Blood
# Approach to hematological disorders

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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
Salinity: 0.9%
Colour: Bright red to deep red
Components of Whole Blood

- **Plasma** (55% of whole blood)
- **Buffy coat**: leukocytes and platelets (<1% of whole blood)
- **Erythrocytes** (45% of whole blood)

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

1. Withdraw blood and place in tube
2. Centrifuge
Components of blood

Composition

Plasma
- 55%
  - Water
    - 90%
  - Solids & Gases

Cells
- 45%
  - WBC
    - 4000-11000/mm³
  - RBC
    - 4.8–5.4 million/mm³
  - Platelets
    - 1.5-4 lakh/mm³
BLOOD is composed of:

- Plasma
  - Water
  - Ions
  - Organic molecules such as:
    - Amino acids
    - Proteins
    - Glucose
    - Lipids
    - Nitrogenous waste
  - Trace elements and vitamins
  - Gases such as:
    - CO₂
    - O₂

- Cellular elements
  - Red blood cells
  - White blood cells include:
    - Lymphocytes
    - Monocytes
    - Neutrophils
    - Eosinophils
    - Basophils
  - Platelets
Components of plasma (90% water rest solids)

- **Solids**
  - Plasma Proteins: 6-8 gm/dL
    - Albumin: 3-4 gm/dL
    - Globulin: 2.5-4 gm/dL
    - Fibrinogen: 0.3 gm/dL
  - Regulatory & protective proteins
    - Hormones
    - Antibodies
    - Enzymes
  - Organic and inorganic substances
    - Waste material
    - Nutritive material
    - Ions
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) polyacrylamide 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

1. Denature sample with sodium dodecylsulfate

   SDS-coated proteins

2. Place mixture of proteins on gel, apply electric field

   Partially separated proteins

3. Stain to visualize separated bands

   Decreasing size

SDS is an ionic detergent that binds stoichiometrically to number of peptide bonds, thus separates on basis of size (MW).
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

(A) Ion-exchange Chromatography
- Positively charged bead
- Bound negatively charged molecule
- Free positively charged molecule

(B) Gel-filtration Chromatography
- Porous beads
- Retarded small molecule
- Unretarded large molecule

(C) Affinity Chromatography
- Bead with covalently attached substrate
- Bound enzyme molecule
- Other proteins pass through
Separation procedures...

- Immunoelectrophoresis
- ELISA (Enzyme linked immunosorbent 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

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 an 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

Apply sample

20 min

Separating serum proteins by electrophoresis

Albumin

alpha-globulin

beta-globulin

gamma-globulin
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, $\alpha$ and $\beta$ globulin, fibrinogen
B lymphocytes in lymph nodes, bone marrow: $\gamma$ 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 transcytosis 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
- $\alpha_2$ macroglobulin: Inhibitor of serum endoprotease
- Caeruloplasmin: Transports copper
- Haptoglobin: Transports free hemoglobin
- Transferrin: Transports iron
- Fibrinogen: Precursor of fibrin
- C3 & C4: Proteins of complement system
- $\alpha$ 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\textsuperscript{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: $\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^\cdot + \text{OH}^-$
\( \alpha_1 \)- ANTITRYPsin
\( (\alpha_1\text{-antiproteinase}) \)

- 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

Proteolytic 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

Tissue injury or infection

Local inflammation

Activated macrophage

IL 1 and TNF α

Systemic acute phase response

Fever

Hepatocyte

Acute phase reactants
Acute phase reactant response

- **Activated macrophage**
  - IL-1
  - TNF
  - IL-6

- **Hepatocyte**
  - CRP
  - SAA
  - Hp
  - Fibrinogen
Acute phase proteins

- α1 antitrypsin
- Fibrinogen
- Complement
- Haptoglobin
- C-reactive protein
Mammalian embryonic blood formation

**Day 1**
Fertilization

**Day 2**
2 Cell Stage

**Day 3**
Morula

**Day 4**
Early Blastocyst

**Day 5**
Late Blastocyst

**Day 12**
Maternal and Trophoblast Vessels

Human embryo at various stages
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

- Red Blood Cells
  - Red Blood Cells
- White Blood Cells
  - Lymphocyte
  - Monocyte
  - Eosinophil
  - Basophil
  - Neutrophil
- Platelets

Diagram showing the structure and components of red bone marrow.
Bone marrow consists of blood cells in different stages of development and supporting tissue known as the **stroma** (mattress).

- Mature blood cells squeeze through the endothelium to reach the circulation.
- Platelets
- Fragments of megakaryocyte break off to become platelets.
- Reticular cell
- The stroma is composed of fibroblast-like reticular cells, collagenous fibers, and extracellular matrix.
- Stem cell
- Neutrophil maturation
- Reticular fiber
- Red blood cell maturation
- Venous sinus
- Reticulocyte expelling nucleus
- Macrophage
- Monocyte
- Lymphocyte
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 pluripotent cells were progressively more committed to a single lineage
• 1961 (Till & Mc Culloch) demonstrated that single cells were capable of establishing nodules of hemopoietic growth in spleens of irradiated mice & that such colonies displayed multilineage differentiation
Developmental models for hematopoiesis

Diagram showing hemoglobin switch between embryonic, fetal, and adult sites.
Terminology of stem cells

- Lymphoid Line
  - erythroid
  - granulocyte
  - megakaryocyte

- Non-lymphoid line (myeloid)
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

15-20 µm
Nucleus: Big
Hb: absent

Nucleus: size ↓
Nucleoli: absent
Hb: absent

10-14 µm
Nucleus: Size ↓
Hb: Starts appearing

7-10 µm
Nucleus: cart wheal-pyknotic
Hb: increases
Erythropoiesis

Progenitors → Erythroblast Precursors → Erythrocytes

HSC → BFU-E → CFU-E → ProE → BasoE → PolyE → OrthoE

Bone marrow

Colony formation (in vitro)

Hemoglobin accumulation
Nuclear condensation
Decreased cell size
Decreased mRNA
Morphologically identifiable

Bloodstream

Enucleation
Reticulocyte
Q

• Why does the cytoplasm become more eosinophilic as RBCs mature
M:E ratio = 3:1(2.3)
In accelerated erythropoiesis the ratio
• Increases
• Decreases
• Doesn’t change
• None of the above
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)

Side view

Top view

2.0 μm

7.5 μm

Figure 17.3
PBS under low power
PBS under high power

Blood Smear  Leishman

- erythrocytes
- blood platelets
## Mature Erythrocyte

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

Current is measured across an aperture 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 aperture 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 erythrocyte.
• MCHC gives you the average hemoglobin concentration per erythrocyte.
RBC indices

MCV: Average volume of the RBC

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

MCH: Average Hb concentration of a RBC

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

MCHC: Ave. Hb concentration per RBC

\[
MCHC = \frac{Hb(\text{gm/dl}) \times 100}{PCV(\%)}
\]
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 → G-6-P → F-6-P → ATP → F-1,6-P → Ribulose-5-P → DHAP → 3-P-Glyceraldehyde → NADH → 1,3-DPG → 2ADP → 2ATP → 3-PGA → 2-PGA → PEP

→ NADH → NADPH → GSH + O² → GSSH + H₂O

→ NADPH → NADP⁺
ATP production in anaerobic glycolysis
NADPH synthesis
• Normally H₂O₂ 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
2, 3 DPG generation
Role of 2,3 DPG

T state
Taut /Tense

R state
Relaxed
Energy metabolism in RBC

Glucose

- Anaerobic Glycolysis
  - 2 ATP ← 2 ADP
  - G-6-P
  - F-6-P
  - Ga-3-P

- Rapoport-lubering shunt
  - 2,3 diphospho glycerate

- Pentose phosphate pathway
  - CO2
  - Ribose phosphate

Lactate
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 37°C 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.
2. *Intrinsic proteins*

**Examples**

(1). **Multipass (band 3/anion exchanger)** -- Catalyzes reversible exchange of the anions $\text{HCO}_3^-$ (bicarb) and $\text{Cl}^-$ between RBC and plasma. Exchange allows max. transport of $\text{CO}_2$ in blood (as bicarb in solution)

(a) Basic point: Bicarb is much more soluble in plasma than $\text{CO}_2$, so lots of bicarb (but not much $\text{CO}_2$) can be carried in the blood. Therefore need to covert $\text{CO}_2$ to bicarb when want to carry $\text{CO}_2$ in blood; need to do reverse to eliminate the $\text{CO}_2$ (in lungs).

(b) Conversion of $\text{CO}_2$ to bicarb (& vice versa) can only occur *inside* RBC, where the enzyme carbonic anhydrase is. Carbonic anhydrase catalyzes:

$$\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$$
(c). Gases can pass through membranes by diffusion -- CO₂ 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 CO₂ is high, as in tissues, CO₂ 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 CO₂ inside the RBC (reaction above goes to left). Then the CO₂ 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:
• \((0.02 \times 1,100,000) = 22,000\) 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.
Anisocytosis

Variations in erythrocyte size

- **Microcytic**: $MCV = <80 \text{ fL}$ & $\text{size} = <6 \mu\text{M}$
  Eg. iron-deficiency anemias.
- **Normocytic**: $MCV = 80 - 100 \text{ fL}$ & $\text{size} = 6 - 9 \mu\text{M}$
- **Macrocytic**: $MCV = >100 \text{ fL}$ & $\text{size} = >9 \mu\text{M}$
  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, IL-3, IL-6, GM-CSF, G-CSF, SCF

GM-CSF
EPO

GM-CSF
IL-5

IL-3
IL-4

Blood stem cell
Myeloid stem cell
Lymphoid stem cell

Proerythroblast
Myeloblasts
Monoblast
Megakaryoblast

Promonocyte
Promegakaryocyte

Early erythroblast
Late erythroblast

Eosinophilic Basophilic
myelocyte myelocyte

Neutrophilic myelocyte

Neutrophilic band cell

Eosinophil
Basophil
Neutrophil

Monocyte
Platelets
B Lymphocyte
T Lymphocyte

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)
- Macrophages: Degrading hemoglobin → Free iron
- Hemoglobin Red Cells
- Transferrin–Fe
- Plasma
- Tissues: Ferritin, Hemosiderin, Heme, Enzymes
- Blood loss—0.7 mg Fe daily in menses
- Fe^{2+} absorbed (small intestine)
- Fe excreted—0.6 mg daily

Pinocytosis
Iron metabolism

Why is iron required?
Hemoglobin: 65%
Myoglobin: 4%
Cytochromes
Cytochrome oxidases
Peroxidase
Catalase
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

- Fe in gut lumen
- Body Fe
- Normal
- Unaccepted Fe
- Fe deficient
- Fe overload
- Fe deficient
Copper: Promotes absorption, mobilization & utilization of iron

Vitamin C
Folic acid
Vitamin B12
Regulation of RBC production

**Diagram:**

- Hematopoietic Stem Cells
  - Proerythroblasts
    - Red Blood Cells
      - Tissue Oxygenation
        - Factors that decrease oxygenation:
          1. Low blood volume
          2. Anemia
          3. Low hemoglobin
          4. Poor blood flow
          5. Pulmonary disease

- Kidney
- Erythropoietin
  - Decreases
Erythropoietin Mechanism

Start

Stimulus: Hypoxia due to decreased RBC count, decreased availability of O₂ to blood, or increased tissue demands for O₂

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

(a) Hemoglobin

(b) Iron-containing heme group

Figure 17.4
Life Cycle of Red Blood Cells

1. Low O₂ levels in blood stimulate kidneys to produce erythropoietin
2. Erythropoietin levels rise in blood
3. Erythropoietin and necessary raw materials in blood promote erythropoiesis in red bone marrow
4. New erythrocytes enter bloodstream; function about 120 days
5. Aged and damaged red blood cells are engulfed by macrophages of liver, spleen, and bone marrow; the hemoglobin is broken down

Hemoglobin

Heme

Globin

Iron stored as ferritin, hemosiderin

Amino acids

Iron is bound to transferrin and released to blood from liver as needed for erythropoiesis

Billirubin is picked up from blood by liver, secreted into intestine in bile, metabolized to stercobilin by bacteria and excreted in feces

Food nutrients, including amino acids, Fe, B₁₂, and folic acid are absorbed from intestine and enter blood

Raw materials are made available in blood for erythrocyte synthesis
Sites of erythropoiesis
Genes regulating hemoglobin synthesis

Genes: \( \zeta \), \( \epsilon \), \( GyA\gamma \), \( \alpha \), \( \beta \), \( \delta \)

Chains: \( \zeta \), \( \epsilon \), \( \alpha \), \( \beta \), \( \delta \)

Haemoglobins: \( \gamma_2\gamma_2 \), \( \epsilon_4 \), \( \alpha_2\epsilon_2 \), \( G_y \), \( A_\gamma \), \( A \), \( \alpha_2\gamma_2 \), \( \alpha_2\beta_2 \), \( \alpha_2\delta_2 \)

Portland 1, Gower 1, Gower 2, F, A, A_2
Normal hemoglobin types

In the embryo
- Gower 1 (ζ2ε2)
- Gower 2 (α2ε2)
- Hemoglobin Portland (ζ2γ2)

In the fetus
- Hemoglobin F (α2γ2)

In adults:
- Hemoglobin A (α2β2) The most common with a normal amount over 95%
- Hemoglobin A₂ (α₂δ₂) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- Hemoglobin F (α₂γ₂) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells

Elevated in persons with sickle-cell disease.
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 A1 ($\alpha_2\beta_2$): Appears in 5th month of IUL
- Hemoglobin A2 ($\alpha_2\delta_2$): makes up 3% of adult hb
Developmental profile of hemoglobins
Variant forms of hemoglobin which cause disease

• Hemoglobin H (β₄) - tetramer of β chains, which may be present in variants of α thalassemia
• Hemoglobin S (α₂βₛ²) - β-chain gene, causing a change in the properties of hemoglobin which results in sickling of red blood cells.
• Hemoglobin C(α₂βᶜ₂) - 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
[13] Decrease in sialic acid
Mechanism of red cell destruction

Average 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

Heme
- Heme oxygenase
  - Biliverdin+iron+CO
    - Reductase
      - Bilirubin
        - (Plasma levels: <1mg/dl)
          - Conjugated/Unconjugated

Globin
- Amino acids
  - Excreted via lungs
Hepatic handling of bilirubin

3 steps
1. Uptake
2. Conjugation
3. Excretion

Blood

B-Alb

Alb

1

GST

BG

2

Bilirubin glucuronide

3

BG

Large intestine

Stercobilinogen

Feces 80%

E H circulation 20%

Regurgitation
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 $> 2.5 \text{ mg/dL}$
First site where it is detected: sclera
Types:
• Hemolytic
• Hepatic
• Obstructive
## Types of jaundice

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<thead>
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<th>Jaundice</th>
<th>Hemolytic</th>
<th>Hepatic</th>
<th>Obstructive</th>
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<tr>
<td>Fecal stercobilinogen</td>
<td>Increased</td>
<td>Decreased</td>
<td>Absent</td>
</tr>
<tr>
<td>Urinary urobilinogen</td>
<td>Increased</td>
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<td>Absent</td>
</tr>
<tr>
<td>Urinary bilirubin</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Liver function test</td>
<td>Normal</td>
<td>Impaired</td>
<td>May be impaired</td>
</tr>
</tbody>
</table>
Van den Berg test: Principle

Conjugated bilirubin + diazo reagent

Reddish violet coloured compound

Appears Immediately

Direct positive

Obstructive jaundice

Doesn’t appear immediately + Alcohol

Appearance of reddish violet colour

Indirect positive

Hemolytic jaundice
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<tr>
<td>Van den Bergh test</td>
<td>Indirect</td>
<td>Biphasic</td>
<td>Direct</td>
</tr>
</tbody>
</table>
Physiological jaundice

Neonatal jaundice
More common in premature & LBW babies
Appears on 2-3\textsuperscript{rd} 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

A change in pH or temperature causes the curve to shift. This curve shift represents ease of oxygen loading or unloading.
Left shift
- Decreased temp
- Decreased 2-3 DPG
- Decreased [H+]
- CO

Right shift (reduced affinity)
- Increased temp
- Increased 2-3 DPG
- Increased [H+]
**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 pO₂.
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, chloionychia
- 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

Glossitis & 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

<table>
<thead>
<tr>
<th></th>
<th>Normochromic</th>
<th>Hypochromic</th>
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</thead>
<tbody>
<tr>
<td>Normocytic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrocytic</td>
<td></td>
<td></td>
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<tr>
<td>MCV &gt; 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcytic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV &lt; 80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Normochromic
- Hypochromic

- Normocytic: Normal size and normal color
- Macrocytic: Normal size, increased color
- Microcytic: Decreased size, normal color

Conditions:
- Recent blood loss
- All hemolytic anemias except thalassemia
- Aplastic anemia
- Endocrine abnormalities
- After chronic hemorrhage

- Megaloblastic anemia: Secondary to liver disease
- Iron deficiency anemia
- Thalassemia
Signs & symptoms of blood loss anemia

**Acute blood loss**
- >30% loss suddenly: 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
• Methemoglobinemia
• Jaundice: Unconjugated hyperbilirubinemia
• Hemosiderinuria
Specific types of hemolytic anemias

Spherocytosis
Thalassemia
Sickle cell anemia
Extracorpuscular defect
Erythroblastosis
Mechanical trauma
Malaria
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
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
Thalassemia

- 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 thalassemia

Alpha Globin Gene Deletion
Two Genes per Chromosome

**Scenario 1**

**Scenario 2**

Hemoglobin H Disease

Hydrops Fetalis (fatal in utero)
Beta Thalassemia

- mutations on chromosome 11
- results in *excess of alpha globins*
Beta Thalassemia Trait ($\beta^0/\beta$)

- 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 $\beta^0/\beta^0$

- 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 disease
- HbS/β⁺: Sickle beta plus thalassemia
- HbS/β⁰: Sickle beta zero thalassemia
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 preceded 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

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<tr>
<th>Chronic hemolysis</th>
<th>Vaso-occlusive</th>
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<td>• Anemia</td>
<td>• Pain syndrome</td>
</tr>
<tr>
<td>• Pigment gall stones</td>
<td>• Acute chest syndrome</td>
</tr>
<tr>
<td>• Aplastic episodes</td>
<td>• Priapism</td>
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<tr>
<td>• Jaundice</td>
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<tr>
<td>• Delayed growth</td>
<td>• Retinopathy</td>
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<tr>
<td></td>
<td>• Avascular hip necrosis</td>
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<tr>
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<td>• Spleenic sequestration</td>
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<td>• Leg ulcers</td>
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<td>• Chronic nephropathy</td>
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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

- Fatigue
- Jaundice
- Back pain
- Arm and Leg pains
- Hand Swelling
- Joint pain
- Foot swelling
- Breathlessness
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
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 deficiency
  Defective globin synthesis: Thalassemia
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\(_{12}\)

Stomach

- Pepsin

Duodenum

- R binders
- R-B\(_{12}\) complex
- Pancreatic protease

Ileum

- Vit. B\(_{12}\)
- Intrinsic factor
- Vit. B\(_{12}\)-IF complex
- Absorbed in ileum
Folic acid

- Folic acid deficiency (can develop quickly)
  
  Inadequate intake
  
  Increased requirement: pregnancy, infancy, malignancy, hemodylasis
  
  Malabsorption: Tropical sprue, phenytoin, barbiturates
  
  Impaired metabolism: Methotrexate
Folic acid/ pteroylglutamic acid

- Animals cannot synthesise folates
- Source: Plants (Polyglutamate conjugates)

Diet → Intestine → Intestinal cell

Polyglutamates → Monoglutamates → Tetrahydro folate

Carboxy peptidase → Folate reductase
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
- Glycine
- Serine
- Methyl THF
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
• Ineffective 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\textsubscript{12} levels
• Methyl malonic acid excretion in urine
• Dramatic response on parenteral administration of Vit. B\textsubscript{12}
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\textsubscript{12}** is usually well below 160 ng/L, which is the lower end of the normal range. Serum vitamin B\textsubscript{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

Histidine \rightleftharpoons_{THF} FIGLU \rightleftharpoons_{THF} Glutamate

Oral challenge of Histidine
Increased urinary excretion of FIGLU in folate deficiency as well as B12 deficiency
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. lettuce, 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
  ➢ Polyphenols 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

Heme + apoprotein $\rightarrow$ Acid & protease $\rightarrow$ Hemin $\rightarrow$ Heme oxygenase (enterocyte) $\rightarrow$ Free Fe$^{2+}$ pool

Ferritin $\uparrow$ Apoferritin

Hemopexin bound Fe (Plasma)
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

Fe in gut lumen

Unaccepted Fe

Normal

Body Fe

Fe deficient

Fe overload

Fe deficient
Iron

- Bilirubin (excreted)
  - Macrophages
    - Degrading hemoglobin → Free iron
  - Hemoglobin
  - Red Cells
    - Blood loss—0.7 mg Fe daily in menses
  - Fe²⁺ absorbed (small intestine)
  - Fe excreted—0.6 mg daily
- Tissues
  - Ferritin
  - Hemosiderin
  - Heme
    - Enzymes
- Plasma
  - Transferrin—Fe

Pinocytosis
Iron comes from the diet.

Fe absorbed by active transport.

Transferrin protein transports Fe in plasma.

Liver stores excess Fe as ferritin.

Bone marrow uses Fe to make hemoglobin (Hb).

Spleen converts Hb to bilirubin.

Liver metabolizes bilirubin and excretes it in bile.

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
• Pregnancy 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
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