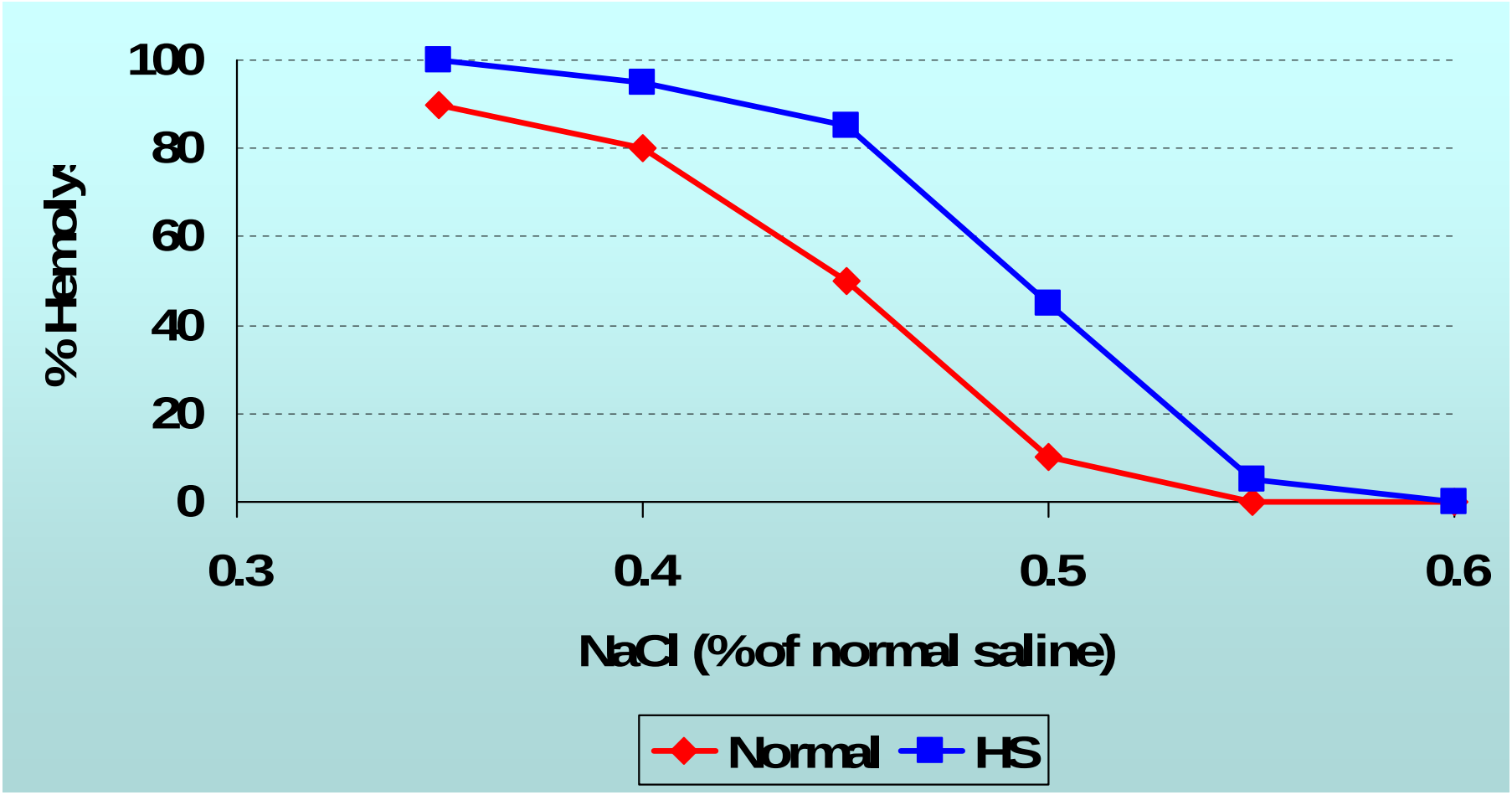


Spleenic architecture




Normal
Increased fragility
arteriole
capillary
venule

Increased osmotic fragility in hereditary spherocytosis

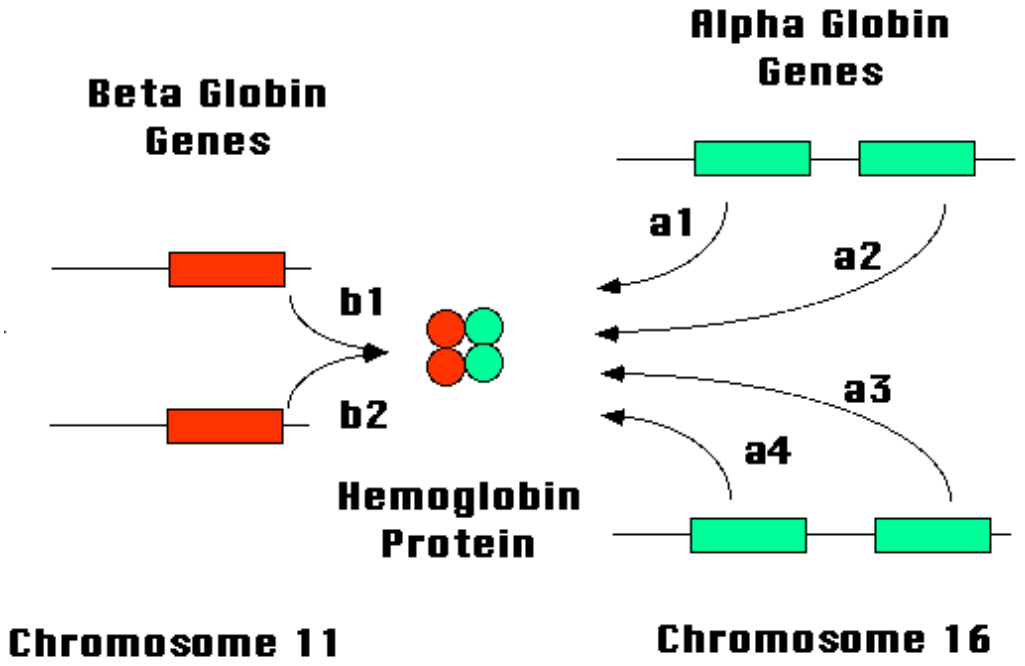


Lab investigations

- Anemia of increased destruction
  - Normocytic, normochromic anemia
  - Shortened RBC survival
  - Reticulocytosis - Response to increased RBC destruction
  - Absent haptoglobin

Thallasemia

- Beta
- Alpha



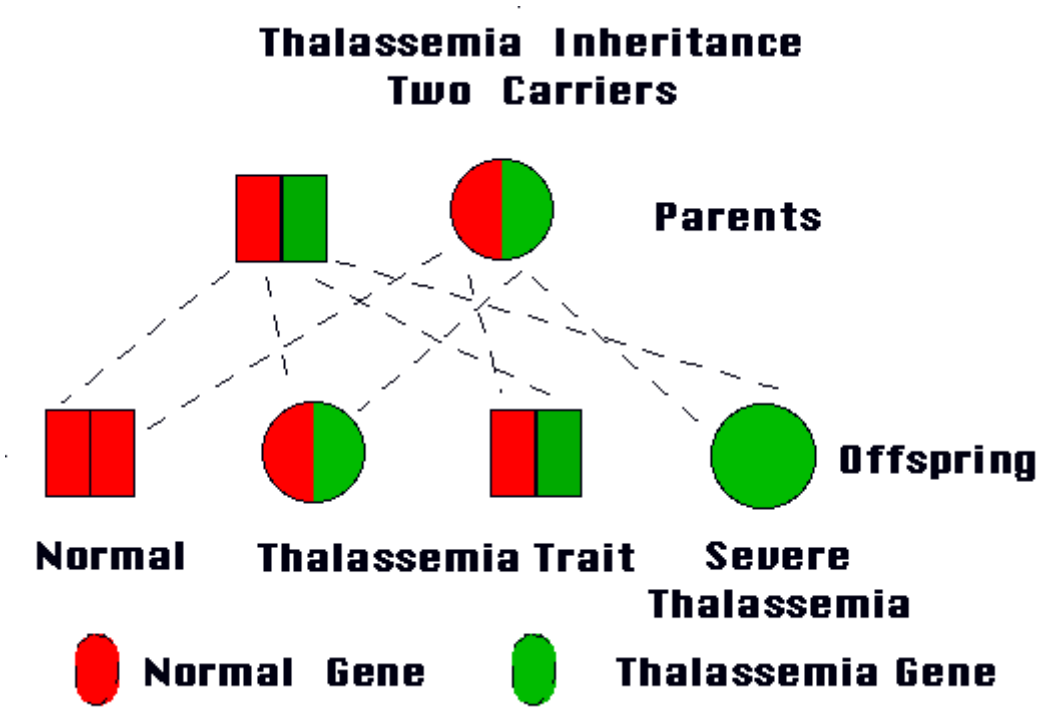
Thalassemia

- Genetic blood disorder resulting in a mutation or deletion of the genes that control globin production.
- Normal hemoglobin is composed of 2 alpha and 2 beta globins
- Mutations in a given globin gene can cause a decrease in production of that globin, resulting in deficiency
- aggregates become oxidized → damage the cell membrane, leading either to hemolysis, ineffective erythropoiesis, or both.
- 2 types of thalassemia: alpha and beta.

Demographics

- The thalassemia gene may be maintained in the human population, in part because of the greater immunity of heterozygous individuals against malaria and is found in parts of the world where malaria is common
- These include Southeast Asia, China, India, Africa, and parts of the Mediterranean.

Inheritance of thalassemia



Alpha Thalassemia

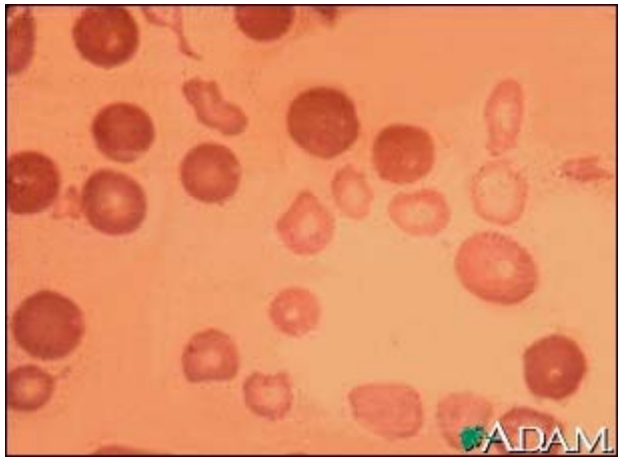
- mutation of 1 or more of the 4 alpha globin genes on chromosome 16
- severity of disease depends on number of genes affected
- results in an *excess of beta globins*

Silent Carriers (heterozygotes ++/+-)

- 3 functional alpha globin genes
- No symptoms, but thalassemia could potentially appear in offspring

Alpha Thalassemia Trait (++/--)

- 2 functional globin genes
- results in smaller blood cells that are lighter in colour
- no serious symptoms, except slight anemia



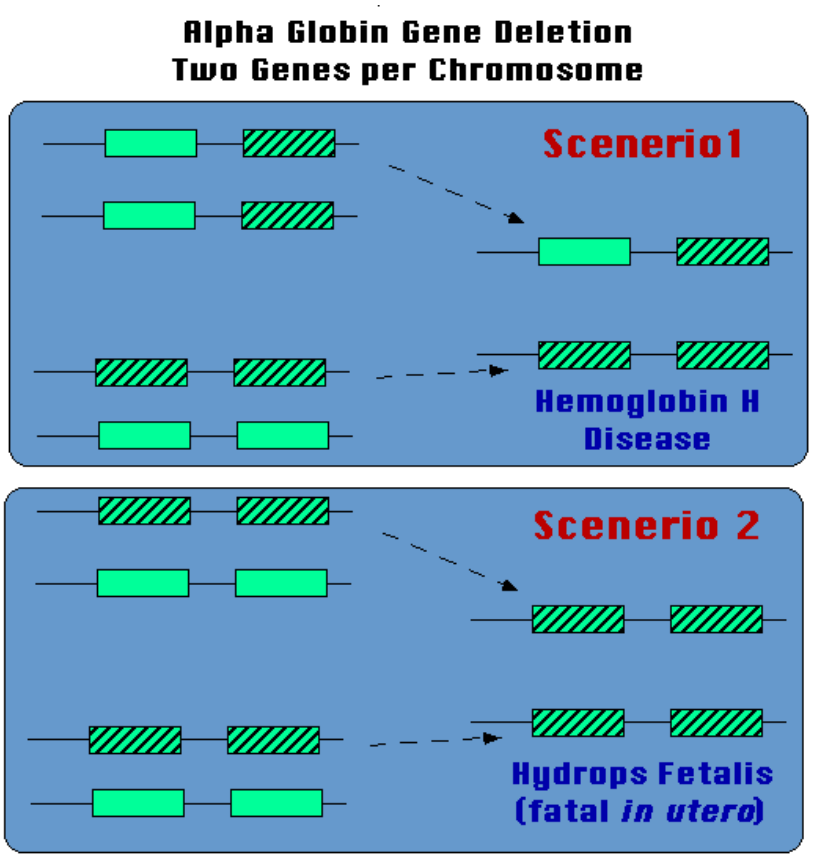
Hemoglobin H Disease (+-/--

- 1 functional globin gene
- results in very lightly coloured red blood cells and possible severe anemia
- hemoglobin H is susceptible to oxidation, therefore oxidant drugs and foods are avoided
- treated with folate to aid blood cell production

Alpha Thalassemia Major (--/--

- no functional globin genes
- death before birth (embryonic lethality): Hydrops fetalis, Hb Bart

Alpha thallasemia



Beta Thalassemia

- mutations on chromosome 11
- results in *excess of alpha globins*

Beta Thalassemia Trait (β^0/ β)

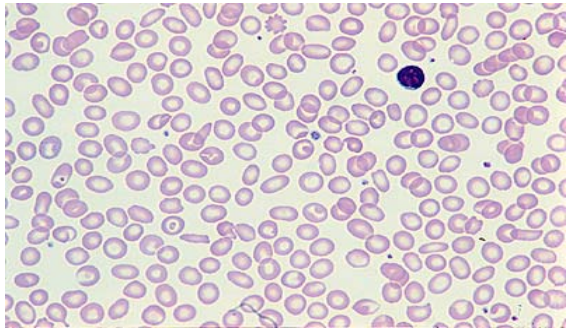
- slight lack of beta globin
- smaller red blood cells that are lighter in colour due to lack of hemoglobin
- no major symptoms except slight anemia
- Beta thalassemia trait is seen most commonly in Mediterranean (including North African, and particularly Italian and Greek), Middle Eastern, Indian, African, Chinese, and Southeast Asian (including Vietnamese, Laotian, Thai, Singaporean, Filipino, Cambodian, Malaysian, Burmese, and Indonesian

Beta Thalassemia Intermedia ($\beta^+/\ \beta^+$)

- lack of beta globin is more significant
- bony deformities due to bone marrow trying to make more blood cells to replace defective ones
- causes late development, exercise intolerance, and high levels of iron in blood due to reabsorption in the GI tract
- if unable to maintain hemoglobin levels between 6 gm/dl – 7 gm/dl, transfusion or splenectomy is recommended

Beta Thalassemia Major β^0/β^0

- complete absence of beta globin
- enlarged spleen, lightly coloured blood cells
- severe anemia
- chronic transfusions required, in conjunction with chelation therapy to reduce iron (desferoxamine)



Clinical features

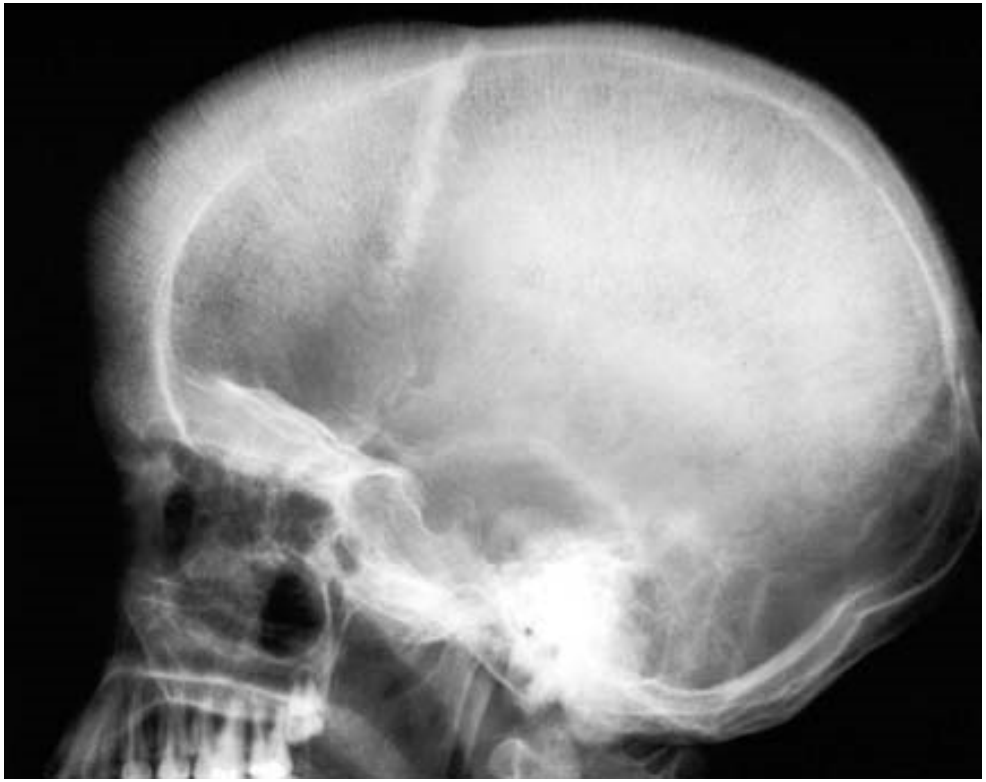
- Severe hypochromic anemia with spleenomegaly and markedly elevated levels of HbF.
- Family studies show both parents as carriers of the beta-thalassemic trait, which is marked by mild, microcytic, hypochromic anemia and high levels of HbA2.
- Early signs and symptoms are associated with the anemia, which is characterized by hypochromic, microcytic red cells with variable numbers of nucleated erythrocytes and reticulocytes.
- Pallor, icterus, and cardiac enlargement occur frequently.

Clinical features

- Marrow hypertrophy and extramedullary hematopoiesis may result in hepatosplenomegaly
- Skull deformities
- Facial deformity: Prominence of malar eminences and mal alignment of teeth, which gives rise to the characteristic "rodent facies."

Clinical features

- Iron overload
- Infections such as hepatitis
- Bone deformities
- Enlarged spleen
- Slowed growth rate
- Heart problems

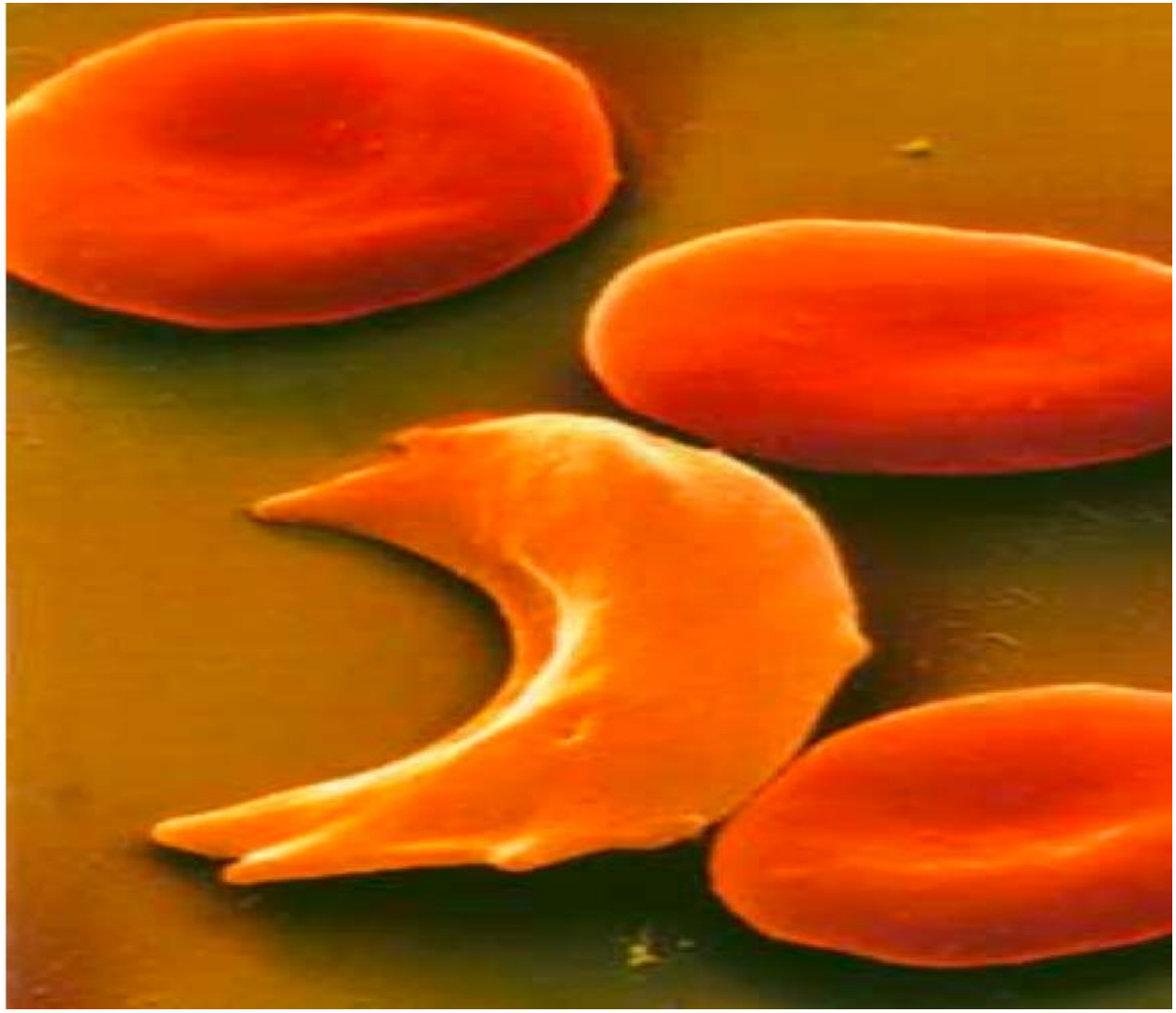


Widening of the calvarium
New bone formation producing a "hair-on-end" appearance

More Permanent Options

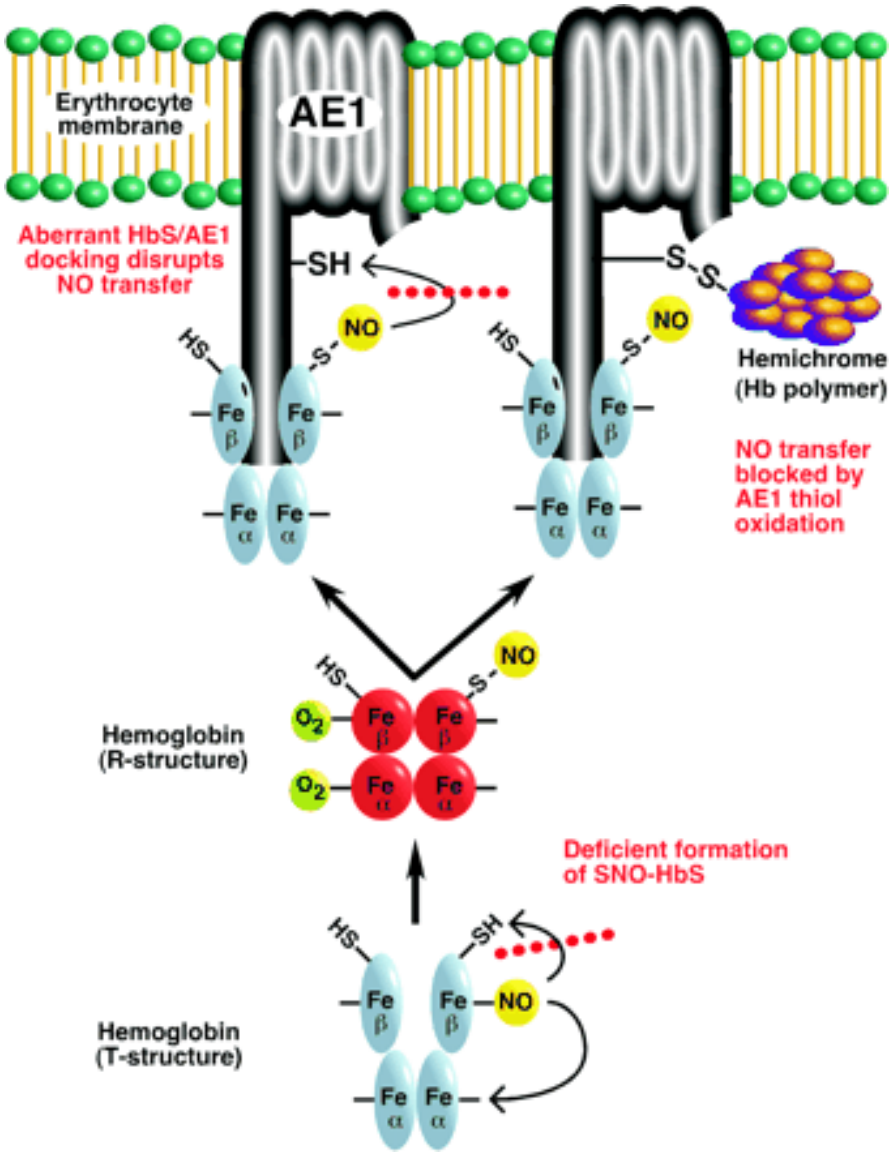
- Bone Marrow Transplants
  - Replacing patient's marrow with donor marrow
  - First performed on thalassemia patient in 1981
  - Difficult, because donor must be exact match for recipient
  - Even a sibling would only have a 1 in 4 chance of being a donor
- Cord Blood Transplants
  - Rich in stem cells
  - Also needs to be an exact match

Sickle cell anemia or HbSS or SS disease



Heterozygous population

- Hb AS : Sickle cell trait
- Hb SC: Sickle Hb C disaease
- HbS/ β^{+} : Sickle beta plus thalassemia
- HbS/ β^{0} : Sickle beta zero thalassemia


Erythrocyte membrane
AE1
Aberrant HbS/AE1 docking disrupts NO transfer
SH
NO
HS
S
Fe
β
α
S-S
Hemichrome (Hb polymer)
NO transfer blocked by AE1 thiol oxidation
Hemoglobin (R-structure)
O2
Deficient formation of SNO-HbS
Hemoglobin (T-structure)

Signs & symptoms

- Anemia
- Crisis
  - Vaso-occlusive
  - Sequestration
  - Aplastic
  - Hemolytic
- Complications

Vaso-occlusive crisis

- Due to sickled RBCs which obstruct blood flow and lead to ischemia in several organs
- Bone, lung, spleen, brain, spinal cord, digits
- Painful dactylitis is the first manifestation

Spleenic sequestration crisis

- Acute painful enlargement of the spleen
- May occur in the liver also
- An emergency

Aplastic crisis

- Transient arrest of erythropoiesis resulting in reticulocytopenia
- Typically preceeded by fever & upper respiratory or GIT infection
- Often due to Parvo virus B19 infection

Hemolytic crisis

- Increased rate of hemolysis with fall of hemoglobin but increase in reticulocyte count
- Usually accompany a painful crisis

Complications of sickle cell disease

Chronic hemolysis

- Anemia
- Pigment gall stones
- Aplastic episodes
- Jaundice
- Delayed growth

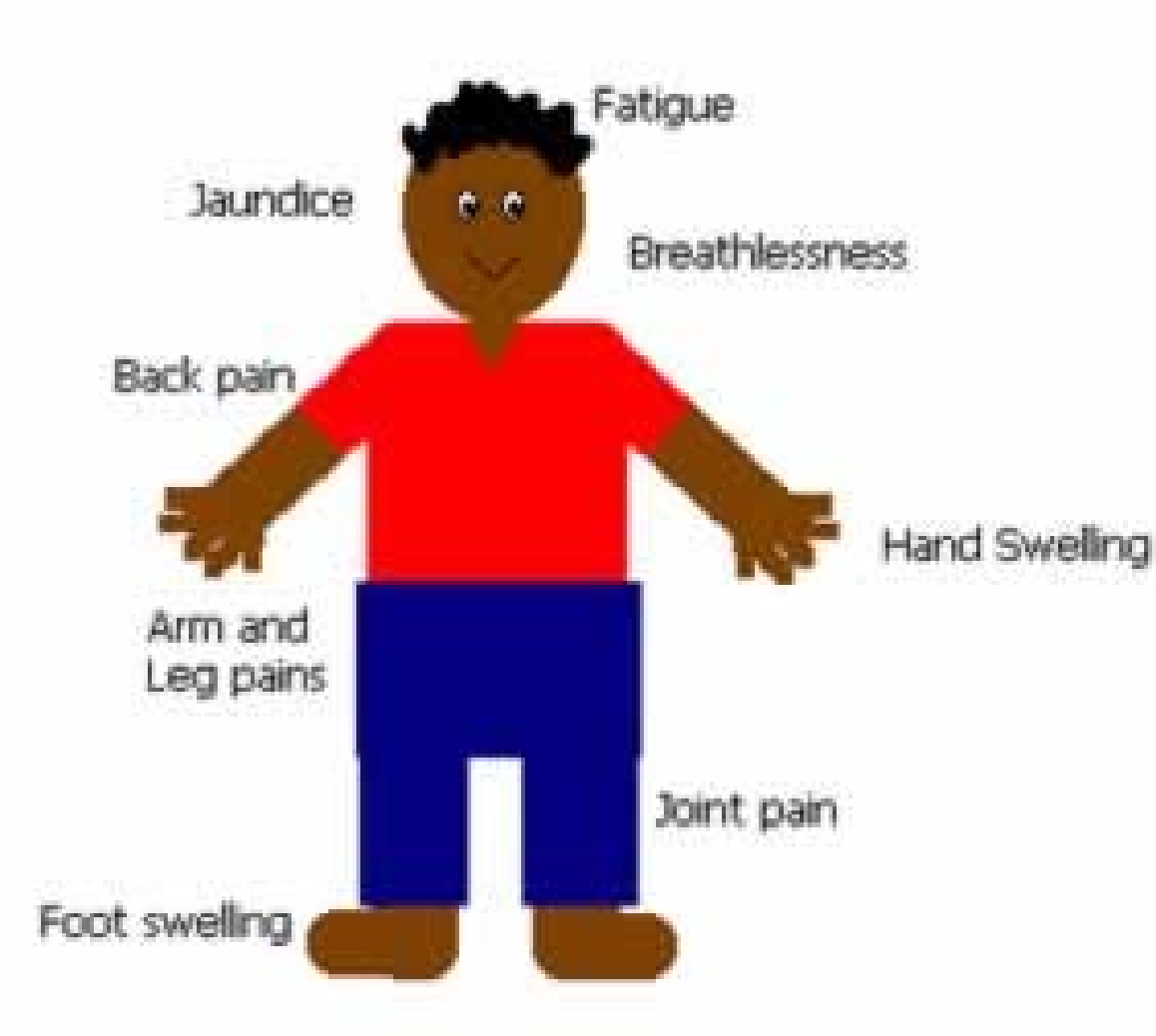
Vaso-occlusive

- Pain syndrome
- Acute chest syndrome
- Priaprism
- Stroke
- Retinopathy
- Avascular hip necrosis
- Spleenic sequestration
- Leg ulcers
- Chronic nephropathy

Early symptoms & complications

- Typically in 1st year of life
- Dactylitis
- Fever
- Pain in chest, abdomen, limb & joints
- Enlargement of, heart, Liver, spleen
- Frequent URI
- Chronic anemia as child grows older

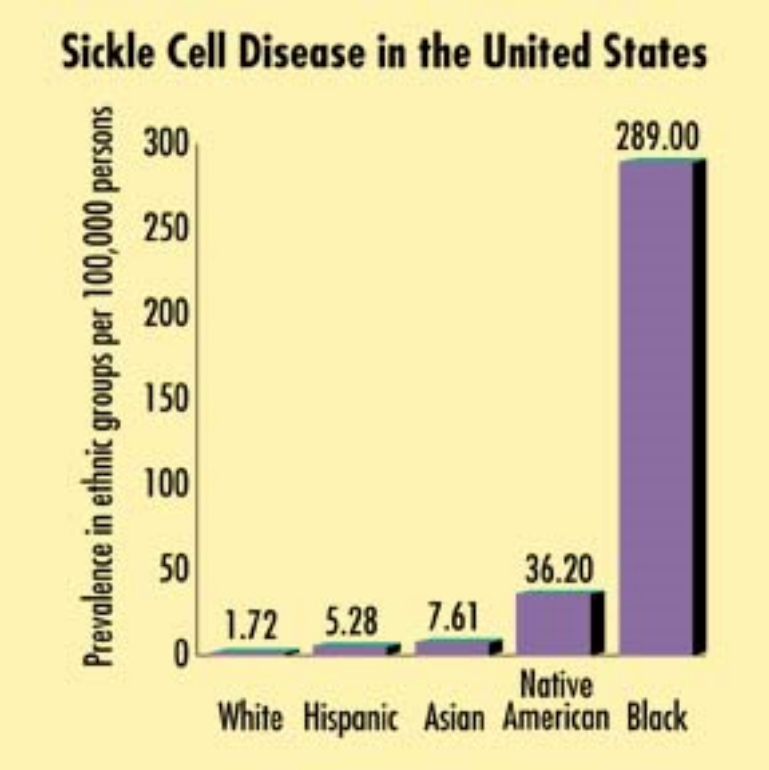
Clinical features of sickle cell disease



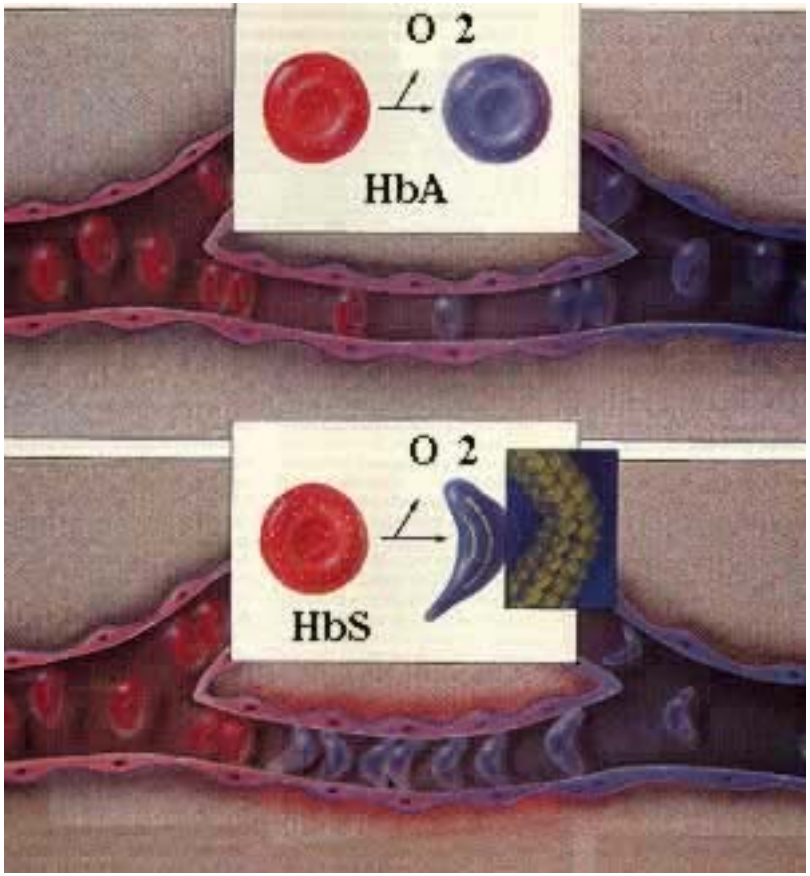
Sickle cell anemia

- Symptoms experienced throughout childhood and adulthood can include: fatigue, breathlessness, jaundice, paleness, susceptibility to infections, hand and foot swelling, painful joints, hands, arms, legs, and back, chest syndrome (pain in the chest wall), priapism (prolonged, painful erections), anemia and “pain crises

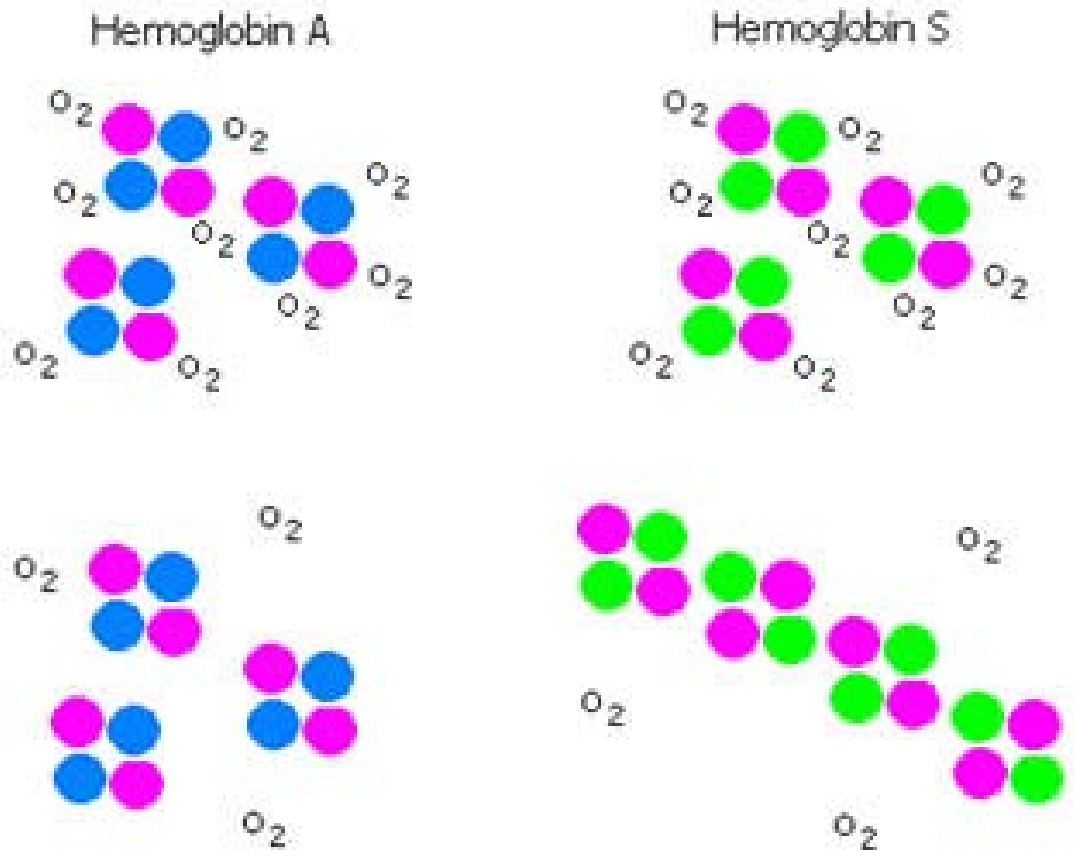
Prevalence of disease



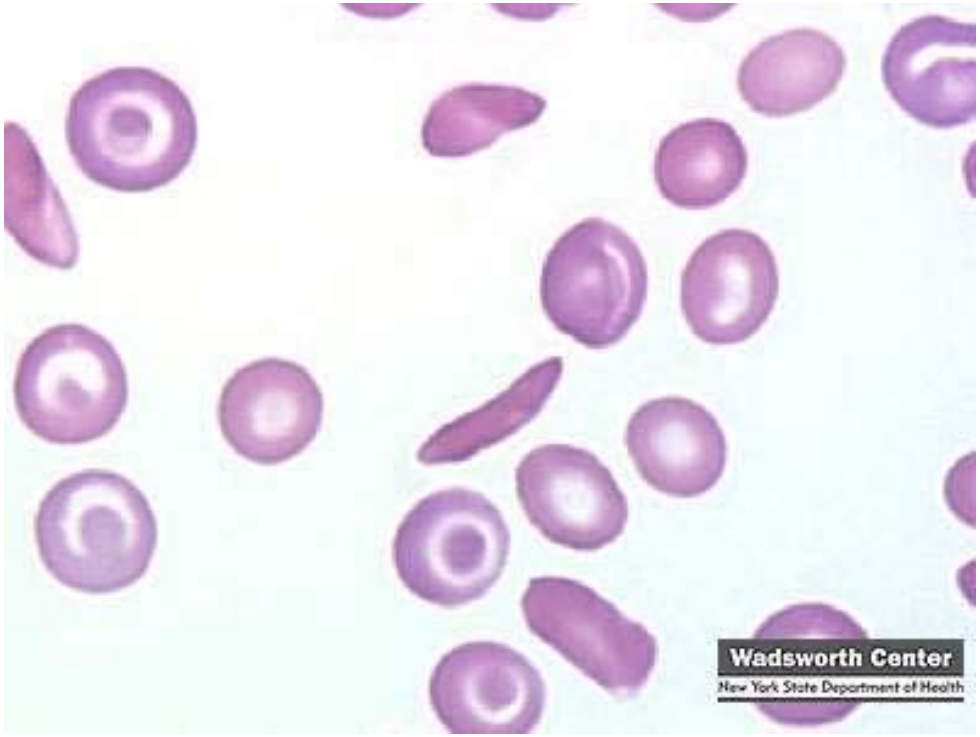
Sickling of RBC in circulation



Polymers of Hemoglobin S



Sickle cell



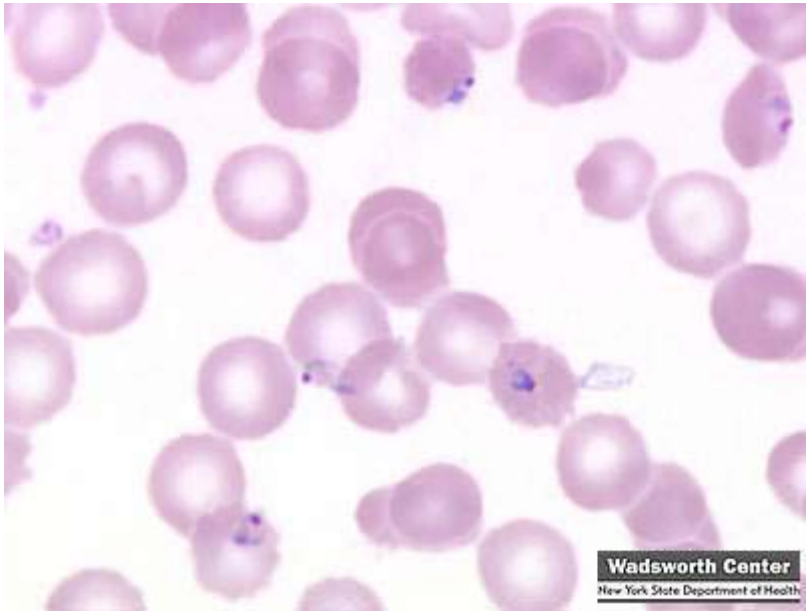
Prevention

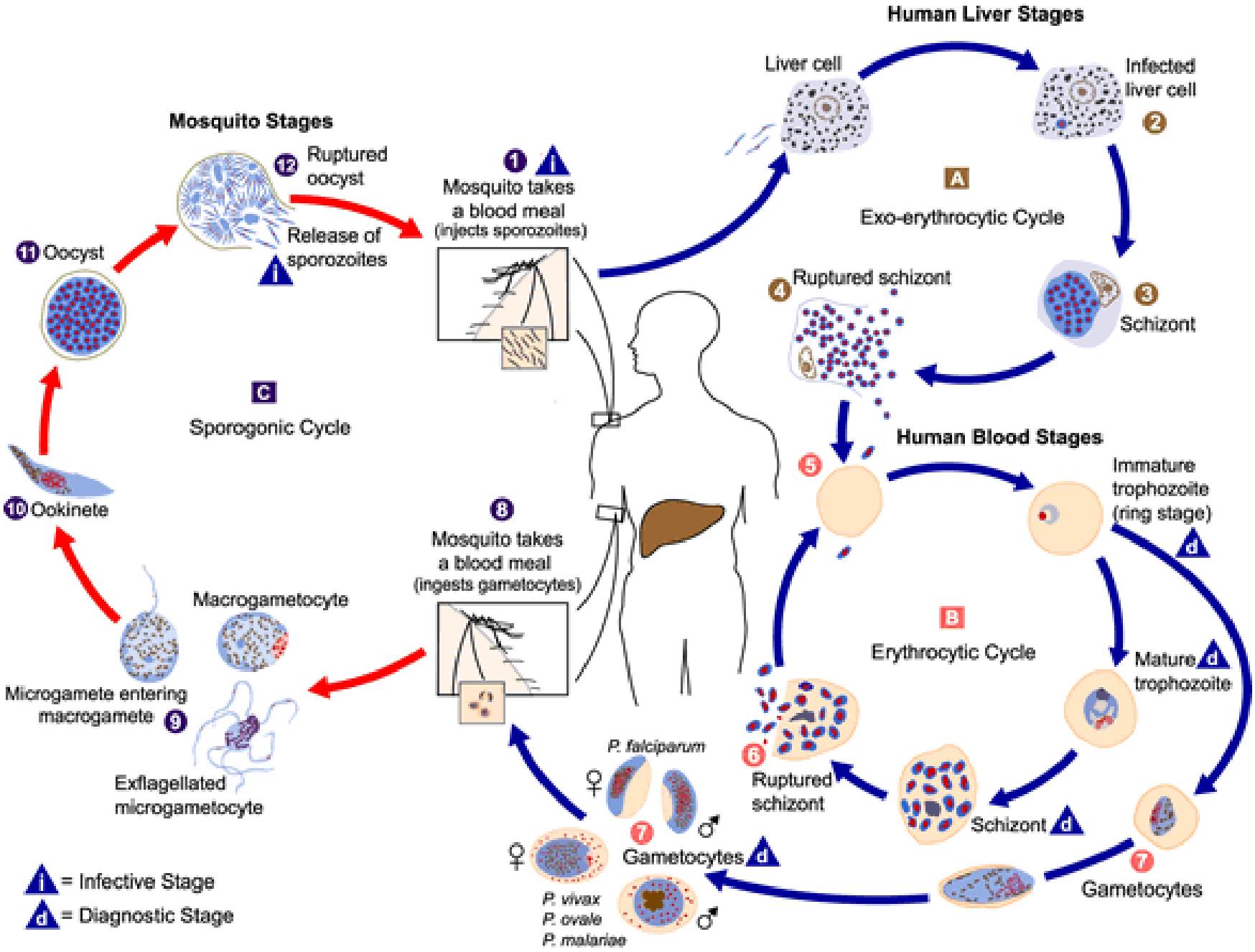
- Daily folic acid supplement
- Daily Pencillin till 6 yrs
- Plenty of water
- Avoid over exertion & stress
- Plenty of rest
- Regular check ups

Treatment

- Analgesia for painful episodes
- IV antibiotics
- Vaccination
- Correction of NO deficit
- Induce HBF synthesis
- Bone marrow transplantation
- Gene therapy

Malarial parasite within RBC




Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
Ruptured schizont
4
3
Schizont
Mosquito Stages
12 Ruptured oocyst
1
Mosquito takes a blood meal (injects sporozoites)
Release of sporozoites
11 Oocyst
C
Sporogonic Cycle
Human Blood Stages
5
Immature trophozoite (ring stage)
d
10 Ookinete
8
Mosquito takes a blood meal (ingests gametocytes)
B
Erythrocytic Cycle
Mature trophozoite
Macrogametocyte
Microgamete entering macrogamete 9
Exflagellated microgametocyte
P. falciparum
6
Ruptured schizont
Schizont
7
Gametocytes
Gametocytes
P. vivax
P. ovale
P. malariae
= Infective Stage
= Diagnostic Stage

Malaria and sickle cell anemia

- It is likely that *P. falciparum*, the parasite responsible for malaria, decreases oxygen in red blood cells it infects.
- As a result of low oxygen concentrations, hemoglobin S within cells polymerizes, forming a sickled cell.
- These cells are then marked for cell death since they are unhealthy, and the parasite-infected cells are destroyed before they can cause harm

Anemia due to impaired production

- Disturbance of proliferation & differentiation

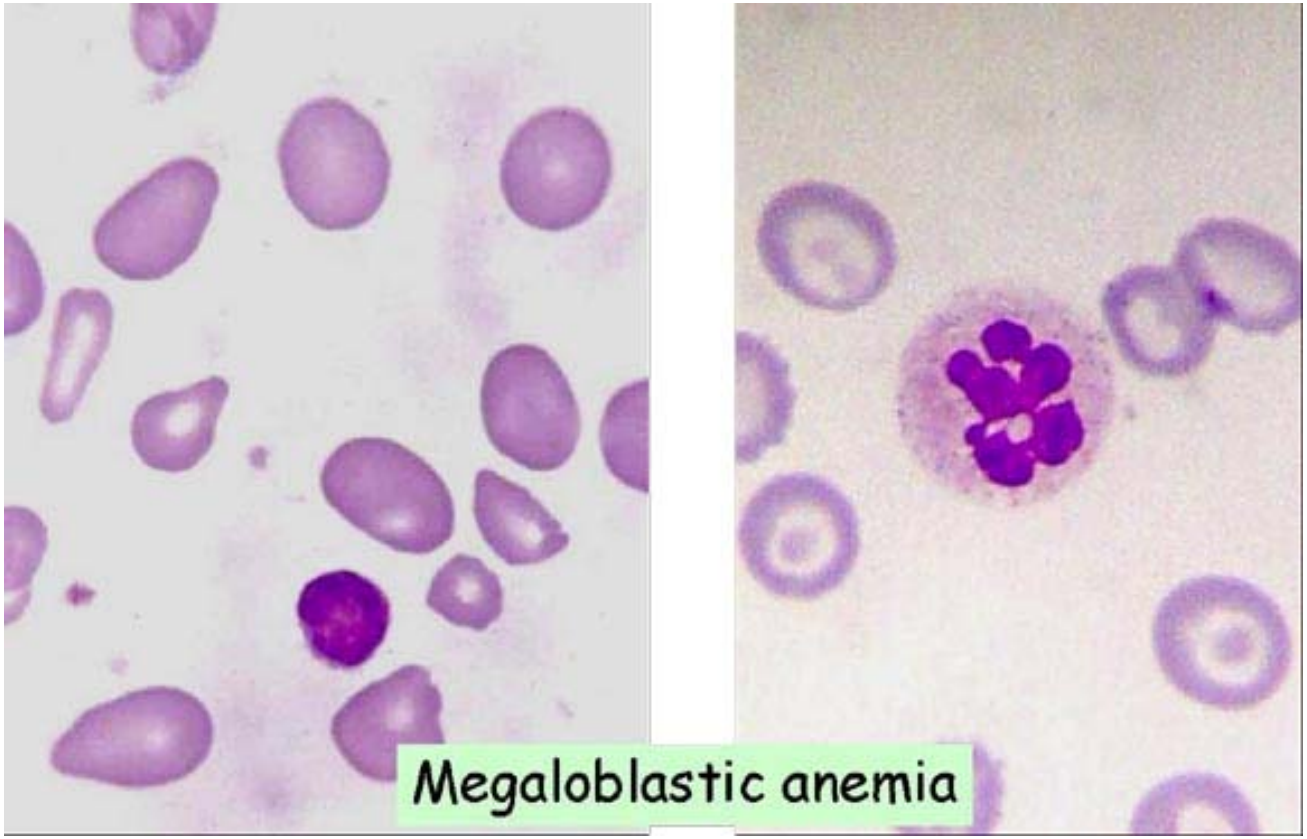
  Aplastic anemia, anemia of renal failure, anemia of endocrine disorders

- Disturbance of proliferation & maturation

  Defective DNA synthesis: Megaloblastic anemia

  Defective heme synthesis: Iron defficiency

  Defective globin synthesis: Thalassemia



Megaloblastic anemia

Classification of megaloblastic anemia

- Cobalamine deficiency

Inadequate intake : rare

Malabsorption:

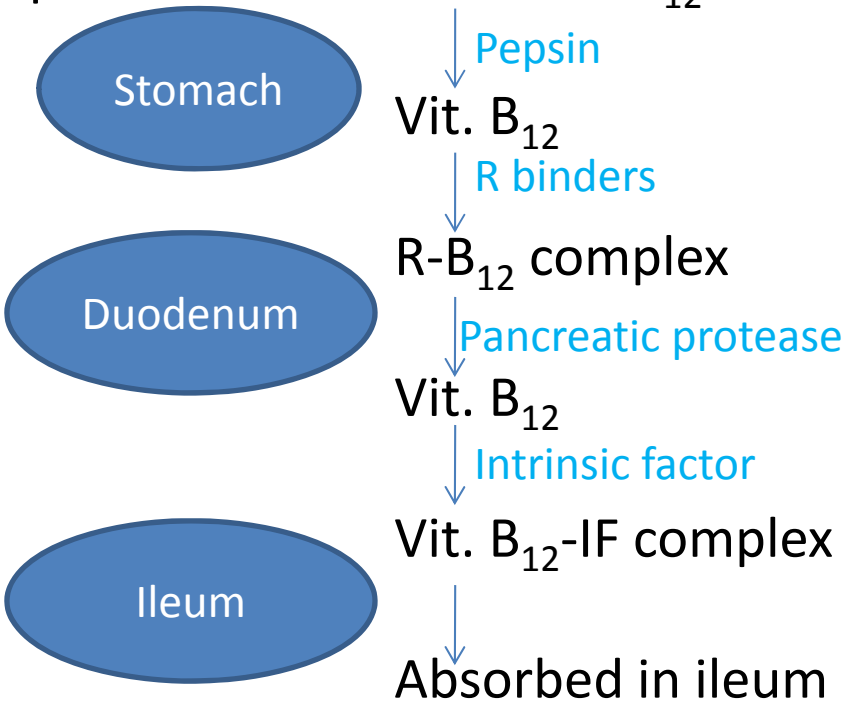
Decreased intrinsic factor — Pernicious anemia
Gastrectomy
Congenital absence

Disorders of terminal ileum: tropical sprue, regional enteritis

Competition for cobalamine: fish tape worm, blind loop syndrome

Vitamin B_{12} absorption

- Daily requirement 2-3 µg
- Source: Animal products
- Absorption: Protein bound Vit. B_{12}



Folic acid

- Folic acid defficiency (can develop quickly)

  Inadequate intake

  Increased requirement: pregnancy, infancy, malignancy, hemodylasis

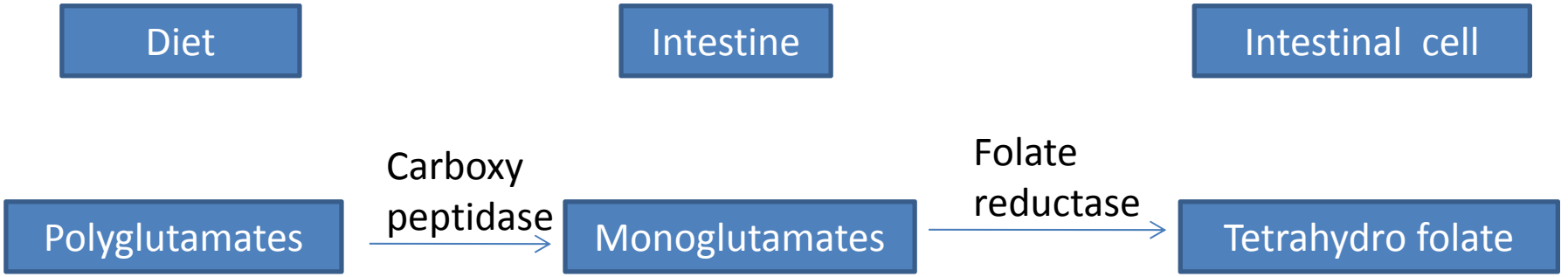
  Malabsorption: Tropical sprue, phenytoin,

  barbiturates

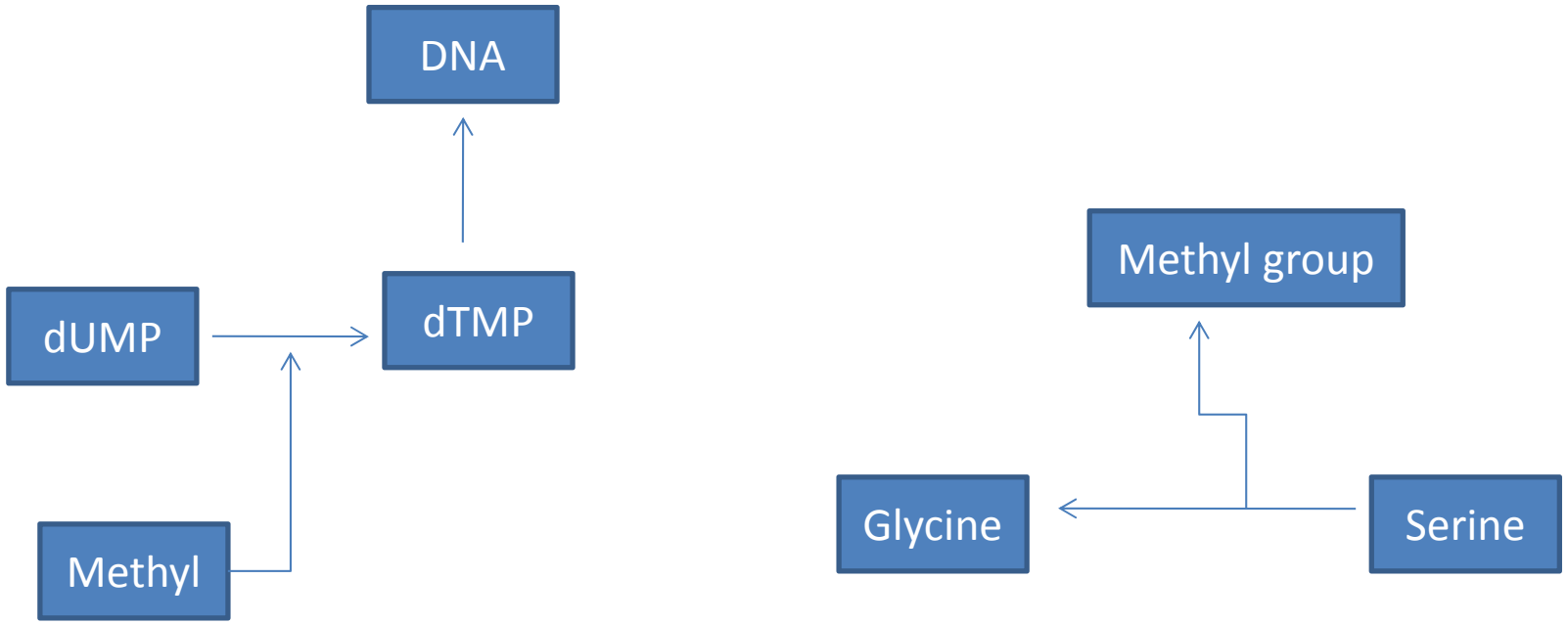
  Impaired metabolism: Methotrexate

Folic acid/ pteroylglutamic acid

- Animals can not synthesise folates
- Source: Plants (Polyglutamate conjugates)

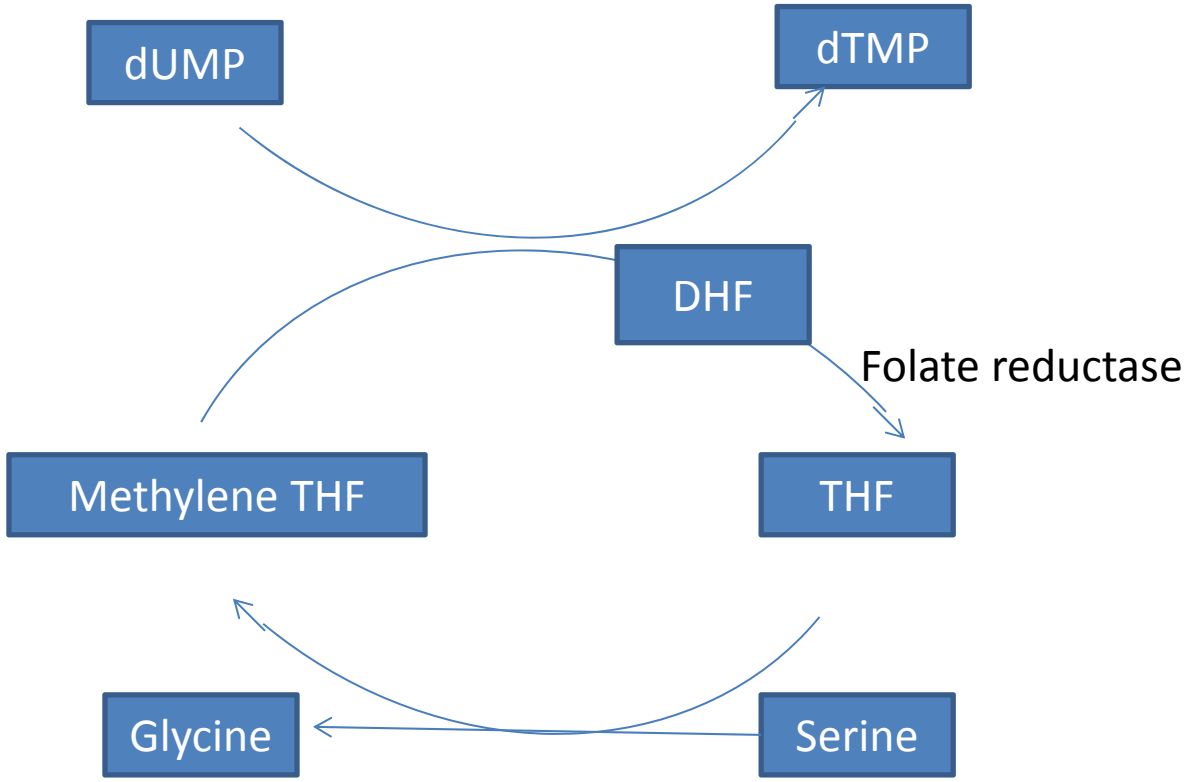


Role of folic acid in DNA synthesis: DNA cycle



THF transfers methyl group from serine to uridine

DNA cycle



Serine transfers methylene group to THF

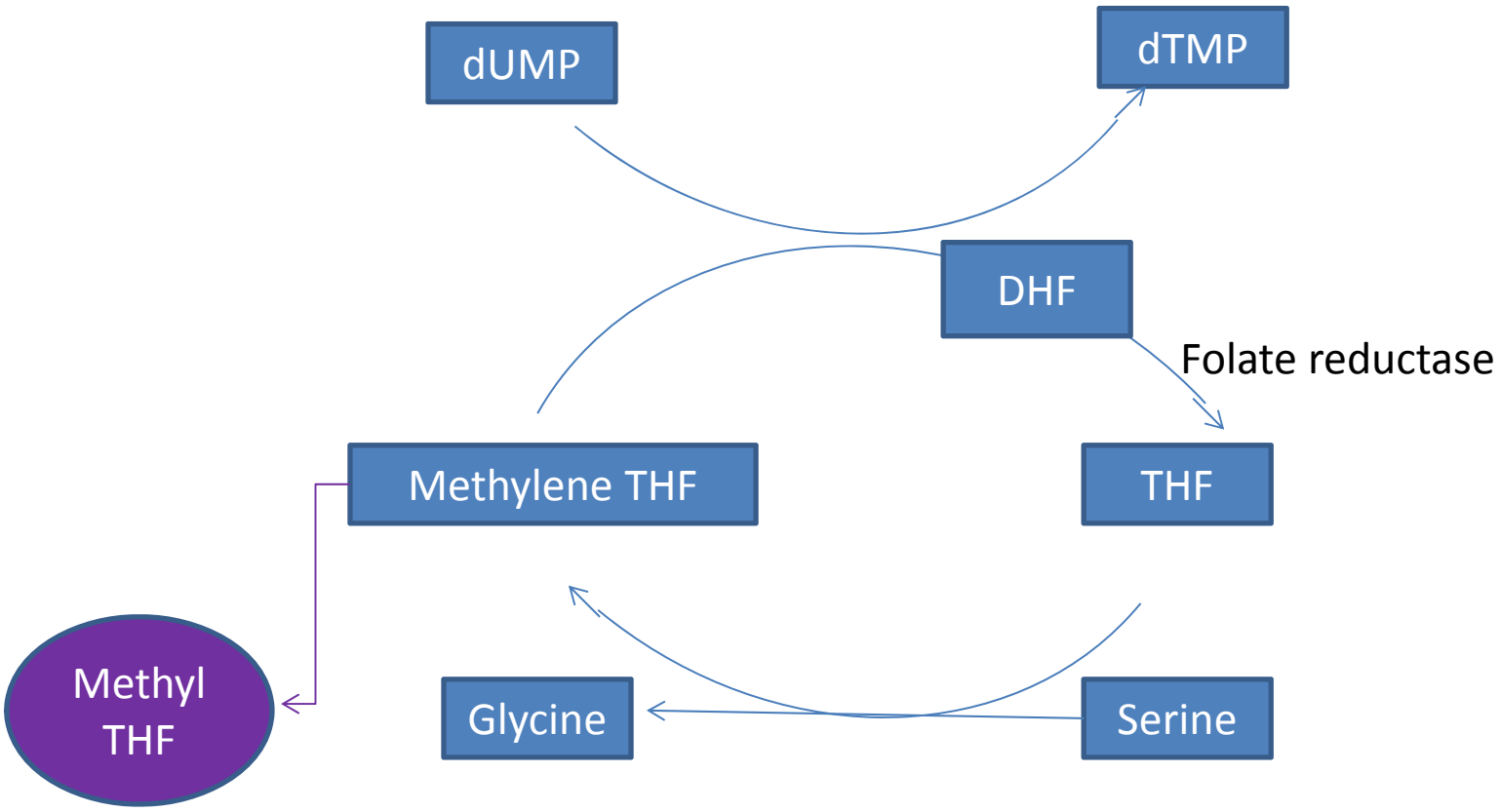
Methylene THF transfers the methyl group to uridine

Regeneration of THF from DHF occurs in the presence of folate reductase

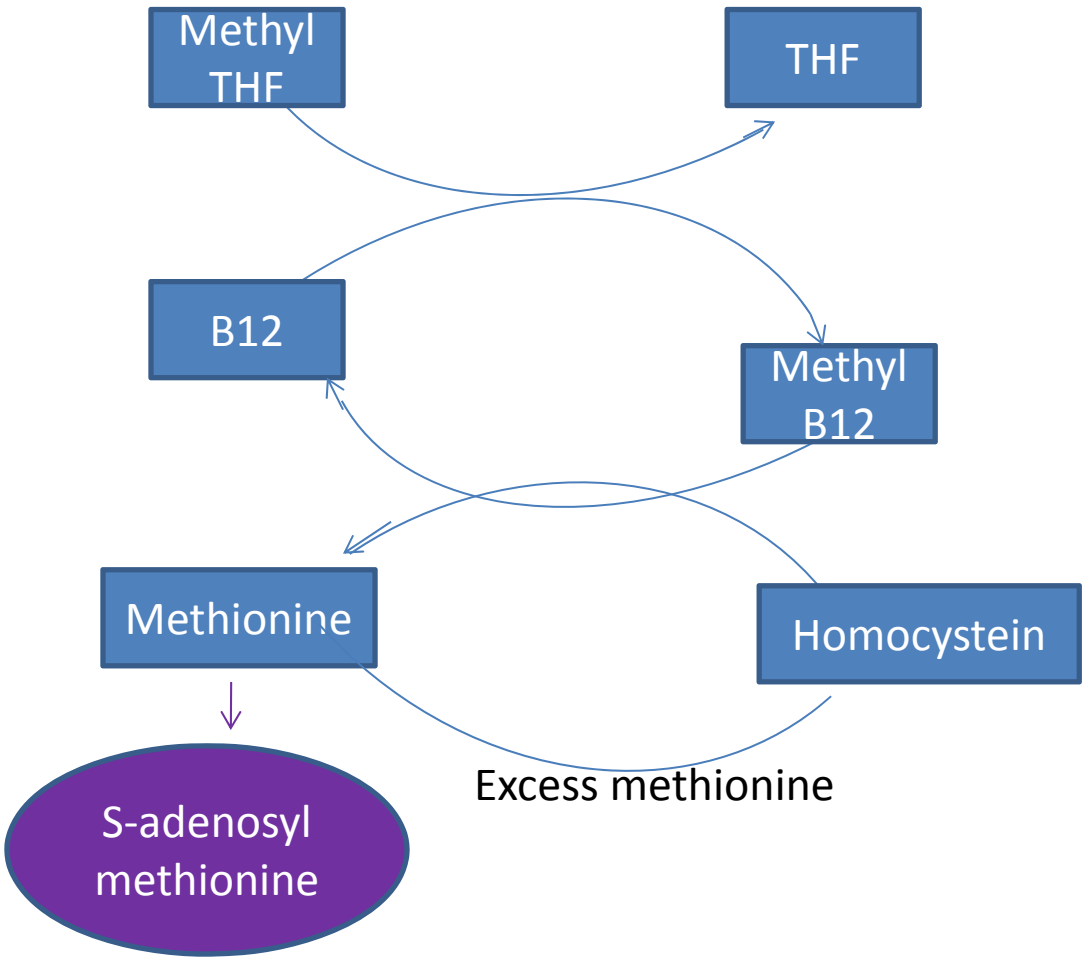
Unlike the methylation cycle, the DNA cycle does not depend on vitamin B12.

Folic acid can thus maintain the supply of intracellular folate required for DNA synthesis. DNA synthesis, and hence cell replication, can therefore take place in people with vitamin B12 deficiency, provided that folic acid is available as a source of folate. This is why, in people with vitamin B12 deficiency, folic acid supplementation will treat the megaloblastic anaemia (due to deficient cell replication), but will not affect the neurological complications which occur as a result of the disruption of the methylation cycle.

Methyl trap


dUMP
dTMP
DHF
Folate reductase
Methylene THF
THF
Methyl THF
Glycine
Serine

Action of B_{12}



Recovery of THF from the methyl trap

S-adenosyl methionine production

The methylation cycle

Depends on both folate and vitamin B12 to produce methionine
An example of a methylation reaction is the methylation of the protein in myelin (the insulation cover on nerves). When this process is interrupted, as it is during vitamin B12 deficiency, one of the clinical consequences is the demyelination of nerves, resulting in a neuropathy, which leads to ataxia, paralysis and, if untreated, ultimately to death.

Another methylation reaction involves the degradation of methionine.

Any excess methionine is degraded to homocysteine.

Homocysteine can be either degraded to form pyruvate which can then be used as a source of energy, or it can be remethylated to form methionine

Vitamin B6 is essential in the former reaction, and vitamin B12 and folate in the latter.

Features common to all forms of megaloblastic anemia

- Anisocytosis
- Normochromic
- Macrocytes(MCV > 100) and oval shaped
- Lower reticulocyte count
- Hypersegmented neutrophil
- Dissociation between nuclear and cytoplasmic maturation

Features common to all forms of megaloblastic anemia….

- Accumulation of megaloblasts in bone marrow
- Inefective erythropoiesis
- Increased hemopoietic destruction

- Leucopenia
- Thrombocytopenia

Pernicious anemia

Immunologically mediated ,autoimmune

destruction of gastric mucosa

Morphological changes in

- Alimentary system

  Tongue: shiny, glazed &“beefy”(atrophic glossitis)

  Stomach: Atrophy of fundic glands
- Bone marrow: Hemosiderosis

Pernicious anemia...

- Classical neurological features
- Poly neuropathy progressively involving the peripheral nerves and the posterior and eventually the lateral columns of the spinal cord (subacute combined degeneration)
- Symmetrical paraesthesiae in the fingers and toes
- early loss of vibration sense and proprioception
- progressive weakness and ataxia
- Paraplegia
- Dementia and optic atrophy

Clinical course of megaloblastic anemia

Insidious in onset

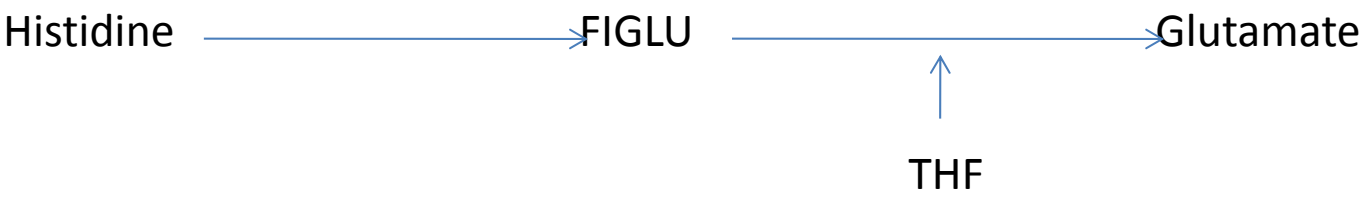
- Moderate to severe megaloblastic anemia
- Leukopenia with hypersegmented neutrophil
- Thrombocytopenia
- Neurological changes associated with involvement of posterolateral spinal cord
- Achlorhydria
- Inability to absorb an oral dose of cobalamine (Schillings test)
- Low Vitamin B_{12} levels
- Methyl malonic acid excretion in urine
- Dramatic response on parenteral administration of Vit. B12

Investigations

- **Haematological findings** show the features of a megaloblastic anaemia
- **Bone marrow** shows the typical features of megaloblastic erythropoiesis
- **Serum bilirubin** may be raised as a result of ineffective erythropoiesis.
- **Serum vitamin B_{12}** is usually well below 160 ng/L, which is the lower end of the normal range. Serum vitamin B_{12} can be assayed using radioisotope dilution or immunological assays.
- **Serum folate level** is normal or high, and the red cell folate is normal or reduced owing to inhibition of normal folate synthesis

Investigations

- FIGLU test



Oral challenge of Histidine
Increased urinary excretion of FIGLU in folate defficiency as well as B12 defficiency

Investigations

- Schilling's test

  Radiolabelled B_{12} orally

  Measuring radioactivity in urine

Folic acid/pteroylmonoglutamic acid

Neurological changes not seen

Prime function: To act as intermediates in transfer of 1 C moieties i.e. methyl & formyl groups to various organic compounds

1 C moieties are used as building blocks in the synthesis of biological macromolecules

- Daily requirement of folic acid 50-200 micro g
- Source: green vegetables i.e. lectuce, spinach, asparagus & broccoli

Fruits: lemons, banana, melons

Polyglutamate form in diet

Absorbed as 5-methyltetrahydrofolate

Iron deficiency anemia

- Most common form of nutritional deficiency in developed & developing countries
- 1.0 ml of blood may be considered to contain 0.5 mg iron

Iron balance

Unique:

Balance achieved by a control of absorption

Absorption

In proximal jejunum

Only 5% of ingested iron is absorbed

Absorption of iron

- Non heme iron

Mainly in Fe^{3+} form

Must be converted to Fe^{2+} before absorption

- Dietary factors enhancing non heme iron absorption

➢ Acsorbate
➢ Meats & fish
➢ Human breast milk
➢ Acidic gastric juice

- Dietary factors inhibiting non heme iron absorption

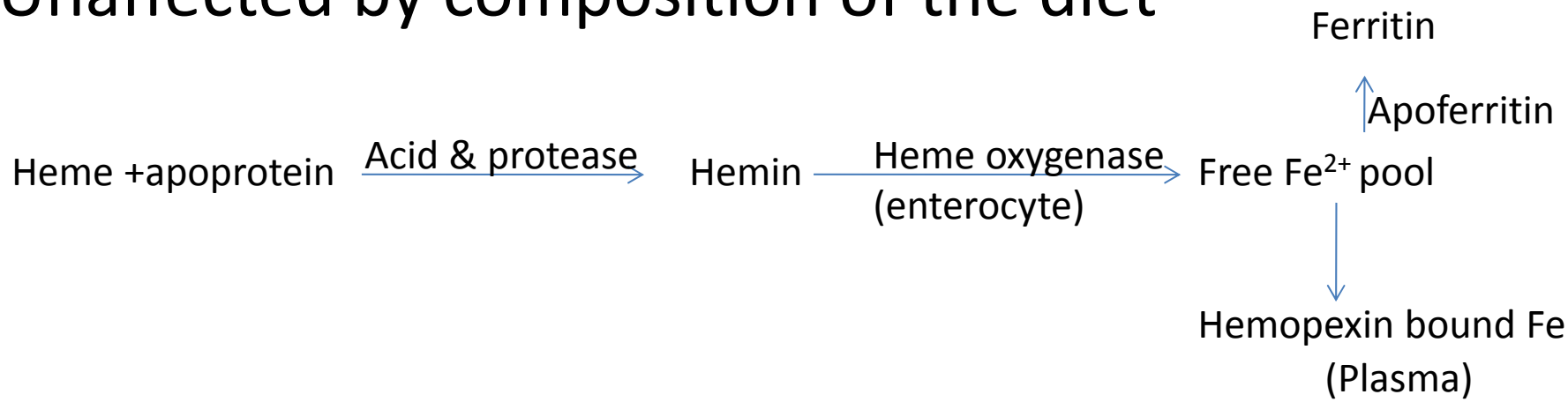
➢ Phytates in grains and vegetable food
➢ Polyohenols in legumes, tea, coffee & wine
➢ Phosphates
➢ Calcium
➢ Egg white & bovine milk proteins

Absorption of iron…

- Heme iron

10 – 15% of iron in non vegetarian diets

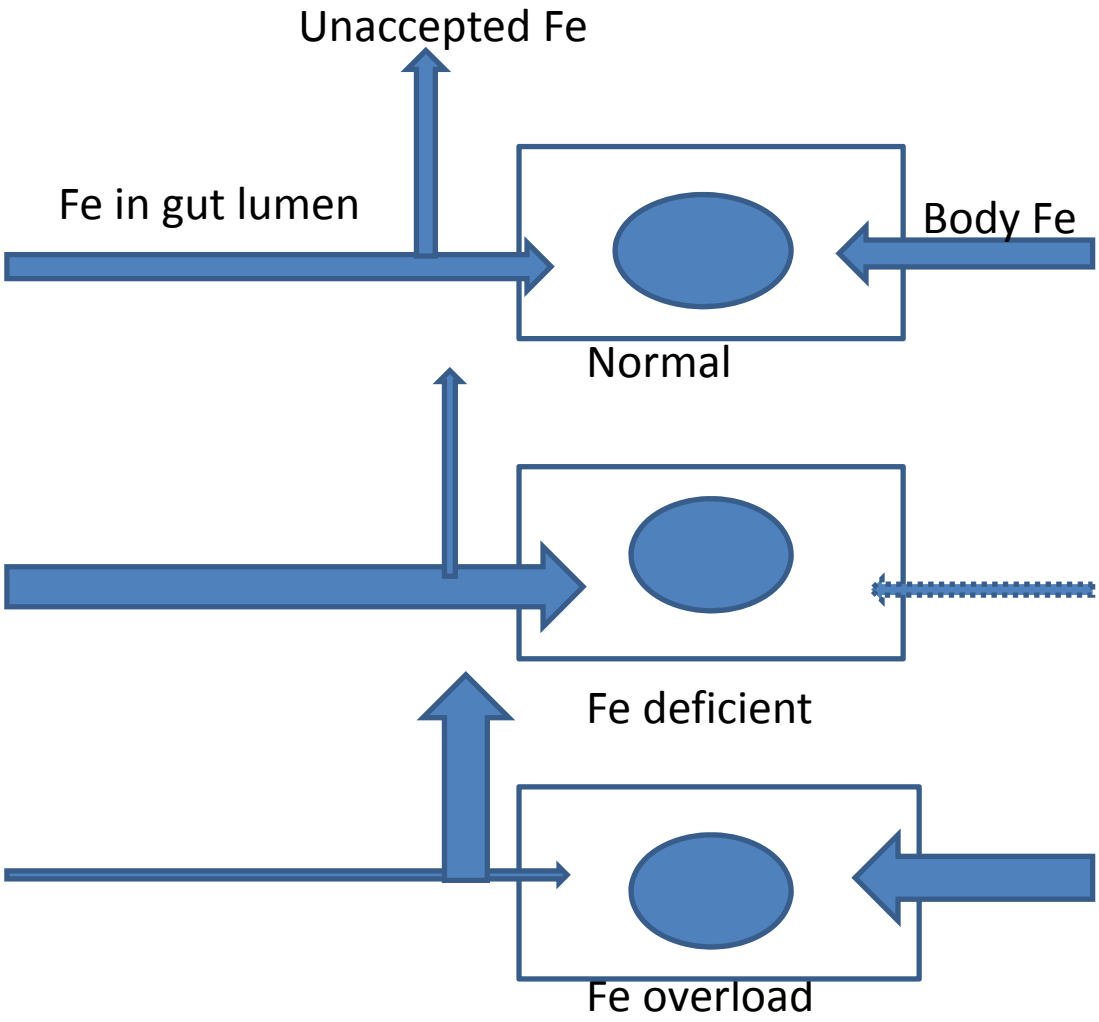
Unaffected by composition of the diet



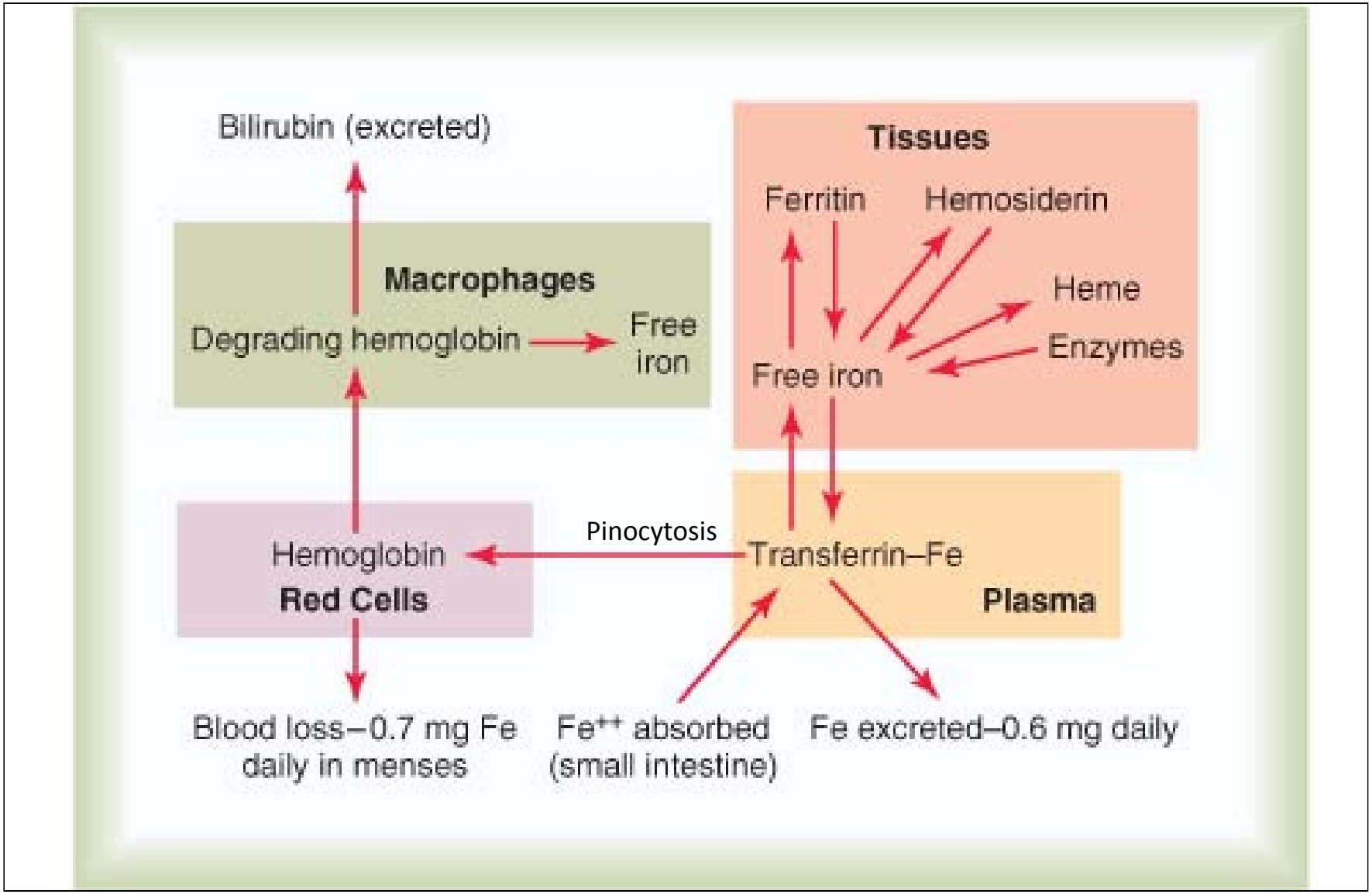
Regulation of mucosal absorption

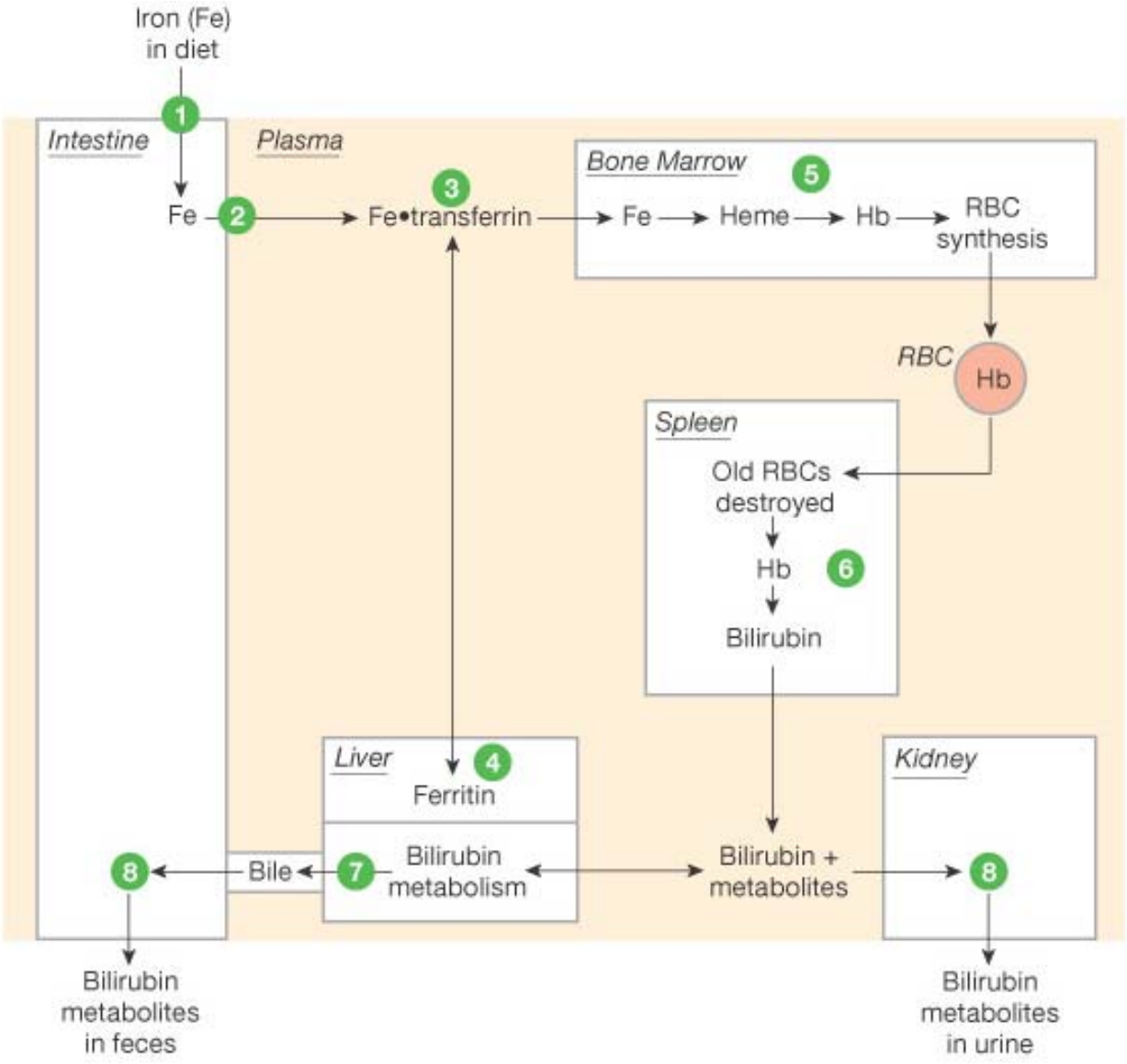
- Intestinal mucosal cell is programmed to absorb iron in proportion to the body's iron requirement esp. rate of erythropoiesis

Modified mucosal block theory



Iron


Bilirubin (excreted)
Tissues
Ferritin
Hemosiderin
Heme
Enzymes
Macrophages
Degrading hemoglobin
Free iron
Free iron
Pinocytosis
Hemoglobin
Red Cells
Transferrin–Fe
Plasma
Blood loss–0.7 mg Fe daily in menses
Fe++ absorbed (small intestine)
Fe excreted–0.6 mg daily



1. Iron comes from the diet.
2. Fe absorbed by active transport.
3. Transferrin protein transports Fe in plasma.
4. Liver stores excess Fe as ferritin.
5. Bone marrow uses Fe to make hemoglobin (Hb).
6. Spleen converts Hb to bilirubin.
7. Liver metabolizes bilirubin and excretes it in bile.
8. Bilirubin metabolites are excreted in urine and feces.

- Total Iron Binding Capacity (TIBC)

Clinically the amount of transferrin is expressed in terms of amount of iron it will bind

- Storage iron proteins

➢ Ferritin

➢ Hemosiderin

Etiology

Negative iron balance

Decreased Fe intake
Inadequate diet
Impaired absorption

Increased Fe loss
GIT bleeding
Excessive menstrual flow
Blood donation
Disorders of hemostasis

Idiopathic hypochromic anemia

Increased requirement

Infancy
Pregnancy
Lactation

Etiology…..

- GIT infection with hookworm: Necator americanus or Ancylostoma duodenale(0.2 ml/worm/day)
- Other worms: Schistosoma mansoni & S. hematobium , Trichuris trichura
- Excessive menstruation: use of > 12 pads /d, Passage of clote > 2 cm diameter after the first day, Duration> 7 days

Etiology….

- Blood donation Each unit of blood donated contains approx. 250 mg of iron
- Pergnancy and lactation : Most of the Fe loss occurs during the third trimester (3-7.5 mg/day)
- Lactation : Daily blood loss 0.5-1.0 mg

Stages in the development of iron deficiency

- Depletion of the iron stores in the hepatocytes and macrophages of spleen, liver and bone marrow
- Decrease in plasma iron content leading to inadequate supply of iron to bone marrow for regeneration of hemoglobin
- Increase in free erythrocyte protoporphyrin and decrease in blood hemoglobin levels

Clinical manifestations

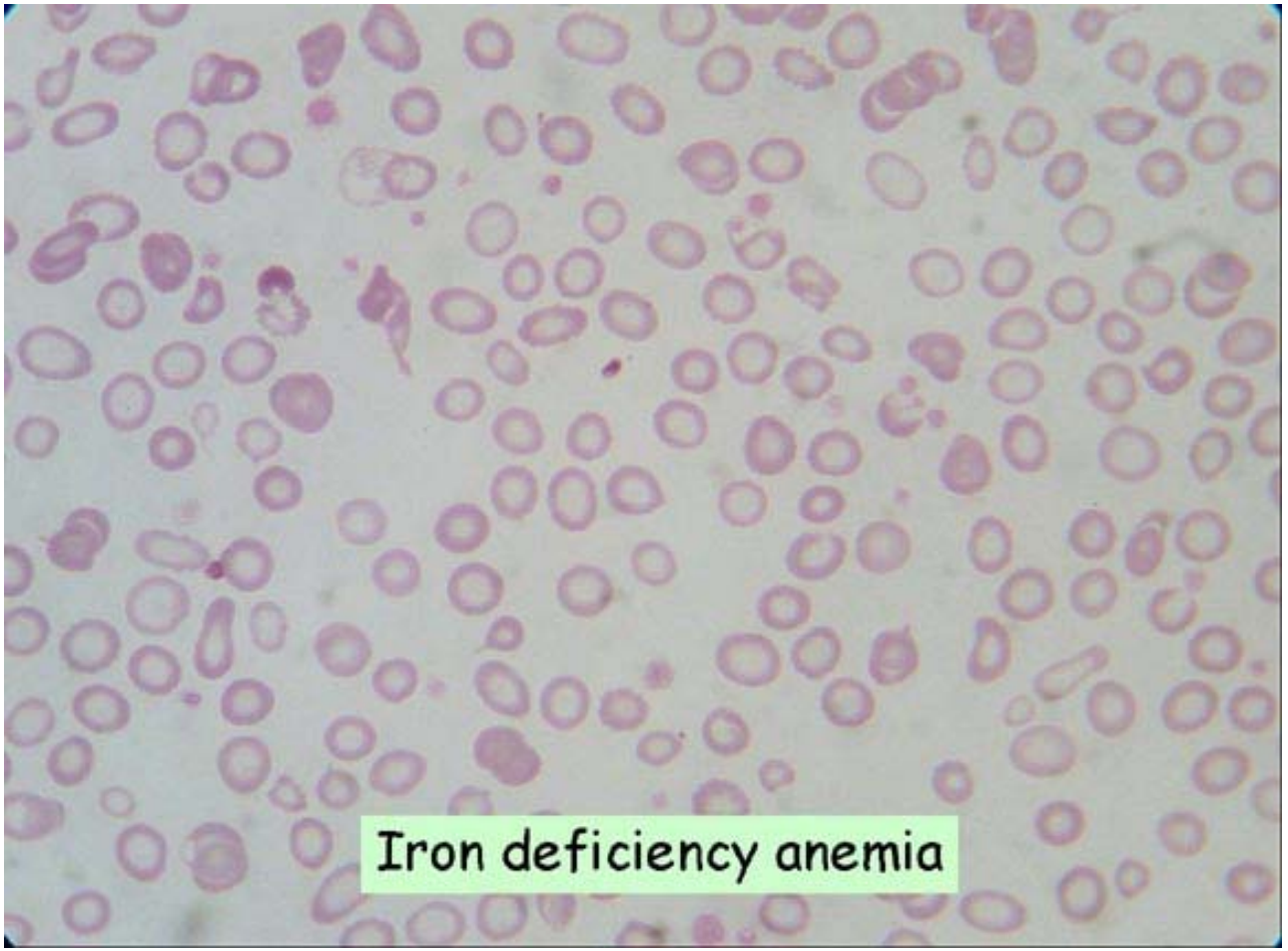
- Growth: Impaired growth in infancy
- Neuromuscular system: Impaired muscular performance as measured by standardized exercise tests
- Epithelial tissue
- Nails: Brittle, longitudinally ridged, thinning, flattening, kolionychia(Spoon shaped nails)
- Tongue & mouth: Atrophy of the lingual papillae, angular stomatitis
- Dysphagia: Difficulty in swallowing solid foods but little problem in swallowing liquids
- Stomach: Presence of gastritis & reduction in gastric secretion

Clinical manifestations…

- Immunity & infection: Defective cell mediated immunity & impaired bacterial killing by phagocytes
- Pica
- Spleen: Enlarged in 10% patients
- Genitourinary system: Frequent disturbances in micturition
- Skeletal system: Changes similar to those found In Thalassemia

Laboratory investigations

- Microcytic hypochromic anemia
- MCV <80 Fl, MCH<25 gm/dl
- Serum iron < 30 micro grams/dL(Normal:50-150)
- Total iron binding capacity: raised
- % saturation of transferrin:<10%(30-50)
- S. ferrritin: <15 microgram/L
- Cigar / pencil RBCs
- With iron treatment, reticulocyte counts increase after 3-4 days, peak at 10 days


Iron deficiency anemia

Aplastic anemia

- Acquired

  Chemical &physical agents: Benzene, radiations, antifolic compounds

  Other causes: Viral infections(Hepatitis, EBV, HIV)
- Familial

  Fanconis anemia, pancreatic deficiency in children

Symptoms & signs

- Anemia , bleeding, fever , infections
- Weakness, fatigue
- Bleeding from the, nose, mouth, GIT
- Ulcerations in the mouth & pharynx

Polycythemia/Erythrocytosis

Increase RBC & Hb levels

Classification

- Relative: Reduced plasma volume
- Absolute

➢ Primary: Abnormal proliferation of myeloid stem cells(polycythemia vera)

➢ Secondary: Lung disease, high altitude, erythropoietin secreting tumour

Polycythemia vera

- Increase RBC
- Increase blood volume
- Increase viscosity
- Increase hematocrit

Effect of polycythemia on function of the cardiovascular system

Cardiac output

Arterial pressure

Colour of skin