THE BLOOD

BLOOD

- One of the largest organs of the body.
- An average 70 kg man has almost 5L blood (5.5 kg).
- Blood circulates throughout the body and supports the functions of all other body tissues.

Blood integrates
tissues and organs
and provide
a special means of
communications.

THE FUNCTIONS OF BLOOD

- Respiration: transport of O_2 and CO_2.
- Transport: hormone, nutrients, metabolic waste.
- Excretion of metabolic wastes to the kidney, lungs and skin.
- Regulation of body temperature by distribution of body heat.
- Defense against infections (WBCs, antibodies).
- Maintenance of acid-base balance.
- Nutrition: transport of absorbed food material.

PHYSICAL PROPERTIES OF BLOOD

- Specific gravity:
  - Whole blood: 1.055 - 1.065
  - Plasma: 1.024 - 1.028
- Viscosity: 5-6 times that of water.
- Mass: 6-8% of the body weight.
- Blood volume: ~ 8% of body weight.
  ~ 86% ml/kg body weight.
  5-6L in adults

  [Infants have a larger blood volume in proportion to body weight than adults].
- Osmotic pressure: 7-8 atmosphere at body temperature.

Composition of Blood

- Formed Elements (45%),

  i- Red blood cells (erythrocytes).
  ii- White blood cells (leukocytes).
  iii- Platelets (thrombocytes)

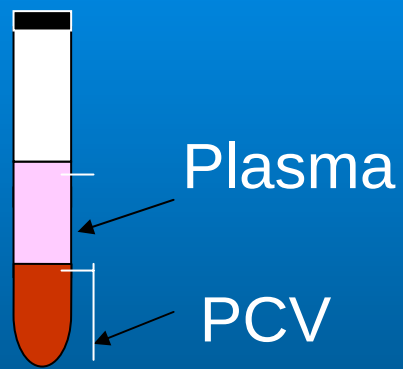
- Fluid medium i.e. the plasma (55%).

Gross composition of Plasma and Blood Cells

Constituents	Plasma	Red blood cells
Water	91-95%	65%
Solid	8-9 %	35%
Protein	6-8 gm %	31-33%
Specific gravity	1.026	

Haematocrit or Packed Cell Volume (PCV)

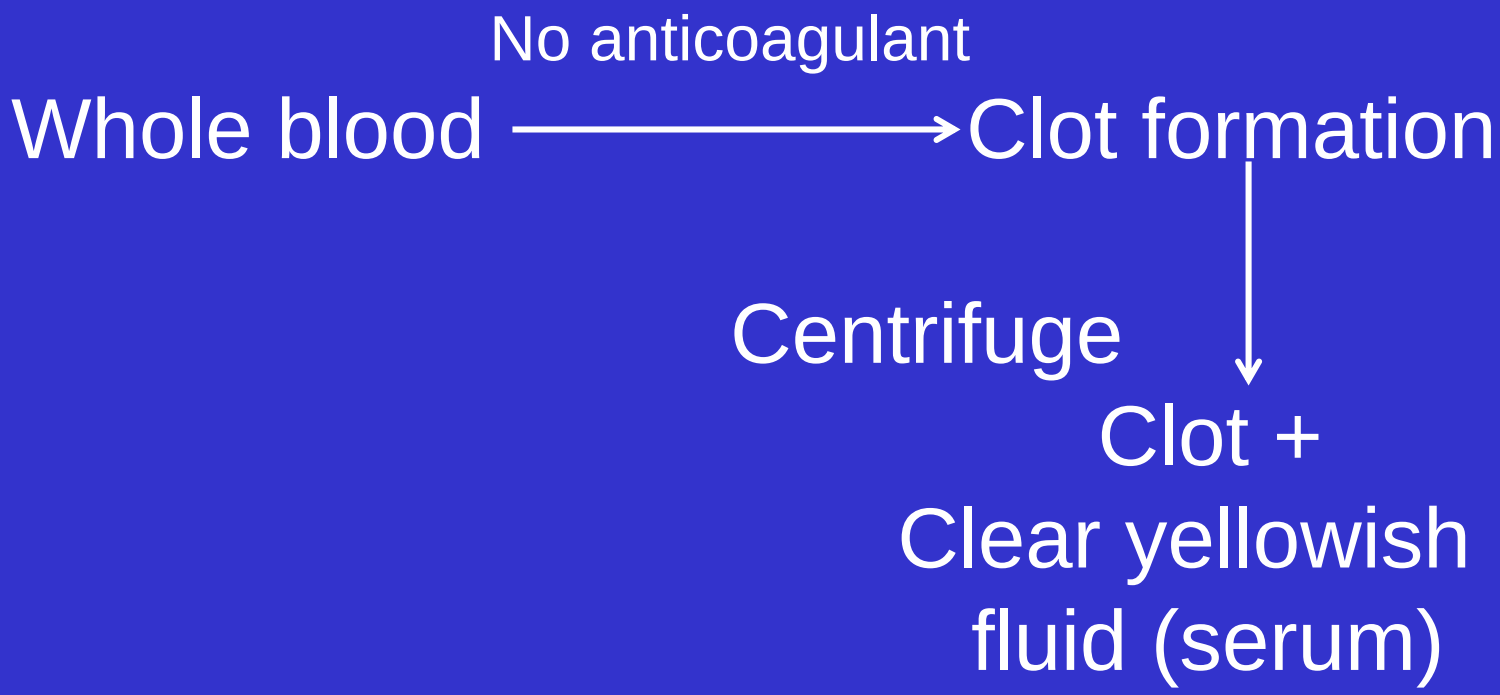
Whole Blood → Anti-coagulant (EDTA, Oxalate, Citrate, heparin) → No clotting → Centrifugation



PCV = 0.45 L/L
= 0.41 L/L

PCV ↓ is anaemia
↑ is polycythaemia

SERUM


No anticoagulant
Whole blood
Clot formation
Centrifuge
Clot +
Clear yellowish
fluid (serum)

ERYTHROCYTE SEDIMENTATION RATE (ESR)

- Rate of Sedimentation of Erythrocytes.
- ESR at 20 ± 3 °C (Westergress Method)

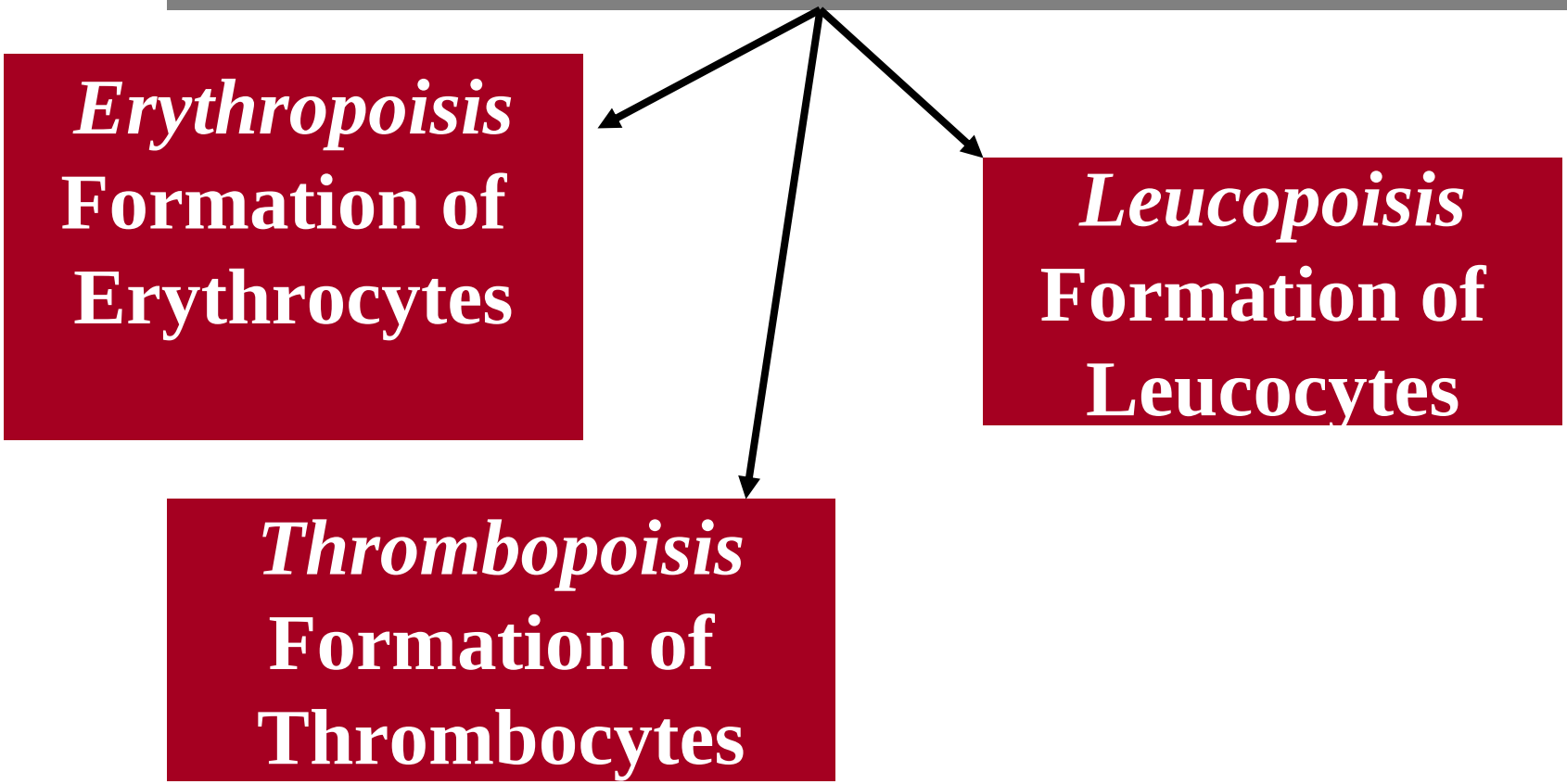
  Male = 0-5 mm

  Female =0-7 mm

- ESR - Non-specific indicator of infection.
- ↑ ESR - For monitoring status of chronic inflammatory diseases.

Haemopoisis

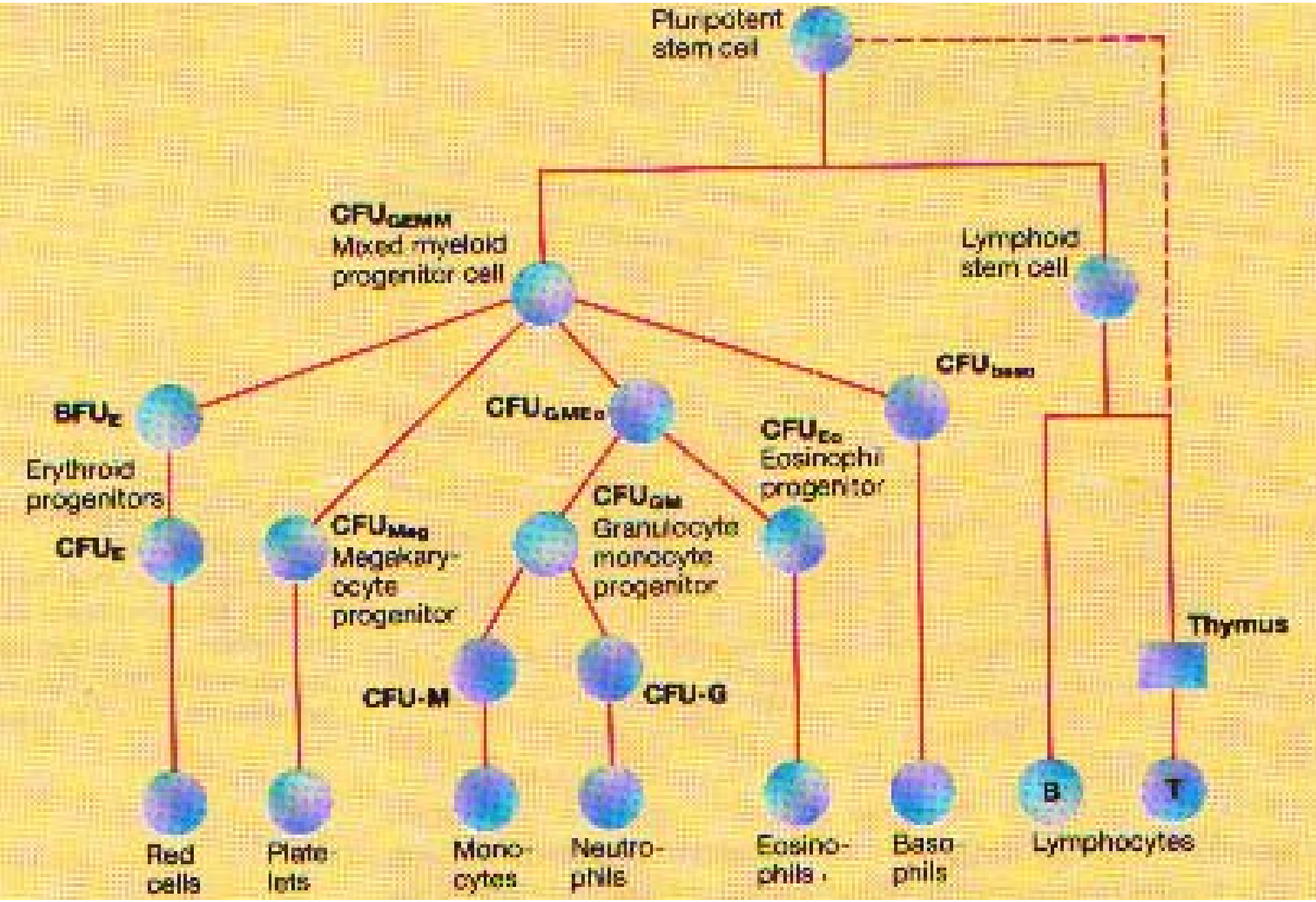
The process of formation of blood.



Process	Product
♦ Erythropoesis	RBC
♦ Leucopoiesis	WBC
♦ Granulopoiesis	Granulocytes
♦ Lymphopoiesis	Lymphocytes
♦ Megakaryocytes	Platelets

Site of Haemopoisis

Fetal Life	
1-2 m	**Yolk Sac**
2-6 m	**Spleen**
1-9 m	**Liver**
from 4 m	**Bone marrow**
At Birth	**Bone marrow**
Adult life	**Bone marrow**


Plurpotent stem cel
CFU-GEMM Mixed myeloid progenitor cell
Lymphoid stem cell
CFU-baso
BFU-E
CFU-GMEo
CFU-Eo Eosinophil progenitor
Erythroid progenitors
CFU-E
CFU-Meg Megakary-ocyte progenitor
CFU-GM Granulocyte monocyte progenitor
Thymus
CFU-M
CFU-G
Red cells
Plate-lets
Mono-cytes
Neutro-phils
Eosino-phils
Baso-phils
B
T
Lymphocytes

Erythropoisis—A two stage differentiation system

Stage 1: From Pluripotent stem cells to committed cells

Stage 2: From committed cells to the recognisable precursors

Stem cell (pluripotent)

Committed stem cells(CFU)

Pronormoblast

Basophil normoblast

Polychromatic normoblast I and II

Orthochromatoblast

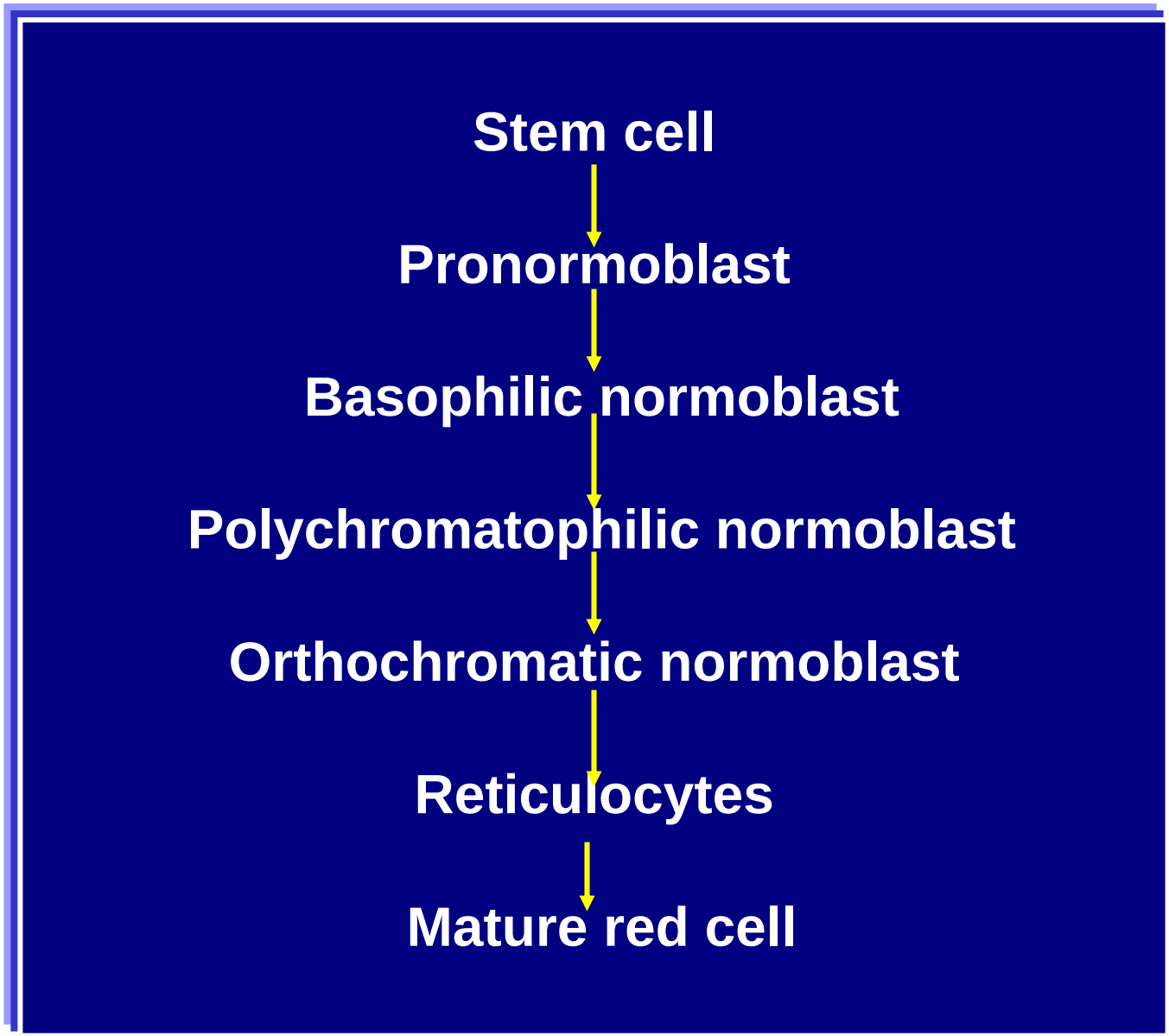
Reticulocytes

Erythrocytes

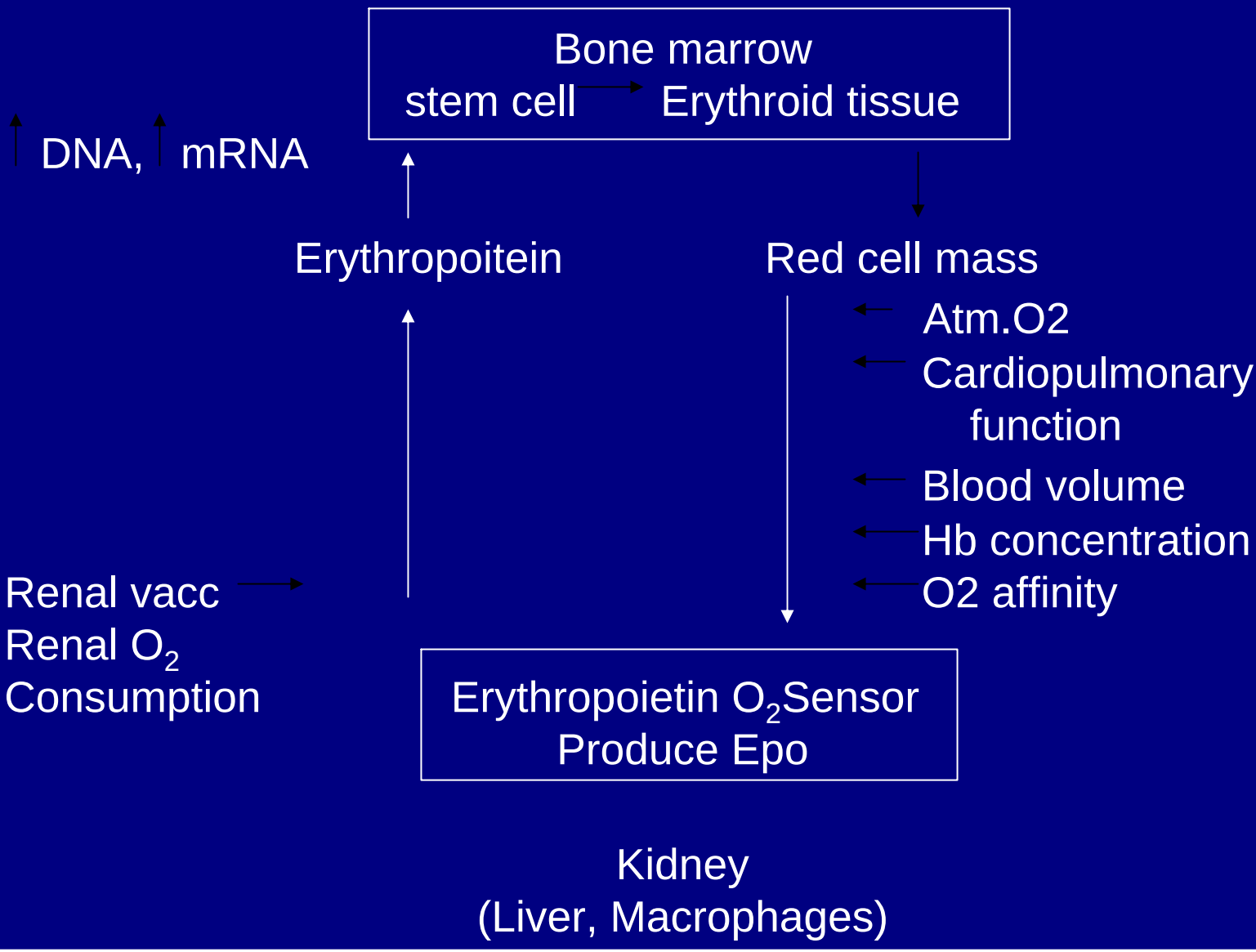
Control of Erythrocyte Synthesis

Cellular hypoxia	Oxygen sensor
↓	
E-Releasing factor	Serum
↓	
Erythropoietin (E)	Kidney
↓	
Erythropoietin (E)	Serum
↓	
Stem cell	Bone Marrow

Erythropoisis



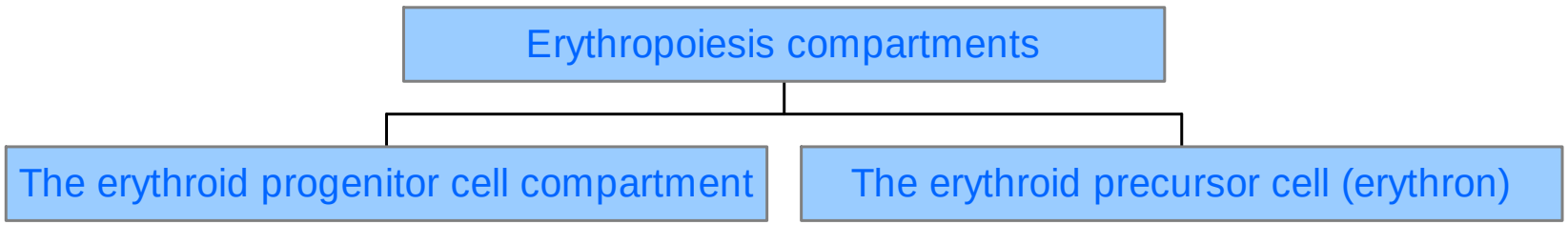
Current model of the feedback circuit which regulate the rate of RBC synthesis to the need for O_2 in the peripheral tissues.
(Surface receptors and intracellular secondary messenger):



Erythropoiesis

- The proliferation and differentiation of cells from pleuripotent noncommitted stem cells of the bone marrow.
- Two main compartments:

Erythropoiesis compartments



1.The erythroid progenitor cells

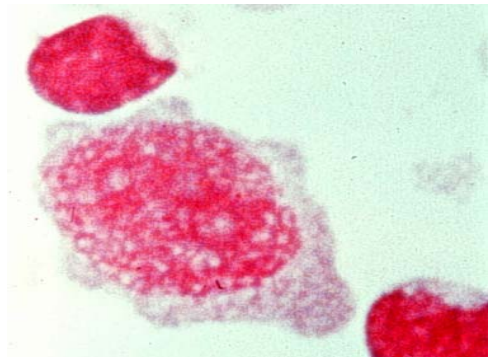
- The earliest recognizable committed progenitor for erythroid cells is the CFU-GEMM.
- The next are the BFU-E.
- The final progenitor cells are the CFU-E.

2. The erythroid precursor cell

- It is the morphologically recognizable erythroid cell within the normal bone marrow.

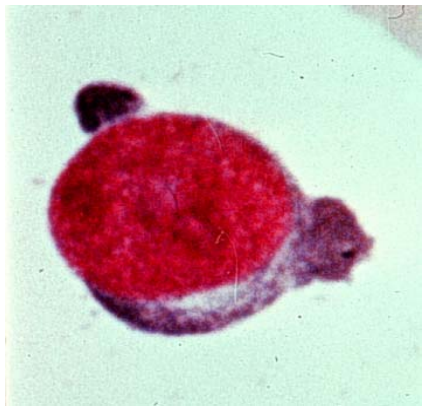
Cells involved in erythropoiesis

- 1.Pluripotent stem cell:
  - Most primitive haemopoietic cell.
  - Extensive capacity to proliferate.
  - Mature into other cell types:
    - Multipotent myeloid stem cell.
    - Lymphoid stem cell.



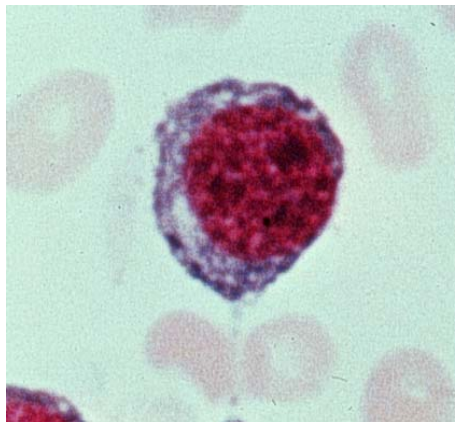
Cells involved in erythropoiesis

- 2. Pronormoblast:
  - earliest recognized cell in erythron.
  - A large cell.
  - Basophilic cytoplasm.
  - Has a large nucleus.
  - High conc. Of m.RNA.
  - 1% of protein is Hb.
  - 1000 receptor/cell for Epo.



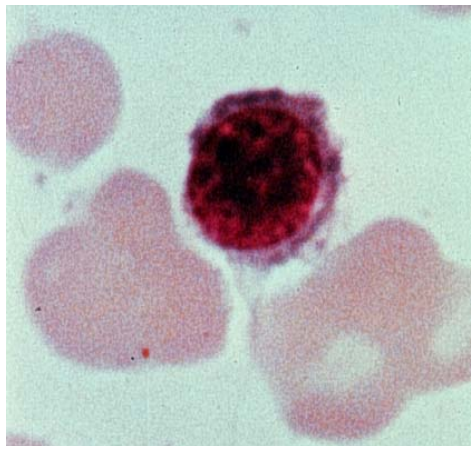
Cells involved in erythropoiesis

- 3. Basophilic normoblast:
  - Nucleolus is lost.
  - The golgi apparatus remains prominent.



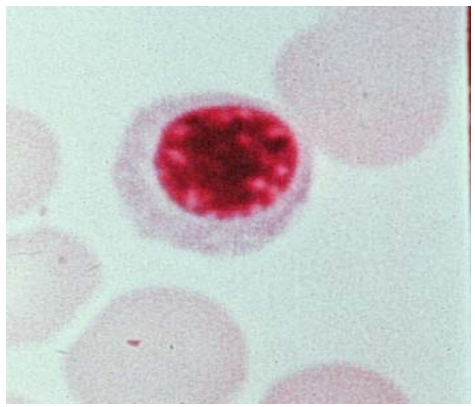
Cells involved in erythropoiesis

- 4. Polychromatophilic normoblast:
  - Hb production.
  - smaller nuclear:cytoplasmic ratio.
  - Chromatin is more clumped and condensed.



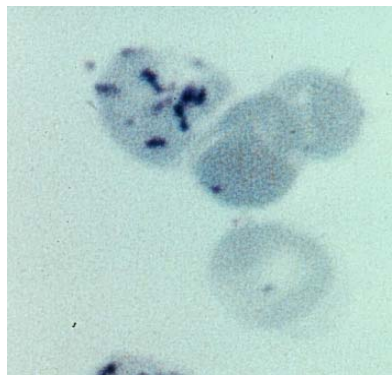
Cells involved in erythropoiesis

- 5. Orthochromatic normoblast:
  - cytoplasm is more eosinophilic.
  - Final nucleated stage.
  - > 300 receptors/cell for Epo.



Cells involved in erythropoiesis

- 6. Reticulocyte:
  - No nucleus.
  - Reticular networkes of polyribosomes.
  - It enters blood stream and circulate for 1-2 days they become mature RBC.
  - Rounder, faintly polychromatic, larger diameter than RBC.
  - 95% of cell protein is Hb.
  - No receptors for Epo.



Maturation

- Maturation of the proerythroblast within the bone marrow to the end of the basophilic erythroblast stages takes about 60 hrs.
- Maturation of the polychromatophilic erythroblast takes about 30 hrs, the late erythrocyte stage about 50 hrs and the reticulocyte in the steady state remains in the bone marrow for 40-48 hrs.

Transcription factors
for differentiation and maturation

- Random process.
- Epo prevent programmed cell death.
- Commitment of haemopoietic cells to the erythroid lineage involves action of several transcription factors including: TAL1, LMO2, and GATA-2.
- Genes for α- and β- chain of Hb are activated and controlled by *cis*-acting DNA sequence.
  - Other specific proteins: glycophorin A and EpoR.
  - Transferrin and genes of haem synthesis are also activated by *cis*-acting mechanism.

Transcription factors
for differentiation and maturation

- *Cis*-acting control DNA is activated by *trans*-acting factor.
- *Trans*-acting NF-E2 binds to specific sequence in locus control region for β-globulin, probably also to α-globulin.
- Other *cis*-acting promoter regions are involved in the regulation of genes coding for enzymes of haem synthesis including: porphobilinogen deaminase, ferrochetalase and δ-amino laevulinic acid synthetase.
- The m.RNA for these factors disappear after proerythroblast stage.

Transcription factors
for differentiation and maturation

- Upon binding Epo, cell surface EpoR dimerizes and activates specific intracellular kinases including: Janus family tyrosine protein kinase-2, phosphoinositol-3 kinase, and mitogen-activated protein kinase, and the RAS pathway.
- Other *trans*-acting DNA binding proteins are the erythroid Kruppel factor and the human stem cell leukemia genes.

Intrautreine erythropoiesis and postnatal changes

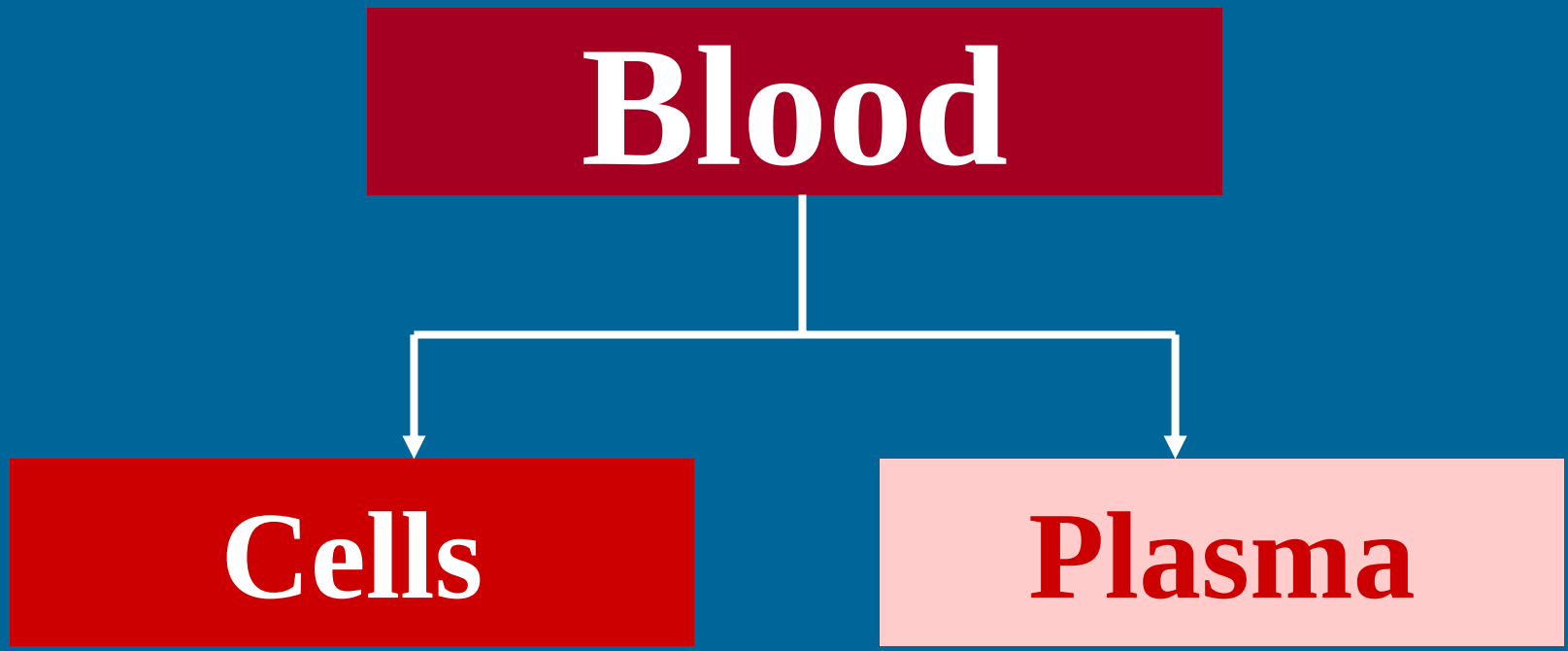
- Erythropoiesis occurs in two distinct waves during embryogenesis:
  - The primitive wave in the extra-embryonic sac of the 14-19 day human embryo.
  - The definitive wave in the fetal liver and spleen are the main sites of erythropoiesis in the 2nd trimester of pregnancy and the fetal bone marrow in the 3rd trimester.

Intrautreine erythropoiesis and postnatal changes

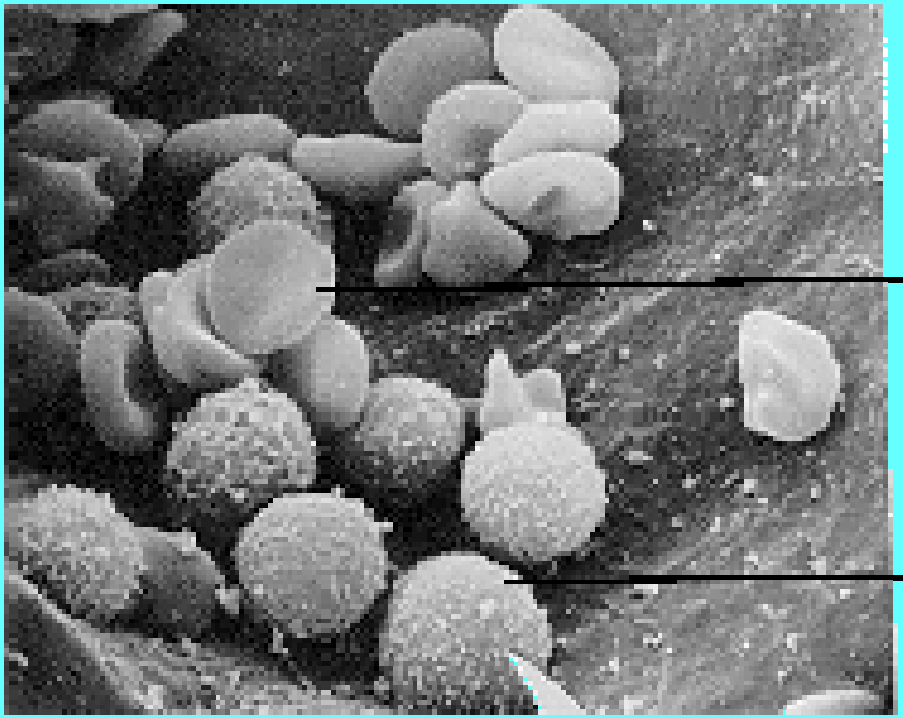
- The placenta activates fetal erythropoiesis by producing factors that stimulate erythropoiesis.
- The major site of Epo gene expression in the fetus is in the kidney.
- After birth, during the 1st 4yrs of life, nearly all the marrow cavities contain red haemopoietic marrow with very few fat cells. By the age of 25yrs the no. of fat cells increase.

Erythropoiesis in adults

- Erythropoiesis occurs within the haemopoietic marrow.
- Pregnancy is characterized by increased erythropoiesis within the maternal and fetal compartments.


Blood
Cells
Plasma

Blood Cells



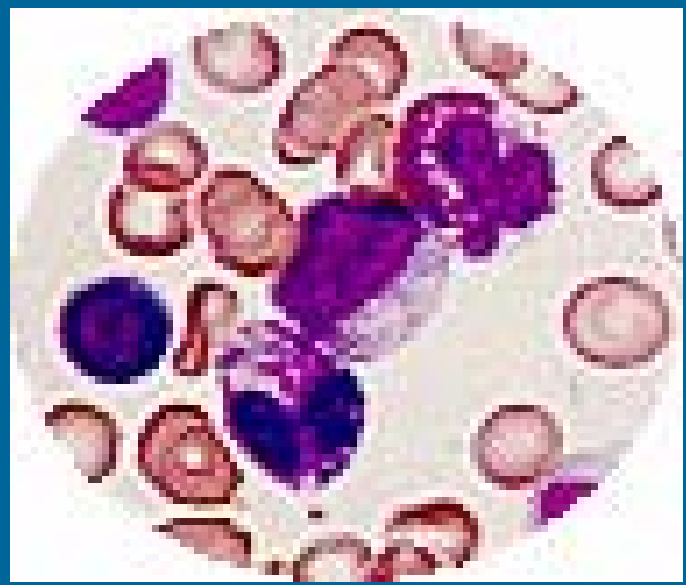
Mammalian blood cells in a small blood vessel

red blood cell

white blood cell

BLOOD CELLS

- Erythrocytes (Red blood cells)
- Leukocytes (White blood cells)
  - Granulocytes
    - Neutrophils
    - Basophils
    - Eosinophils
  - Monocytes
  - Lymphocytes
    - T
    - B
- Megacaryocyte (Platelets)



NORMAL RANGES

RBC

- Men 4.6 – 6.2 x 10^{12}/l.
- Women 4.2 – 5.4 x 10^{12}/l
- Total number of red cells in circulation = 2.5x10^{13}

WBC

- Men and women 5-7 x 10^{9}/l.

Platelets

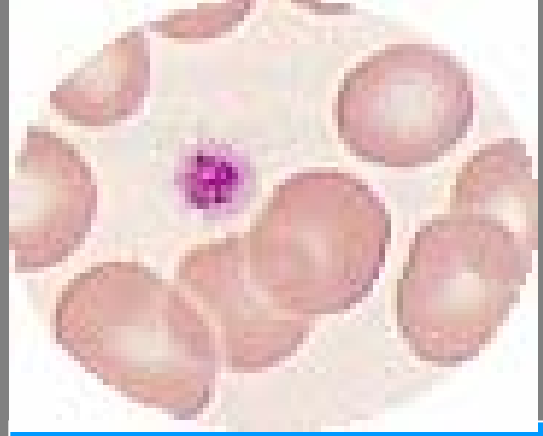
- Men and women 250 x 10^{9}/l

Hb

- Men 14 – 16 g/l
- Women 12 – 16 g/l

PCV (Haematocrit)

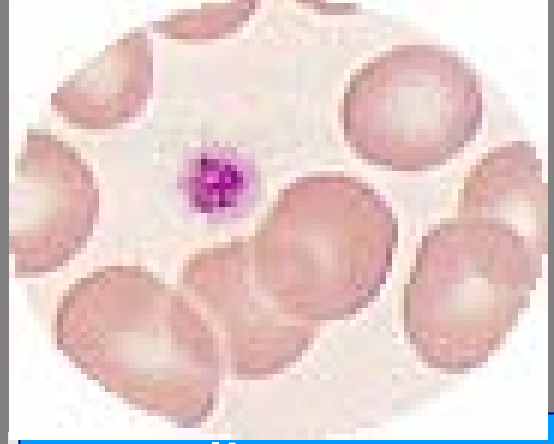
- Men 0.42 – 0.52 l/l
- Women 0.37 – 0.47 l/l



Red blood cells
(Erythrocytes)

- **Biconcave disks:** **Highly specialized**
  - **Diameter** **6 - 9 µm**
  - **Thickness:** **1 - 2 µm**
  - **Volume;** **~ 88 fl.**

- **Deformable -** **i.e. can change shape to transverse smallest blood vessels.**
- **Contain haemoglobin (~ 33%).**
- **No nucleus or mitochondria.**
- **Function: Transport of O_2 and CO_2.**
- **Normal Range:**
  - **$5.5 \pm 1.0 \times 10^{12}$/L**
  - **$4.8 \pm 1.0 \times 10^{12}$/L**



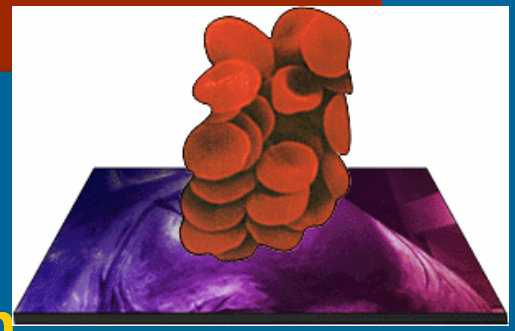
Red blood cells (Erythrocytes)

- Deliver oxygen to tissues and CO_2 from tissues to lungs.
- Synthesis is increased by erythropoietin
- Red cell life span is 120 days.
- Senescent red cells are destroyed by spleen and replaced by juvenile cells released by bone marrow.
- An average 70 Kg adult male produces $2.3x10^6$ red cell/sec.

ERYTHROPOIETIN

- A polypeptide hormone.
- Glycoprotein of 166 a.a. (mol. li 34 Kdl).
- Major regular of human erythropoiesis.
- Synthesized mainly in kidney, released in response to hypoxia and arts on bone marrow.
- Interacts with progenitor of red cells (BFU – E) via specific receptors causing proliferation and differentiation.
- Also interacts with late progenitor cell (CFU – E) to cause proliferation and differentiation.
- Requires cooperation of other factors e.g. interleukin-3 and insulin like growth factor.

ERYTHROCYTE STRUCTURE



- Biconcave shape. Spherical.
- Simple structure:
  - Membrane surrounding cytoplasm.
  - Almost 95% of solutes in cytosal is haemoglobin.
- No intracellular organnels
- Non-nucleated
- Has a cytoskeleton, which plays an important role in determining shape.
- Has deformability due to special structure of cytoskeleton

Erythrocytes Composition:

- **Major cation:** K^+
- **Other cation:** Na^+, Ca^{++}, Mg^{++}
- **Major anion:**
  - Cl'
  - HCO_3'
  - Hb
  - Inorganic phosphate
  - 2,3 diphosphoglycerate

HAEMOGLOBIN

TYPES OF HAEMOGLOBINS

In Adults

Hb	:	~97%	$\alpha_2 \beta_2$
HbF	:	<1%	$\alpha_2 \gamma_2$
Hb A_2	:	2.5 – 3.5%	$\alpha 2 \delta_2$

At Birth

HbF	:	$\beta_2 \gamma_2$
Hb A	:	$\alpha_2 \beta_2$

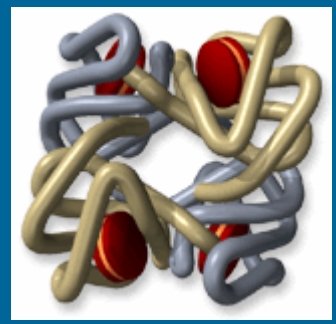
During Embryonic life

Hb Gower 1

Hb Gower 2

Hb Portland

HAEMOGLOBIN IN THE RED CELLS



Haemoglobin

- Major solute in red cells.
- Globular protein
- Conjugated protein: globin + haem.
- Made of 4 subunits (Quarternary structure)

  4 globins + 4 haems $\rightarrow$ haemoglobin.
- Binds O_2 to haem group to form oxyhaemoglobin

  Hb + 4 O_2 $\rightarrow$ Hb $(O_2)4$.

Contd…..

GLOBIN CHAINS OF HAEMOGLOBIN

Amino acids in globin chains

α Globin	141 a.a.
β-like globin chains:	146 a.a.

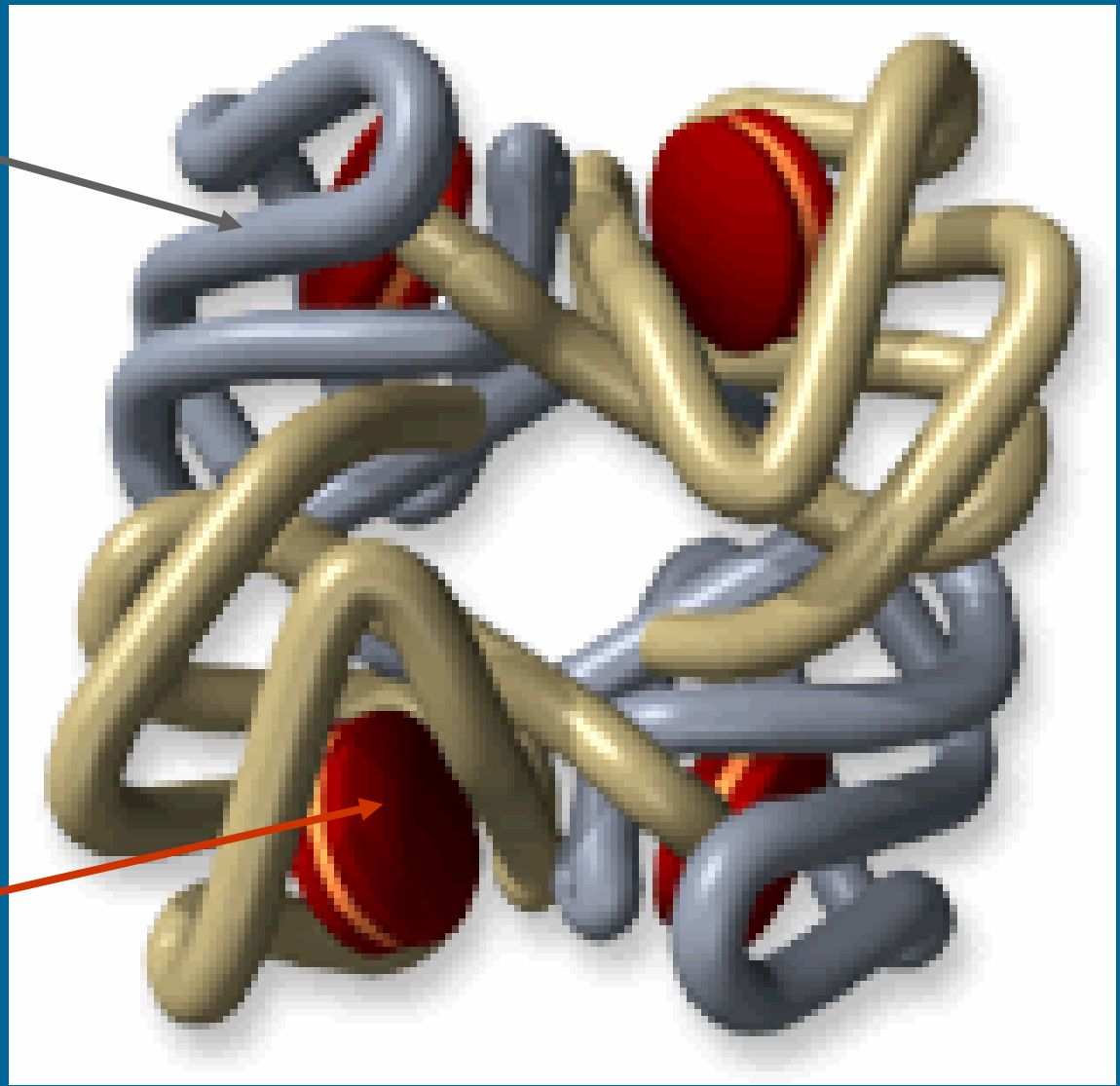
Structure of globin chains

- Globular, compact structure
- ~75% α-helices
- Have a hydrophobic cavity for binding heme.

Haemoglobin

Globin Chains

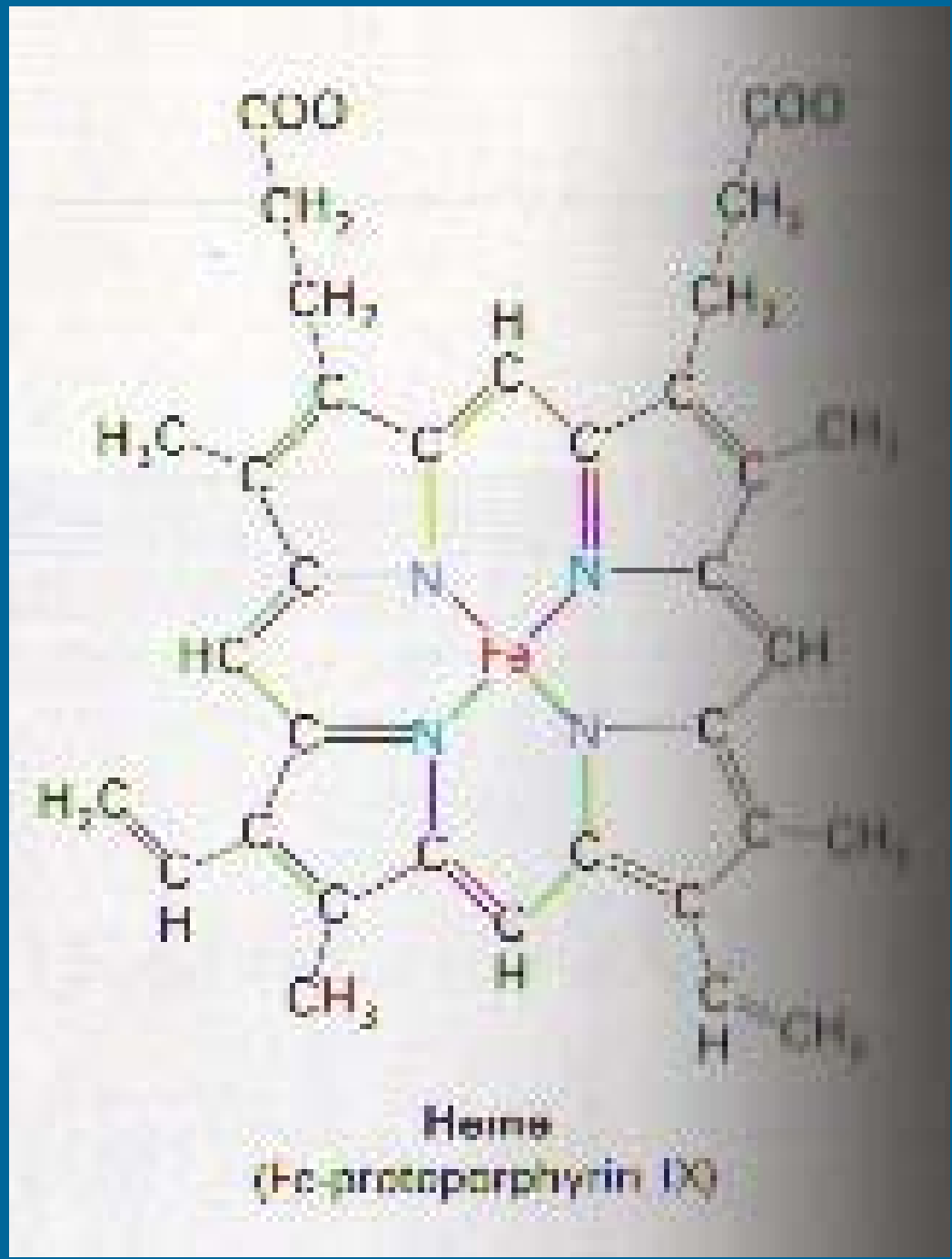
Heam Group



HEME GROUP

- Protoporphyrin IX
- Has tetra pyrolle rings linked together by methylene bridges.
- Fe^{++} coordinates with 4 N of the 4 pyrolle rings:
  - **Bind with coordinate covalent bond to Histidine F8.**
  - **Binds to O_2 between Fe^{++} and His E7.**
- If Fe is oxidized to ferric (Fe^{+++}) the Hb is known as met Hb, which cannot binds O_2.

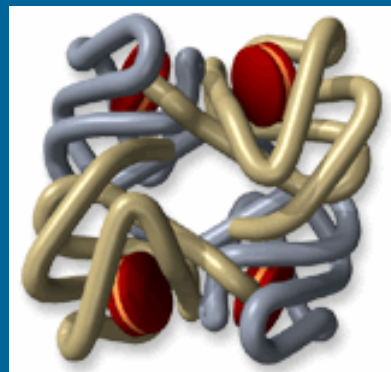
STRUCTURE OF HEME GROUP



HAEMOGLOBIN IN THE RED CELLS

Haemoglobin…..Contd

- Allosteric protein: has 4 O_2 binding sites
- O_2 binding curve of Hb is sigmoidal.
- Shows cooperative effect: i.e. binding of some O_2 molecules makes it easy for other O_2 molecules to bind.
- O_2 affinity of Hb is affected by pO_2, pCO_2, H+, 2,3 DPG.



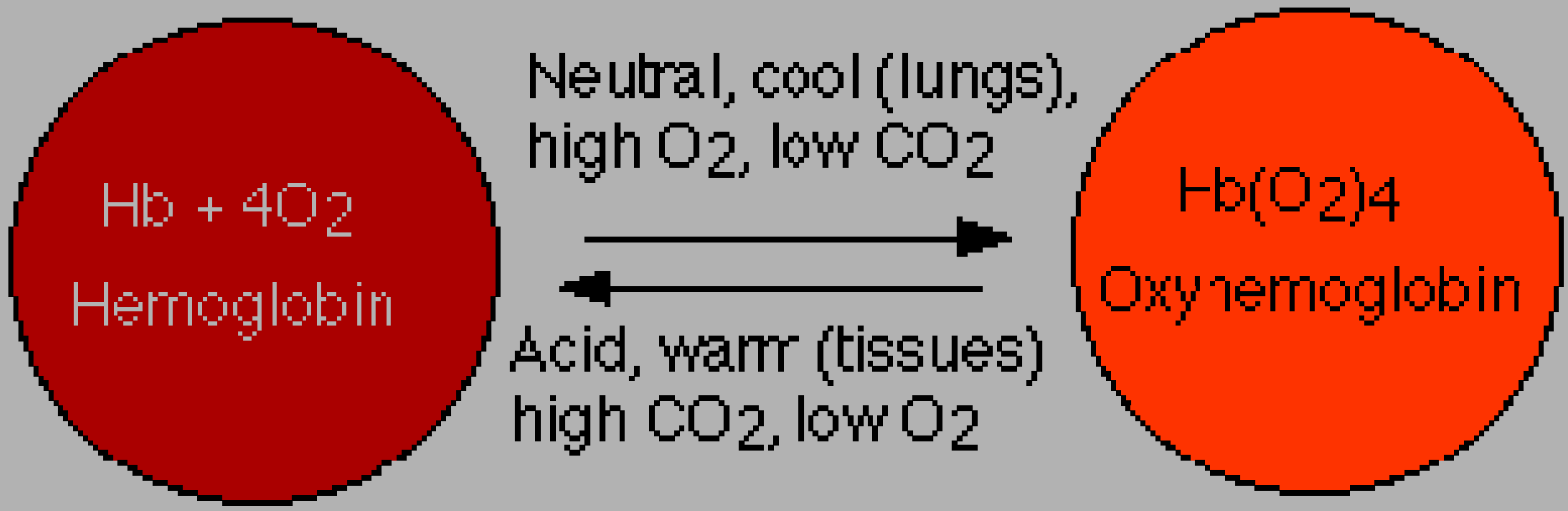
HAEMOGLOBIN IN THE RED CELLS

Haemoglobin…..Contd

- Affinity for O_2 depends on partial pressure of O_2, CO_2, and H+, 2,3 DPG level.
- Binds CO_2 to N-terminal of β-globin chain $\rightarrow$ to form carbamino Hb.
- **Carboxy Hb.**

$$Hb + 4CO \rightarrow Hb(CO)_4.$$

Has high affinity for CO


Hb + 4O2
Hemoglobin
Neutral, cool (lungs),
high O2, low CO2
Hb(O2)4
Oxyhemoglobin
Acid, warm (tissues)
high CO2, low O2

THE BOHR EFFECT

In Lungs:

- High PO_2, $\downarrow H^+$, $\downarrow CO_2$ $\rightarrow$ high affinity of Hb for O_2 (O_2 dissociation curve shifts to left).

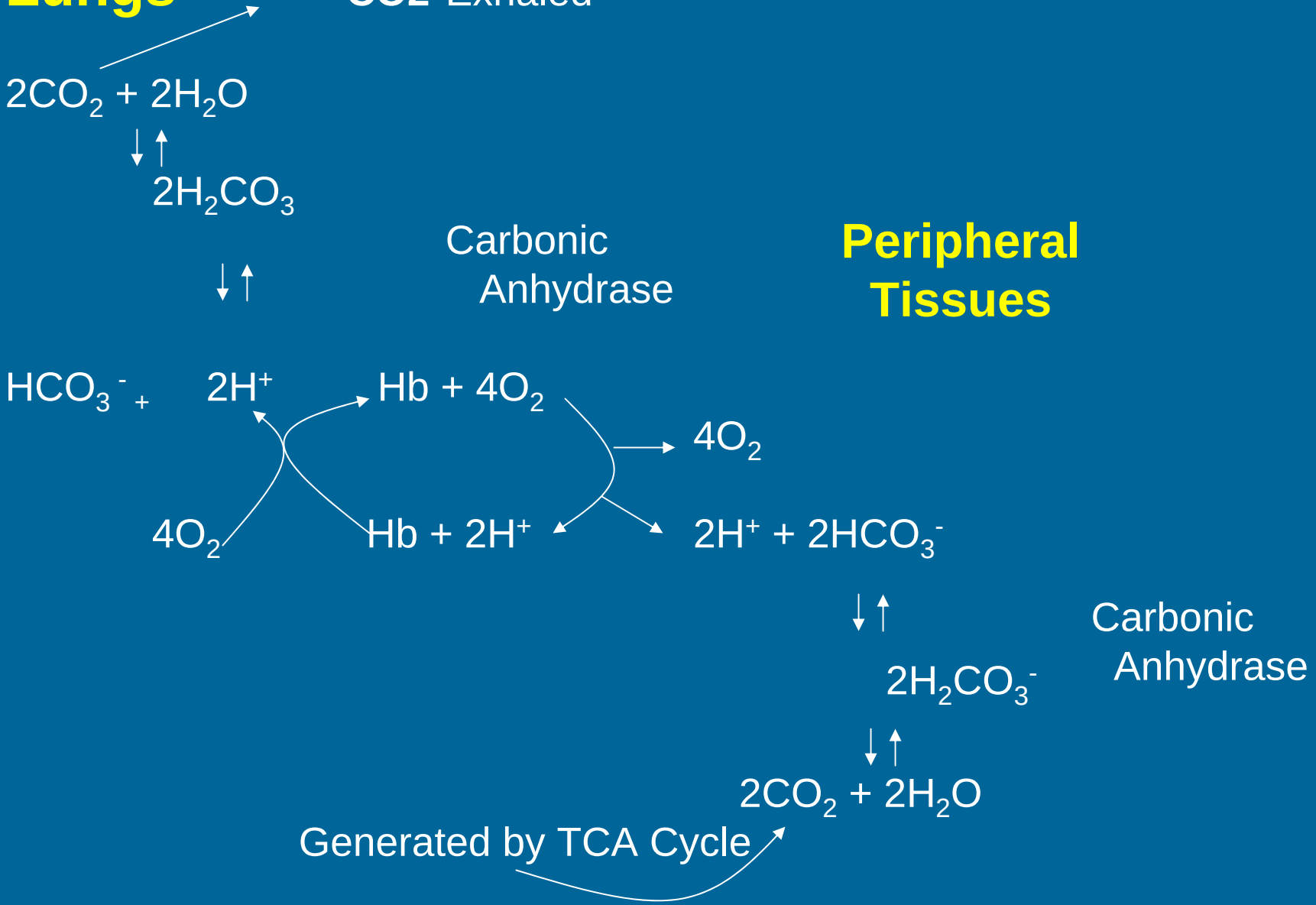
In Tissues

- Low P O_2, $\uparrow$H+, $\uparrow CO_2$, $\uparrow$2,3 DPG $\rightarrow$ Low affinity of Hb for O_2 (O_2 dissociation curve shifts to right)

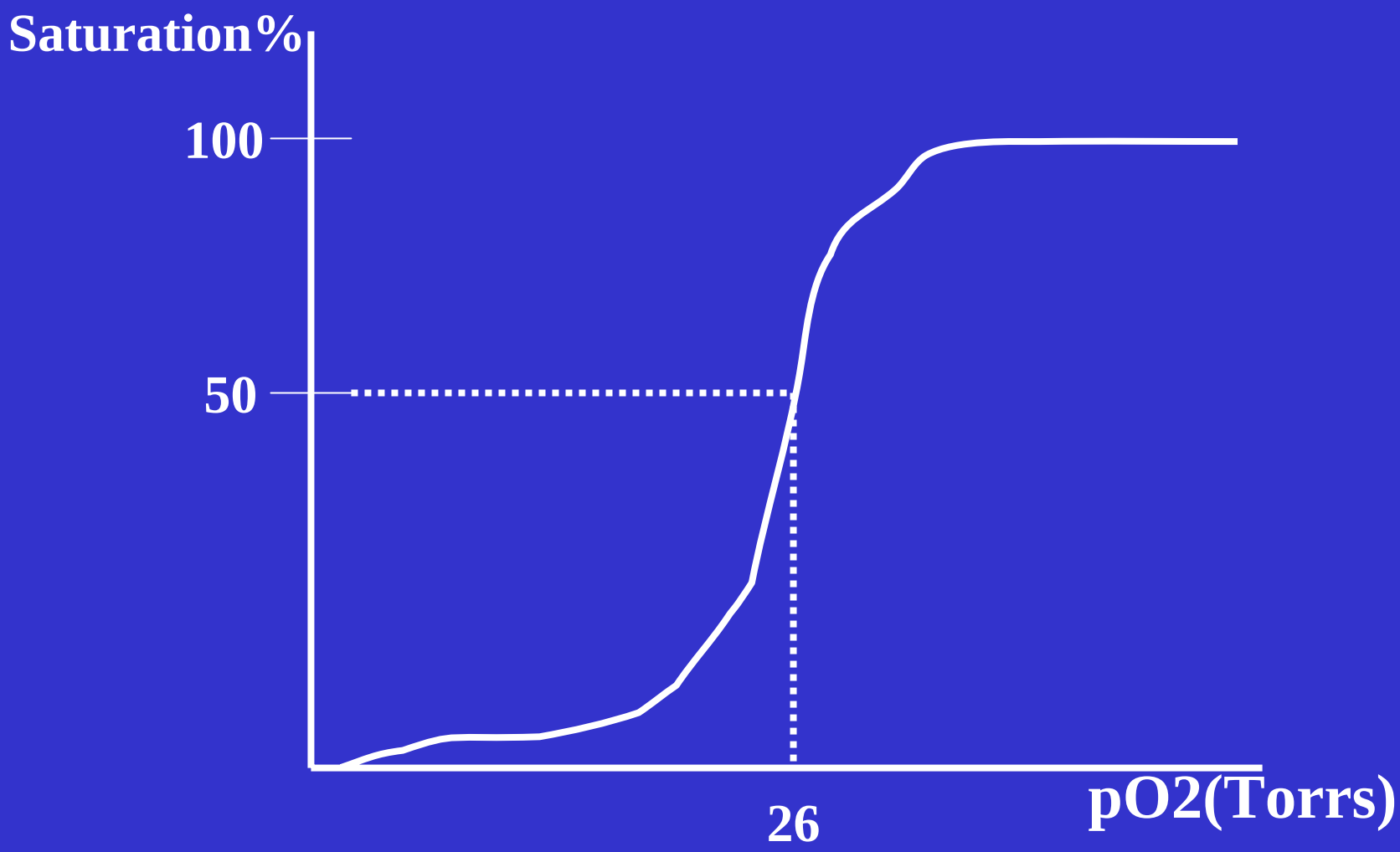
THE BOHR EFFECT

Lungs

CO2 Exhaled



O2 Dissociation curve of Haemoglobin



BINDING OF 2,3 DIPHOSPHOGLYCERATE

- 1 molecule of 2,3 DPG /Hb molecules
- 2,3 DPG binds between 2 β-chains of HbA.
- It is formed from 1,3 DPG (a glycolytic intermediate).
- In peripheral tissues level of 2,3 DPG is high. It binds Hb and decreases affinity for O_2.
- HbF cannot bind 2,3 DPG and has higher affinity for O_2.
  - **O_2 can be transported from mother to fetal blood**

Red Cell Metabolism

SUMMARY OF RED CELL METABOLISM

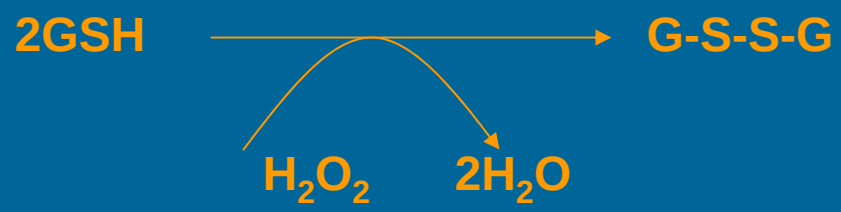
- Highly dependant on glucose as energy source.
- Glucose is metabolized by:
  - Glycolysis (~ 95%)
  - Pentose phosphate pathway (~ 5%)
- Glycolysis produces lactate + ATP
  - 2,3 DPG regulates O_2 affinity of Hb.
- PPP produces NADPH, necessary for keeping red cells in reduced state.
- No synthesis of glycogen, fatty acids, proteins or nucleic acids in red cells

Contd……..

SUMMARY OF RED CELL METABOLISM

……Contd

- Reduced glutathione is important as it keeps the:
  - **red cells and other proteins in reduced state.**
  - **reduces oxidizing radicals (peroxides) generated in red cells**

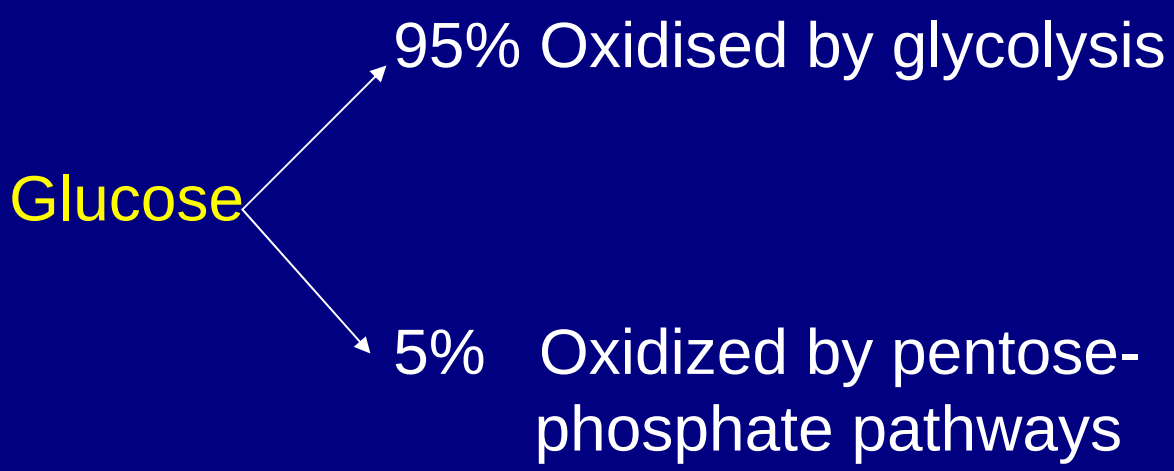


- Iron of Hb is kept in reduced state (Ferrous, Fe^{++}) by NADH-dependant methaemoglobin reductase.

GLUCOSE TRANSPORTERS IN RED CELL MEMBRANE

- Glucose uptake by red cells is by facilitated diffusion.
- Proteins involved in facilitated diffusion of glucose are glucose transporters (~ 2% to membrane protein of RBC).
- Almost 7 different glucose transporters have been identified in different tissue.
- Glucose transporters in red cells membrane are insulin-independent.

Glucose Metabolism in Erythrocytes



The role of glycolysis in the functional requirements of mature red cells:

Function	EMP	PPP
- Maintenance of shape	ATP	
- Membrane structure and Function		GSH
- Regulation of O_2 transport	2,3-DPG ATP	
- Reducing potential	NADP	GSH NADPH

PRODUCTION OF POWERFUL OXIDANT IN RED CELLS DURING METABOLISM

- During metabolism, there is production of:
  - Superoxides (O_2): $O_2 + e \rightarrow O_2$
  - Hydrogen peroxide (H_2O_2)

    $O_2 + O_2 + 2H \rightarrow H_2O_2 + O_2$
  - Peroxyl radicals (ROO)
  - Hydroxyl radicals (OH*)
- These oxidizing radicals are highly reactive molecules and can react with proteins, nucleic acids, lipids and other mol. to alter their structure and produce tissue damage.
- Red cell need several reducing reactions to keep it in reduced state and protect it from damage by oxidizing radicals.

PROTECTION OF RED CELLS FROM HAEMOLYSIS

By:

- Super oxide dismutase

  $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

- Catalase:

  $H_2O_2 + 2H^+ \rightarrow 2H_2O$

- Glutathione

  $2GSH + RO - OH \rightarrow GSSG + H_2O + ROH$

  Glutathione — Oxidised Glutathione

Glucose-6-Phosphate Dehydrogenase (G-6-PD)

- G-6-PD is the first enzyme of the **Pentose Phosphate Pathway**.
- Catalyses the following reaction:

$$\text{G-6-P} + \text{NADP}^{+} \longrightarrow \text{6-Phosphogluconolactone} + \text{NADPH} + \text{H}^{+}$$

- NADPH is necessary for the red cell integrity and stability.
- Co-enzyme for glutathione reductase which converts oxidised glutathione to reduced glutathione. This reduces oxidising radicles and protects red cells from damage.
- **Deficiency of G-6-PD** leads to hemolytic anaemia under oxidative stress(e.g. antimalarial drugs, fava beans, infections, diabetic acidosis)

Other Blood Cells

PLATELETS (Thrombocytes)

- Discoid, anucleated cells with agranular cytoplasm.
  - Diameter = 3 μm
  - Thickness = 1 μm
  - Volume = 7 fl.
- $250x10^9$ platelets/litre.
- Synthesis increased by thrombopoietin.
- Synthesised from megakaryocytes.

Contd…..

PLATELETS (Thrombocytes) Contd...

- Survival in circulation 10-12 days.
- Primary role:
  - in haemostasis: stick to the edges of wounds and form a plug to arrest blood loss.
- Platelets also involved in development of atherosclerosis and hence can lead to thrombosis.

White Blood Cells (Leucocytes)

Two Main Groups:

i. The Phagocytes ----------Play a role in protecting the
a- Granulocytes: the body against infection by
- Neutrophils phagocytosis.
- Eosinophils
- Basophils
b- Monocytes

ii. The Lymphocytes (immunocytes)--Function in protecting.
a- B-Lymphocytes----------Provide humoral immunity.
b- T- Lymphocytes----------Provide cellular immunity.

Total leucocytes: 4.00-11.0x 10^6/l

GRANULOCYTES

- Have numerous lysosomes and granules (secretory vesicles).
- Also known as polymorphonuclear leukocytes (PMN) as they have multilobular nuclei
- Types of granulocytes:
  - Neutrophils,
  - basophils and
  - eosinophils

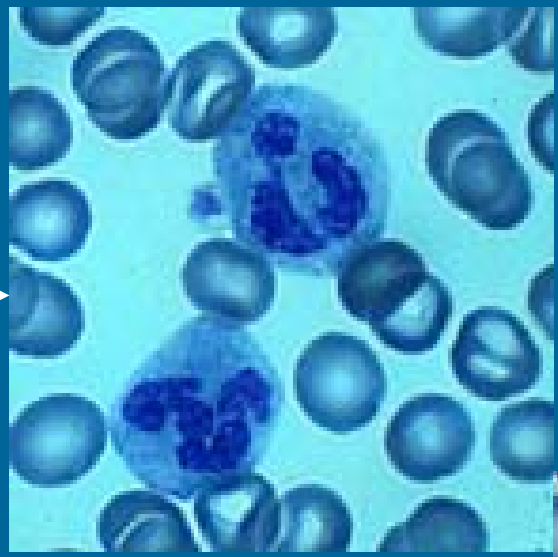
  are distinguished by their morphology and staining properties of their granules.

FUNCTIONS OF GRANULOCYTES

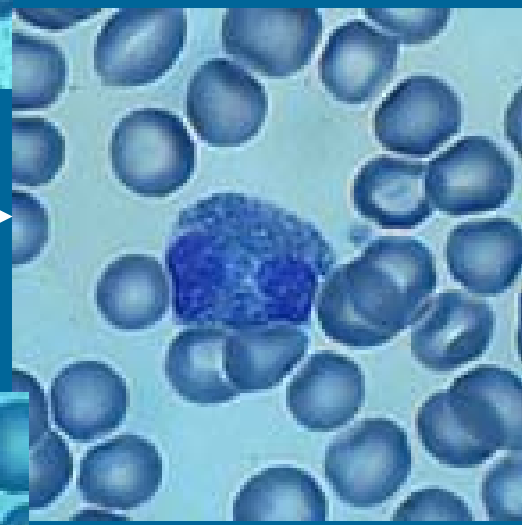
Neutrophils:	Phagocytose bacteria and play a major role in accurate information.
Basophils:	Resemble mast cells and contain histamine and heparin – play a major role in immunologic hypersensitivity reaction.
Eosinophils:	Involved in certain allergic reactions and parasitic infection.

Granulocytes

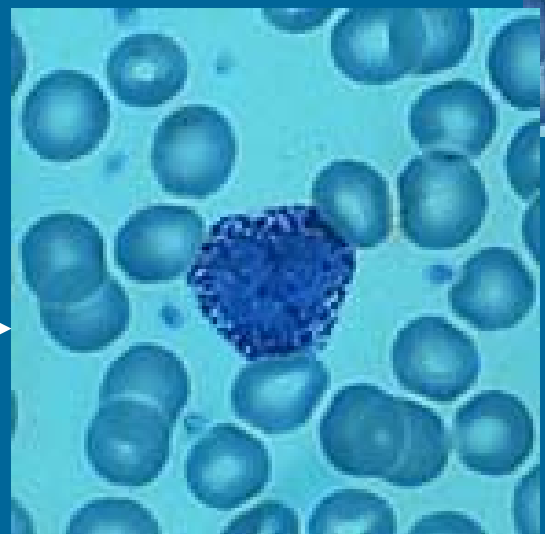
Neutrophils

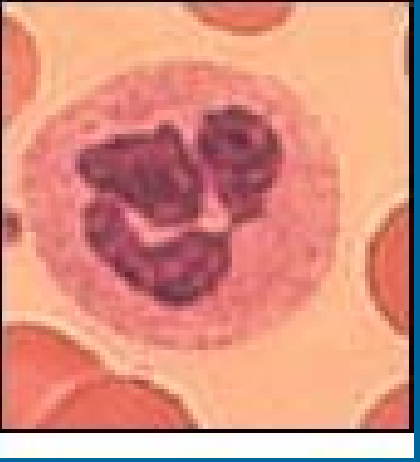


Eosinophils



Basophils





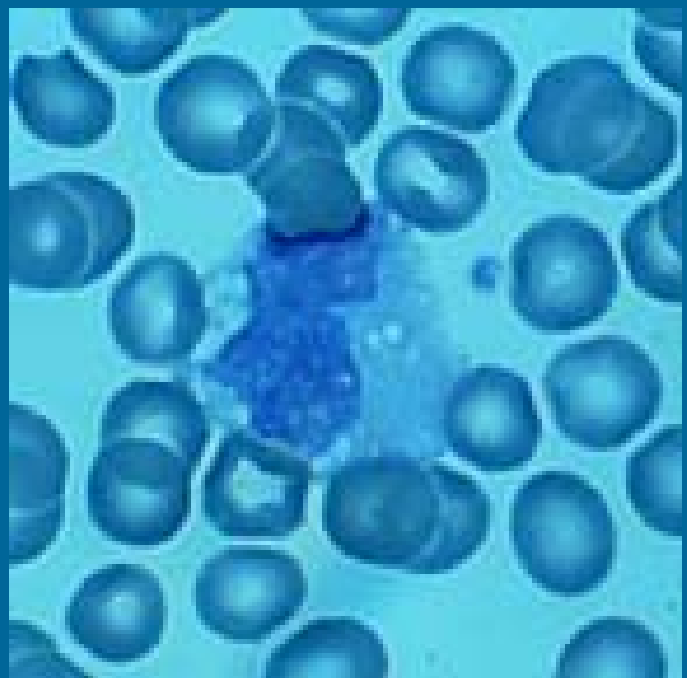
NEUTROPHILS

Responsible for acute inflammatory response

- **Increase vascular permeability**
  - **By:**
    - **Platelet, activating factor (PAF)**
    - **Eicosanoids (various prostaglandins and leukotriens)**
- **Cause entry of activated neutrophils into tissues**
- **Cause Activation of Platelets**
- **Spontaneous subsidence (resolution) of invading organism that have been dealt with successfully**

FUNCTIONS OF MONOCYTES

- Monocytes are precursors of macrophages, which are actively involved in phagocytosis.

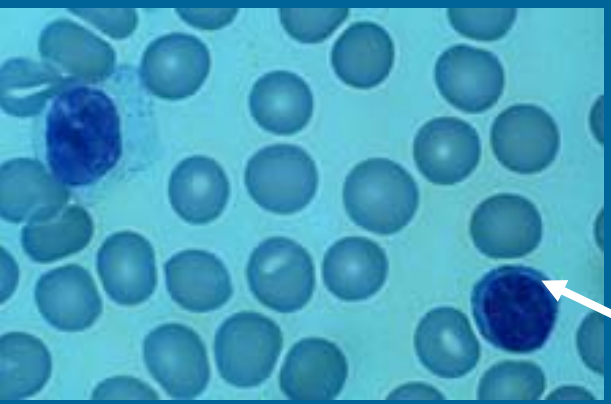


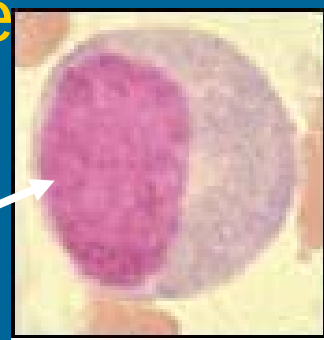
FUNCTIONS OF LYMPHOCYTES

B-Lymphocytes: Synthesize and secrete antibodies (humoral immunity)

T-Lymphocytes: Involved in cellular immune mechanism e.g

- killing virally infected cells and some cancer cells.
- activate B cells to make antibodies.

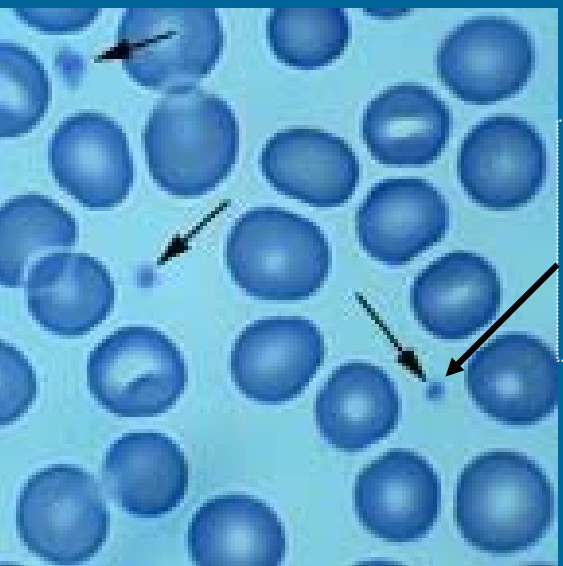




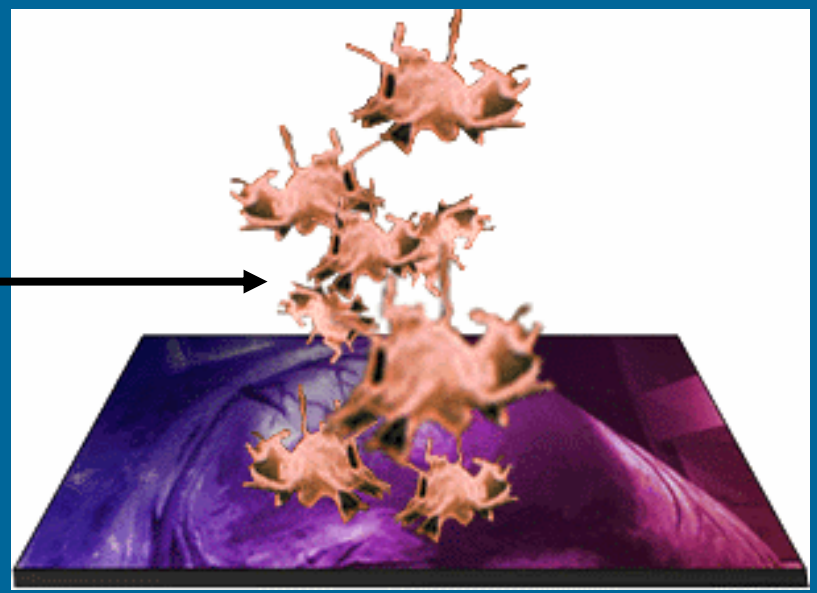
Lymphocytes

PLATELETS

- Involved in coagulation of blood



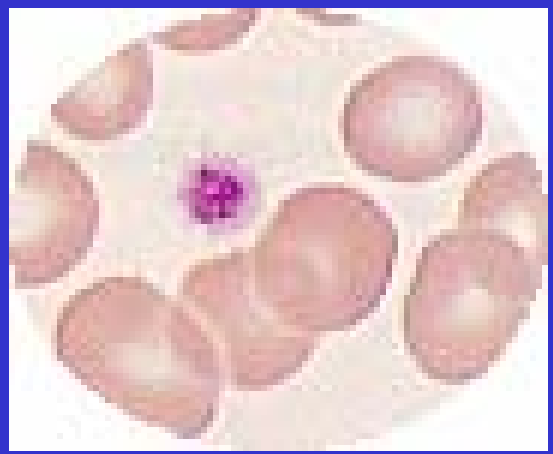
PLATELETS



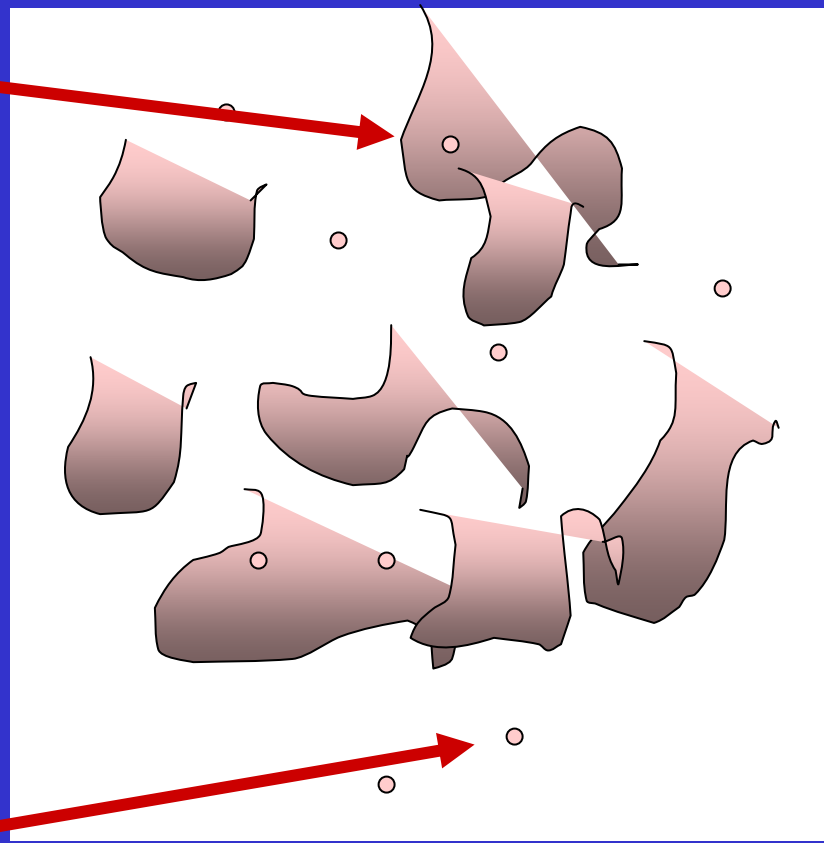
Haemolysis of Erythrocyte

Haemolysis

Haemolysed red cells



>120 days



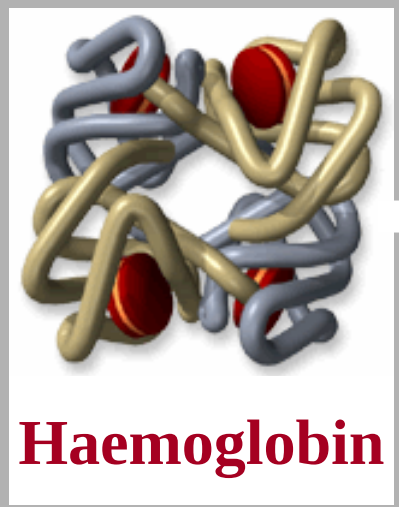
Free Haemoglobin

In Reticuloendothelial cells

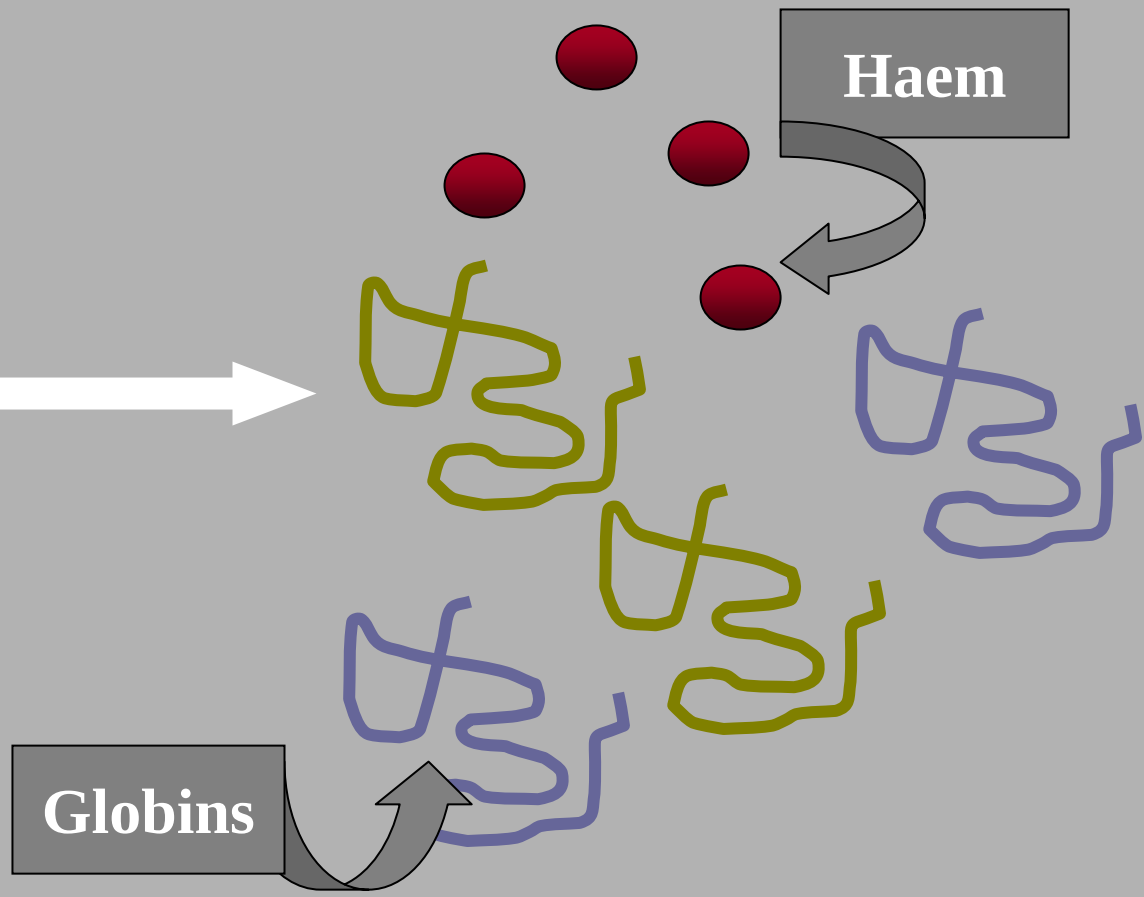
Haemolysis of Erythocytes

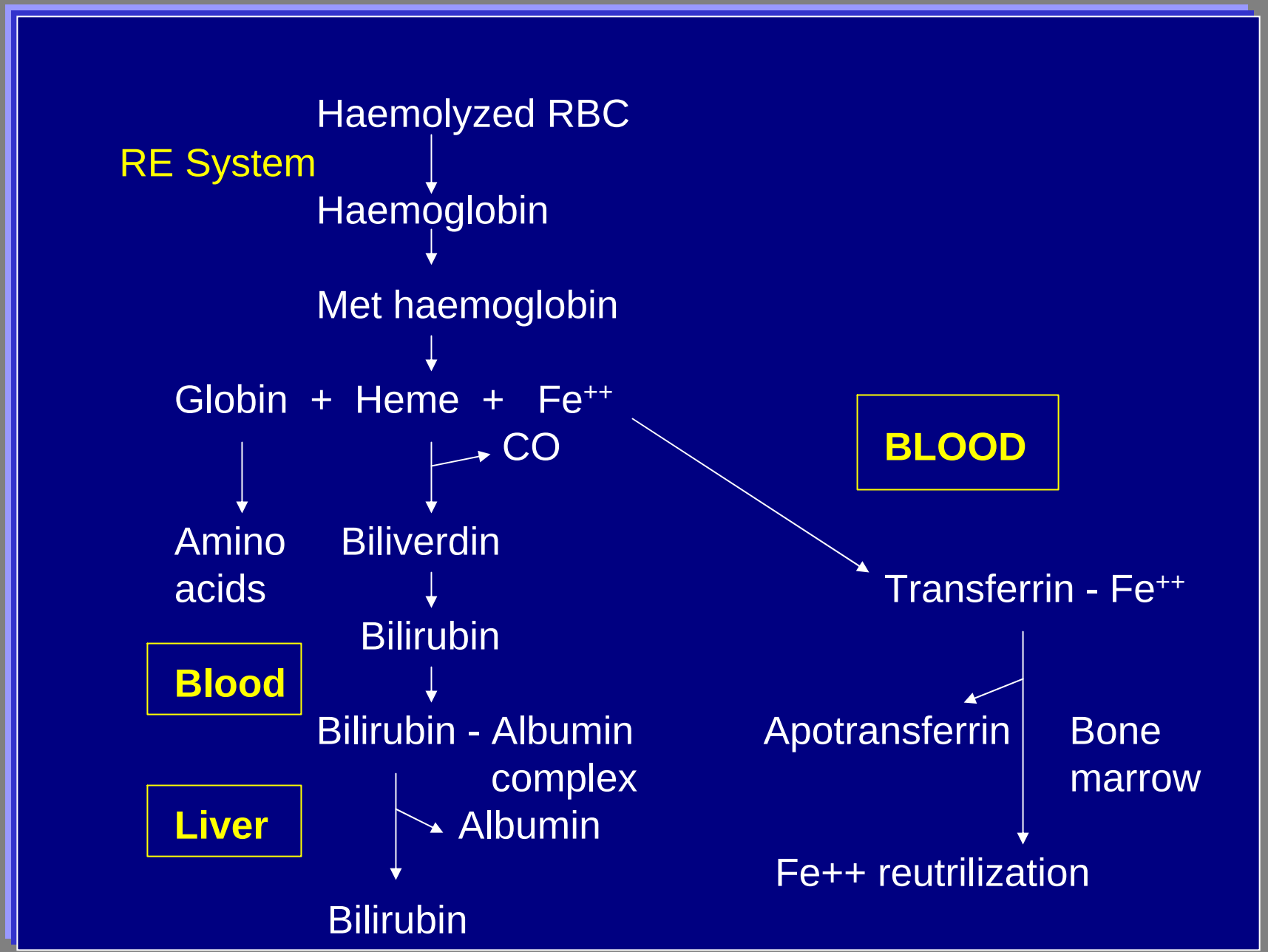
- After a life span of 120 days, erythrocytes are haemolysed
- In:
  - Spleen
  - Bone marrow
  - Other REC
- Signal for haemolysis:
  - Loss or alteration of:
    - Cytoskeleton structure
    - Active ion pump
    - Membrane lipids
    - Membrane glycoproteins
- Most intracellular components are reutilized.

Fate of Haemoglobin



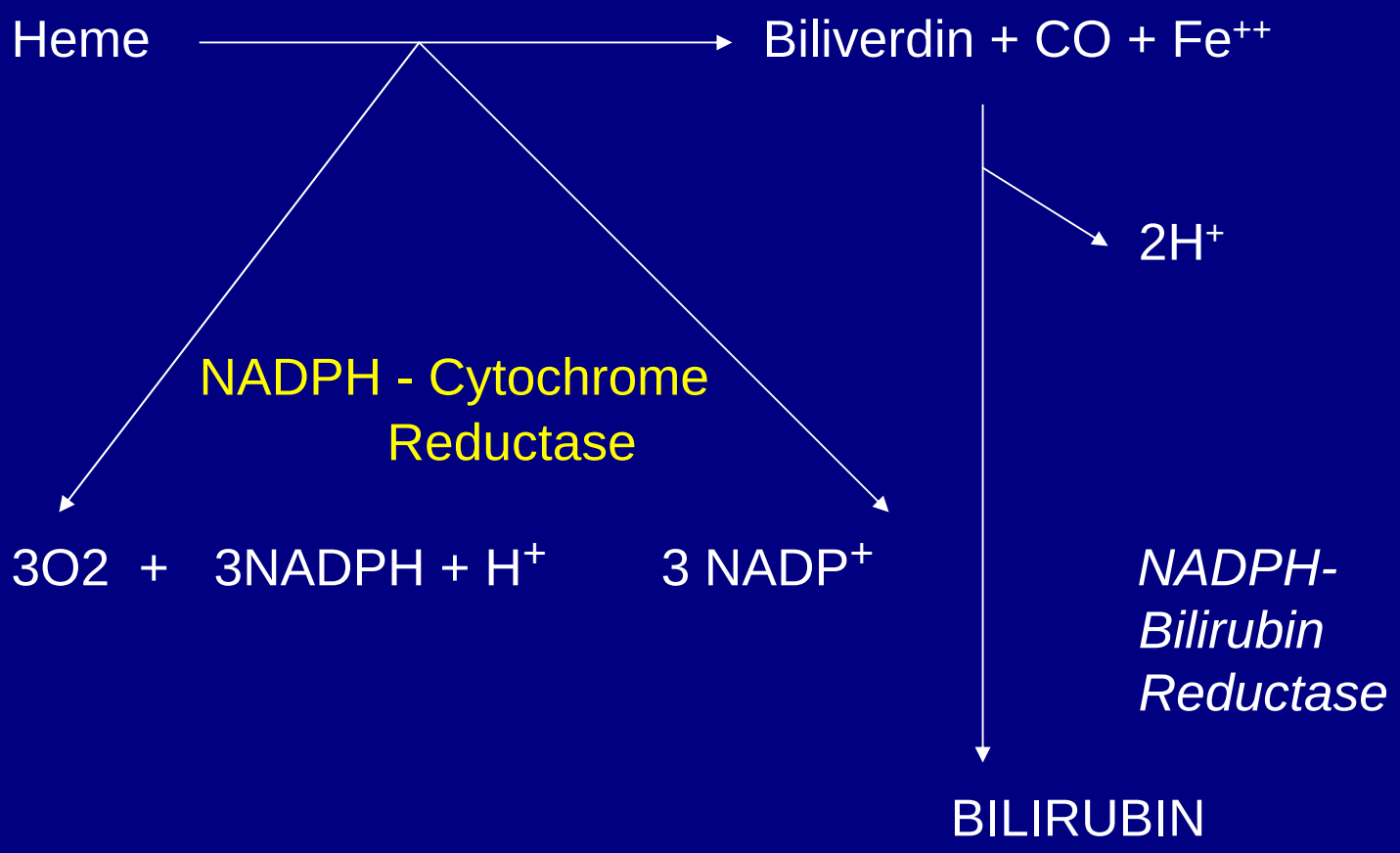
Haemoglobin



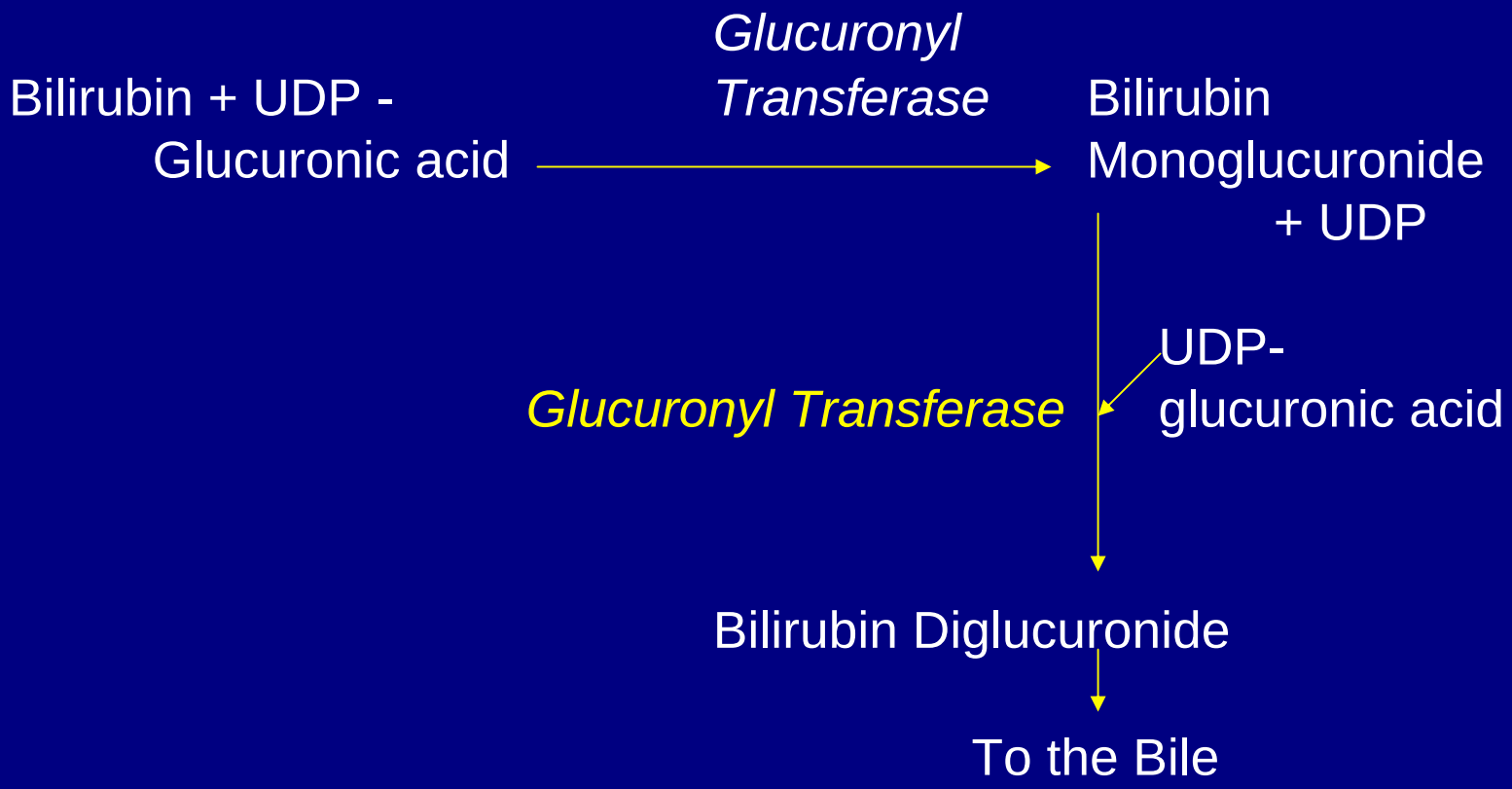

Haemolyzed RBC
RE System
Haemoglobin
Met haemoglobin
Globin + Heme + Fe++
CO
BLOOD
Amino acids
Biliverdin
Transferrin - Fe++
Bilirubin
Blood
Bilirubin - Albumin complex
Apotransferrin
Bone marrow
Liver
Albumin
Fe++ reutrilization
Bilirubin

In R.E.S.

Heme Oxygenase



In Liver


Bilirubin + UDP -
Glucuronic acid
Glucuronyl
Transferase
Bilirubin
Monoglucuronide
+ UDP
UDP-
glucuronic acid
Glucuronyl Transferase
Bilirubin Diglucuronide
To the Bile

Daily excretion of Bile Pigments

250-350 ng Bile Pigment excreted in Feces/day

1-2 mg Bile Pigment excreted in urine/day

Plasma Level of Bilirubin

Total Bilirubin = < 17 µmol/L

Direct Bilirubin = < 2 µmol/L

Disorder of Bile Pigment Metabolism

Causes:

1. An increase load of bilirubin arriving at the liver:
   - due to increased red cell destruction.
   - Absorption of large haematoma.
2. Defective uptake and transport by the liver cells:
   - Gilbert's disease.
3. Disturbance of conjugation:
   - Liver cell destruction.
   - Reduced glucuronyl transferase activity.
     - Neonatal jaundice.
     - Crigler-Najjar Syndrome
     - Gilbert's disease.
4. Disturbance of excretion of conjugated bilirubin:
   - Liver cell destruction.
   - Intra and extrahepatic cholestasis.
   - Dubin-Johnson Syndrome

Jaundice

- Elevation of bile pigments in blood.
- Bile pigments escape into tissues - yellow colouration.
- Due to:
  - ↑ Production of bile pigments.
  - Failure of liver to conjugate and excrete bile pigments.
  - Decreased excretion of bile pigment due to obstructive of bile duct.

Types of Jaundice

- Hemolytic or prehepatic.
- Hepatic.
- Obstructive as posthepatic.
- Congenital non-hemolytic.

Hemolytic Jaundice

- Increase destruction of erythrocytes.

  ↑ Formation of bilirubin

- Elevation of serum bilirubin.

  e.g. - In hemolytic anemia
  - Infection
  - G-6-PD deficiency

Hepatic Jaundice

- Caused by liver dysfunction.
- Results from damage to parenchymal cells.
- Decreased conjugation of bilirubin.

e.g.
- Liver poisons (chloroform phosphorus, CCl 4)
- Toxins.
- Hepatitis virus.
- Engorgement by hepatic vessels in cardiac failure
- Cirrhoses.

Obstructive Jaundice

- Results from blockage of the hepatic or common bile duct.
- Passage of blood into liver cell is normal.
- Conjugation of bilirubin in liver is normal.
- Failure of conjugated bilirubin to be excreted by bile capillaries.
- Bilirubin reabsorbed by hepatic veins and lymphatics.

Congenital Neonatal Hyperbilirubinaemia

- ↓ Activity of glucuronyl transferase in liver.
- ↓ Conjugation and excretion of bilirubin.
- ↑ Unconjugated level in blood.
- Often occurs in neonatal period.
- Treated by phototherapy.

Congenital Hyperbilirubinaemia

- **Gilbert's Disease:**
  - Defective bilirubin transport into liver cells.
  - Occassionally reduced glucuronyl transferase activity.
  - Elevated plasma unconjugated bilirubin (20-35 µmol/L)
  - Harmless.

- **Crigler-Najjar Syndrome:**
  - Deficiency in glucuronyl transferase.
  - Significantly elevated plasma unconjugated bilirubin (350 µmol/L)
  - Hyperbilirubinaemia in first few days of life.
  - Kernicterus in newborn.

Contd…..

Congenital Hyperbilirubinaemia

Contd….

- Dubin-Johnsons Syndrome

  - Defective excretion of conjugated bilirubin.
  - Mildly raised conjugated bilirubin.
  - Bilirubin in urine.
  - Harmless.

Types of Bilirubin present in different Jaundice

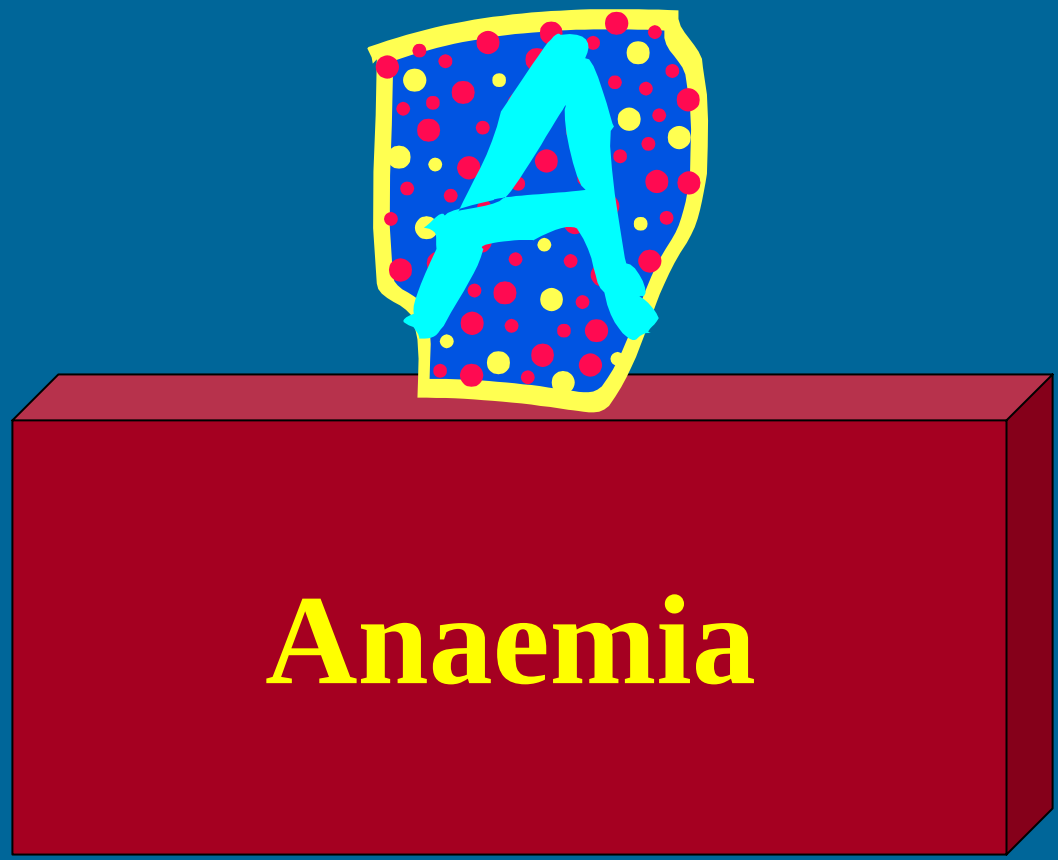
Defect	Types of Bilirubin
- Increased production - Haemolytic disease	Unconjugated bilirubin
- Reduced liver uptake of bilirubin - Drug competition	Unconjugated bilirubin
- Reduced conjugation of bilirubin - Developmental defect - Drug competition - Inherited enzyme defects (Gilbert's disease, Criglar Najjar disease)	Unconjugated bilirubin

Contd….

Types of Bilirubin present in different Jaundice

Contd….

Defect	Types of Bilirubin
- Decrease secretion of conjugated bilirubin - Drug competition - Inherited defects.	Mainly conjugated bilirubin
- Obstruction of biliary tree (cholestasis) - Within the liver (liver cirrhosis, drugs side-effects) - Outside the liver (gallstone, neoplasm)	Mainly conjugated bilirubin


A
Anaemia

Anaemia

- Decrease in the level of:
  - Haemoglobin or/and
  - RBC count or/and
  - PCV (Hematocrit)

Classification of Anaemia

Classified mainly in two ways:

1. According to the morphology of the average red cells.

2. According to the pathophysiologic mechanism of the red cell production.

Classification of Anaemia

By Red Cell Morphology:

An anaemic state with altered or normal red cell morphology i.e. MCV and MCH:

MCV: denotes the mean corpuscular volume of red cells (Normal = 85-100 f/l)

MCH: denotes mean corpuscular hemoglobin (Normal = 27-32 pg).

Mean Corpuscular Haemoglobin (MCH)

- MCH expresses the amount of haemoglobin in red blood cell in picogram (pg).

$$\frac{\text{Hb (g/dl blood)}}{\text{RBC Count X } 10^{12}/\text{l}} = \text{MCH}$$

- Normal range 27-32 pg

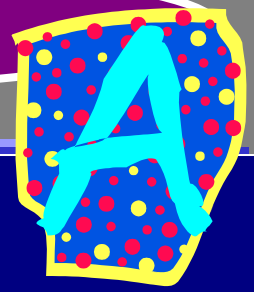
Mean Corpuscular Haemoglobin Concentration (MCHC)

- MCH expresses the amount of haemoglobin in RBC. It is expressed in gm/deciliter.

$$\frac{\text{Hb (g/dl)}}{\text{PCV (l/l)}} = \text{MCHC g/dl}$$

- Normal Range 30-35 g/dl

Classification of Anaemia … Contd.



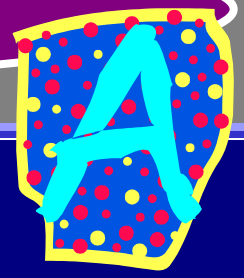
(a) Normocytic Normochromic Anaemia
(MCV = 85-100 fl) (MCH = 31-35 pg)

1. Acute bleeding.

2. Haemolytic anaemia:
   (a) Extracorpuscular defects immune and non-immune.
   (b) Intracorpuscular defects, membrane,and metabolic defects and hemoglobinopathies.
   (c) Combined defects.

3. Marrow failure associated with hypoproliferation of hematopoietic cells:
   (a) Aplastic anaemia.
   (b) Pure red cell aplasia.
   (c) Anaemia of chronic renal failure.
   (d) Anaemia of endocrine disease.
   (e) Toxic depression of bone marrow.

Classification of Anaemia … Contd.



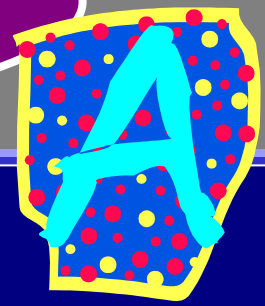
(b) Microcytic - Hypochromic Anaemias
(MCV = < 87 fl) (MCH = < 30 pg)

1. Iron deficiency.
2. Thalassaemias.
3. Sideroblastic anaemia:
   (a) Reflactory.
   (b) Reversible.
   (c) Pyridoxine - responsive.

(c) Macrocytic - Normochromic Anaemias:
(MCV = > 103 fl) (MCH = 31-35 pg)

1. Megaloblastic anaemia:
   (a) Vit. B12 deficiency.
   (b) folic acid deficiency.
   (c) Others.
2. Non-Megaloblastic Macrocytic Anaemia.

Classification of Anaemias (Contd…)



2. According to the pathophysiologic mechanism:

   (A) Increased loss of RBCs
   - Acute bleeding

   (B) Increased destruction of RBCs:
   - Haemolytic anaemia

   (C) Decreased production of RBCs:

   1. Marrow failure associated with hypo-proliferation of hematopoietic cells.
   2. Marrow failure associated with ineffective erythropoiesis.

Causes of Anaemias

1. Dyshaemopoietic anaemias: Due to insufficient blood production.
2. Haemolytic anaemias: Due to excessive intra-vascular destruction.
3. Haemorrhagic anaemias: Due to extravascular blood loss.
4. Anaemias of unknown causes.

Dyshaemotopoietic Anaemias

Deficiency of Factors Essential for Erythropoiesis.

- Iron deficiency.
- Trace metal (copper) deficiency.
- Haemopoietic principle deficiency
  - Extrinsic factor (Vit. B12)
  - Intrinsic factor (in gastric juice)
- Other vitamin deficiencies:
  - Folic acid deficiency.
  - Pyridoxine deficiency.
  - Riboflavin deficiency.
  - Nicotinic acid deficiency.
- Internal secretion deficiency:
  - Thyroid hormone deficiency
  - Pituitary hormone deficiency
- First class proteins deficiency:
  - Milk and milk product.
  - Eggs.
  - Meat proteins.

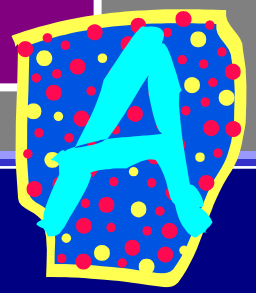
Causes of Deficiency in Factors Essential for Erythropoiesis

- Food Intake Defect - (Nutritional Anaemias):
  - Deficiency of:
    - Proteins
    - Iron and other metals.
    - Vitamin C
    - Vitamin B12
    - Folic acid

- Defect of Digestion - due to impaired gastric function:
  - Achlorhydria
  - Deficiency of intrinsic factor
  - Presence of autoantibodies

- Defects of absorption and transport:
  - Fatty diarrhea, sprue, coeliac disease, diarrhea
  - Transferrin deficiency, ceruloplasmin deficiency.

Causes of Deficiency in the Factors (Contd.)



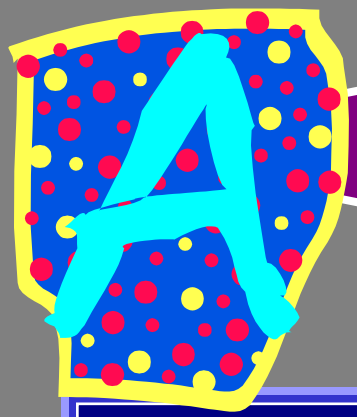
- Defects of storage:
  - Liver damage.
- Failure to utilize the factors essential for haemopoiesis:
  - Failure of iron utilization.
  - Sepsis.
  - Chronic infection (TB, Syphilis)
  - Nephritis.
  - Cachexia of malignant disease.
  - Leukaemia.
  - Liver cirrhosis.

- Toxic and aplastic conditions:
  - Idiopathic aplastic anaemia.
  - Damage by Benzol, X-rays, Radium.

Haemolytic Anaemias

Causes:

- Infections:
  - Sepsis and septicaemia:
    - Streptococcus, Clostridium, Welchi (qas gangrene)
    - Typhoid fever
    - Viral infection
  - Poisons:
    - Chronic lead poisoning
    - Acute lead poisoning
    - Chemicals (phenylhydrazine, saponins)
    - Snake venoms
  - Allergic haemolytic anaemia:
    - Pollens or vegetables



Haemolytic Anaemias (Contd.)

Causes:

- Paroxysmal haemoglobinuria.
  - Intravascular haemorrhage due to cold exertion.
- Hereditary Intracorpuscular Defects:
  - Abnormal haemoglobins (Hb S, Hb C).
  - Thalassaemias (α and β)
  - Enzyme deficiency (G-6-PD and PK deficiency)
- Hereditary abnormalities in corpuscular shape:
  - Congenital haemolytic icterus. (Hereditary spherocytosis)
  - Hereditary Elliptocytosis.
- Hereditary Defects of Unknown cause:
  - Familial non-spherocytic haemolytic anaemia

Haemorrhagic Anaemias

- Acute haemorrhage:
  - Accidents
  - Surgery
- Chronic haemorrhage:
  - Epistaxis, Menorrhagia
  - Haemorrhoids
  - Bleeding duodenal ulcer
- Haemorrhagic disease:
  - Congenital coagulation defects:
    - Haemophilia (Def. of factor VIII)
    - Christmas disease (Def. of factor IX)
  - Acquired coagulation defects:
    - Vitamin K deficiency
    - Liver disease
  - Congenital platelet defects:
    - Familial thrombocytopenia
  - Acquired platelet defects:
    - Irradiation
    - Drugs (cytotoxic drugs)

Anaemias of Unknown Causes

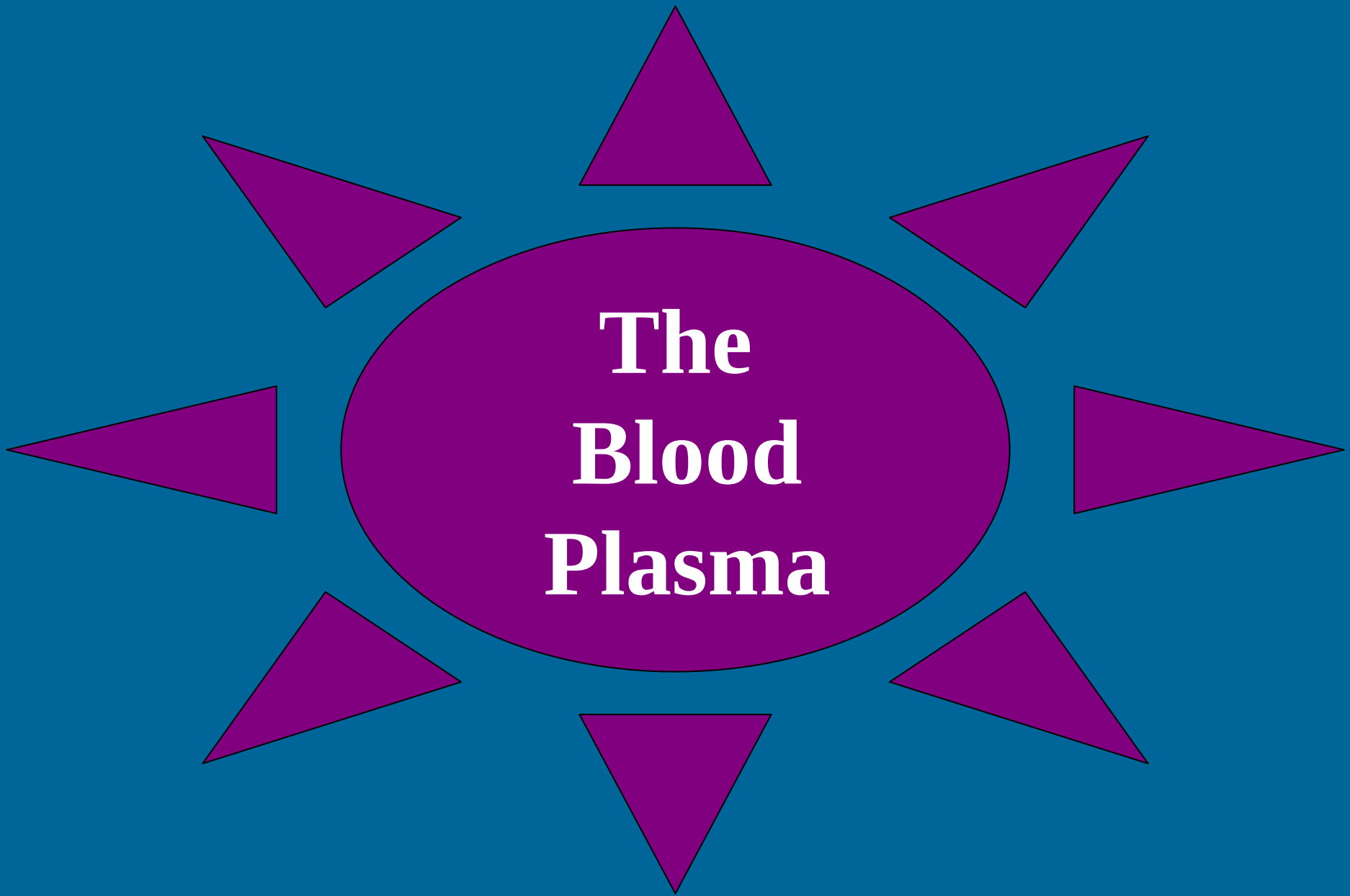
- Refractory anaemias.
- Anaemia secondary to other diseases.
- Anaemia due to exertion.

Pernicious Anaemia

(Addisonial Megaloblastic Anaemia)

- Most common megaloblastic anaemia.
- Due to the absence of intrinsic factor in the gastric juice (atrophy of gastric mucosa).
- Intrinsic factor is needed for absorption of Vit. B12.
- Vit. B12 necessary for Haematopoises.
- ↓ Intrinsic factor → ↓ Vit. B12 absorption →

  Ineffective erythropoiesis → Megaloblastic red cells


The
Blood
Plasma

The Blood Plasma

-Contains 91-95% water.

- Solutes in plasma range from 5-9%
- Proteins are the major solute in the plasma and their level ranges from 6-8 gm %.

Principal Inorganic Constituents of Human Blood Plasma

Anions	Concentration meq/Liter	Cations	Concentration meq/Liter
Total	142-150	Total	142-158
Bicarbonate	24-30	Calcium	4.5-5.6
Chloride	100-110	Magnesium	1.6-2.2
Phosphate	1.6-2.7	Potassium	3.8-5.4
Sulfate	0.7-1.5	Sodium	132-150
Iodine,total	8-15*	Iron	50-180*
Protein bound	6-8*	Copper	80-160*

*These concentrations are in micrograms per 100 ml.

Principal Non-Protein Organic Constituents of Human Blood Plasma

Constituents	Normal Range
Non-Protein N :	25 - 40
Amino acid N	4 - 8
Amino acids	36 - 65
Bilirubin	0.2 - 1.4
Creatine	0.2 - 0.9
Creatinine	1 - 2
Uric acid	2 - 6
Carbohydrates:	
Glucose	65 - 90
Fructose	6 - 8

Contd...

Principal Non-Protein Organic Constituents of Human Blood Plasma

Constituents	Normal Range
Organic acids:	
Citric acid	1.4 - 3.0
α-ketoglutaric acid	0.2 - 1.0
Lactic acid	8 - 17
Lipids:	
Total lipids	285 - 675
Neutral fat	80 - 240
Cholesterol, total	130 - 260
Phosphoglyceride:	
Total	150 - 250

Plasma Proteins

TOTAL PLASMA PROTEINS

- **The normal serum protein level is 63-83 g/L.**
- **The type of proteins in serum include:**
  a. **Albumin**
  b. **Globulins**

     α– globulin: **α1 & α2–globulins**
     β– globulin: **β1 & β2 globulins**
     γ– globulins

  c. **Fibrinogen**
- **Under different pathological conditions the protein levels depart from the normal range.**

Functions of Plasma proteins

- **Transport**: e.g.
  - Transferrin transports iron.
  - Ceruloplasmin transports copper.
  - Albumin transports fatty acids, bilirubin calcium, many drugs etc.
  - Transcortin transports cortisol and corticosterone
  - Retinol binding protein transports retinol.
  - Lipoproteins transport lipids.
  - Haptoglobin transports free haemoglobin.
  - Thyroxin binding globulin transports thyroxin.

Functions of Plasma proteins (contd)

- **Osmotic regulation**:

  - Plasma proteins are colloidal and non-diffusable and exert a colloidal osmotic pressure which helps to maintain a normal blood volume and a normal water content in the interstitial fluid and the tissues.

  - Albumin content is most important in regulation of colloidal osmotic or oncotic pressure.

  - Decrease in albumin level results in loss of water from blood and its entry into interstitial fluids causing edema.

- Catalytic function (enzymes):

  - e.g lipases for removal of lipids from the blood.

Functions of Plasma proteins

(contd)

- **Protective function**:

  - Immunoglobulins combine with foreign antigens and remove them.

  -Complement system removes cellular antigens.

  - Enzyme inhibitors remove enzymes by forming complexes with them. e.g. $\alpha 1\overline{\ }$ antitrypsin combines with elastase, trypsin and protects the hydrolytic damage of tissues such as lungs.

  - Some proteins increase during acute phase and protect the body. E.g. $\alpha 1$ antitrypsin, $\alpha 2$ macroglobulins

Functions of Plasma proteins (contd)

- Blood clotting:

  - Many factors are involved in clotting mechanism and prevent loss of excessive amount of blood. e.g. clotting factors IX, VIII, thrombin, fibrinogen etc.

  - An excess of deficiency leads to a disease. e.g hemophilia, thrombus formation. —

- Anticoagulant activity (thrombolysis):

  - Plasmin breaks down thrombin and dissolves the clot

- Buffering capacity:

  - Proteins in plasma help to maintain acid-base balance.

Specific Functions of some proteins

PROTEIN	PLASMA CONC. (g/L)	FUNCTION
Pre-albumin	0.3	Binds T3 & T4
Albumin	40.0	Transport, colloid oncotic pressure
α1- globulin : α1- antitrypsin	3.0	Anti proteinase
α2- globulins		
ceruloplasmin	0.4	Copper transport
haptoglobulin	1.2	Binds haemoglobin
α2-macroglobulin	3.0	Transport, anti-proteinase

Contd.....

Specific Functions of some proteins

PROTEIN	PLASMA CONC. (g/L)	FUNCTION
β- Globulins		
Transferrin	2.5	- Iron - transport
Hemopexin	1.0	- Binds haem
Plasminogen	0.7	- Fibrinolysis
Fibrinogen	4.0	- Haemostasis
γ - Globulin		
IgA	0.9-4.5	-Ig in external secretions
IgM	0.7-2.8	- First Ab synthesised
IgG	8-18.0	-Main classes of antibody
IgE		- Involved in allergy
IgD		

MEASUREMENT OF PROTEIN FRACTIONS

- The protein fraction in plasma can be separated and estimated using the following methods:
  - Zone electrophoresis
  - Immunochemical methods
  - Chemical methods
  - Ultracentrifugation

CHARACTERIZATION, MEASUREMENT AND ISOLATION OF PLASMA PROTEINS

- Physical Techniques
  1. Ultracentrifugation (analytical or Sedimentation velocity ultracentrifuge) at 60,000 per.min. (Refractive index the boundary between the solvent and the protein is visualized by an optical system - called Sehlieren System).

Advantage

Most useful for the determination of the mol. wt of proteins

Disadvantage

High cost of each analysis and poor resolving capacity (when applied to whole serum or plasma)

CHARACTERIZATION, MEASUREMENT AND ISOLATION OF PLASMA PROTEINS

- Electrophoresis

  Protein in aqueous solution are charged groups (e.g. carboxylic (Asp. Glu), amino groups (Lys, Arg), they can be separated under an electric field using various stabilizing media.

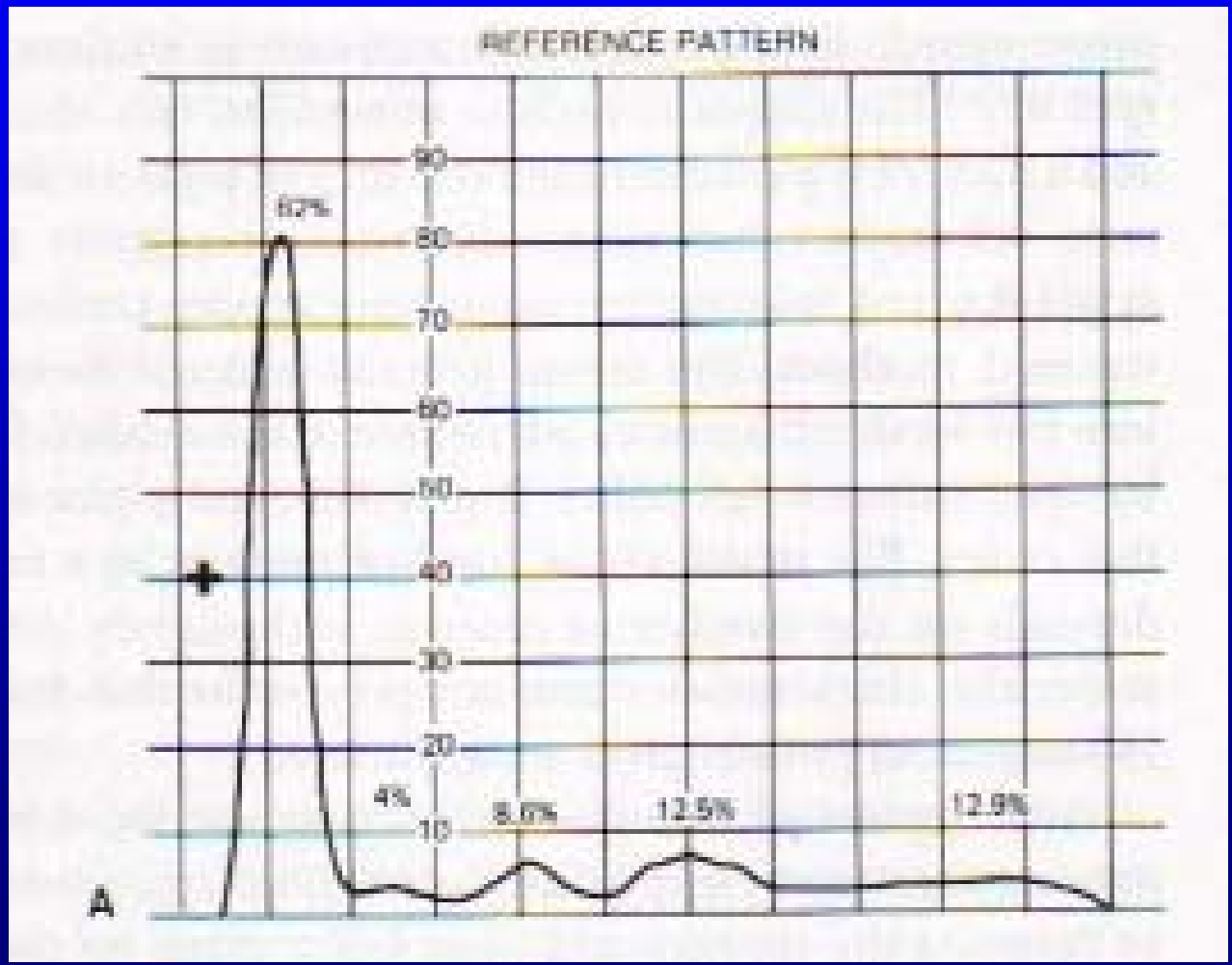
  N.B. Amino groups undergo ionic dissociation at alkaline pH and carboxylic undergo dissociation at acid pH. Most proteins are -ve at pH 8.6. The pH at which +ve charges equal to -ve charges is characteristic for a protein and is called isoelectric point PI).

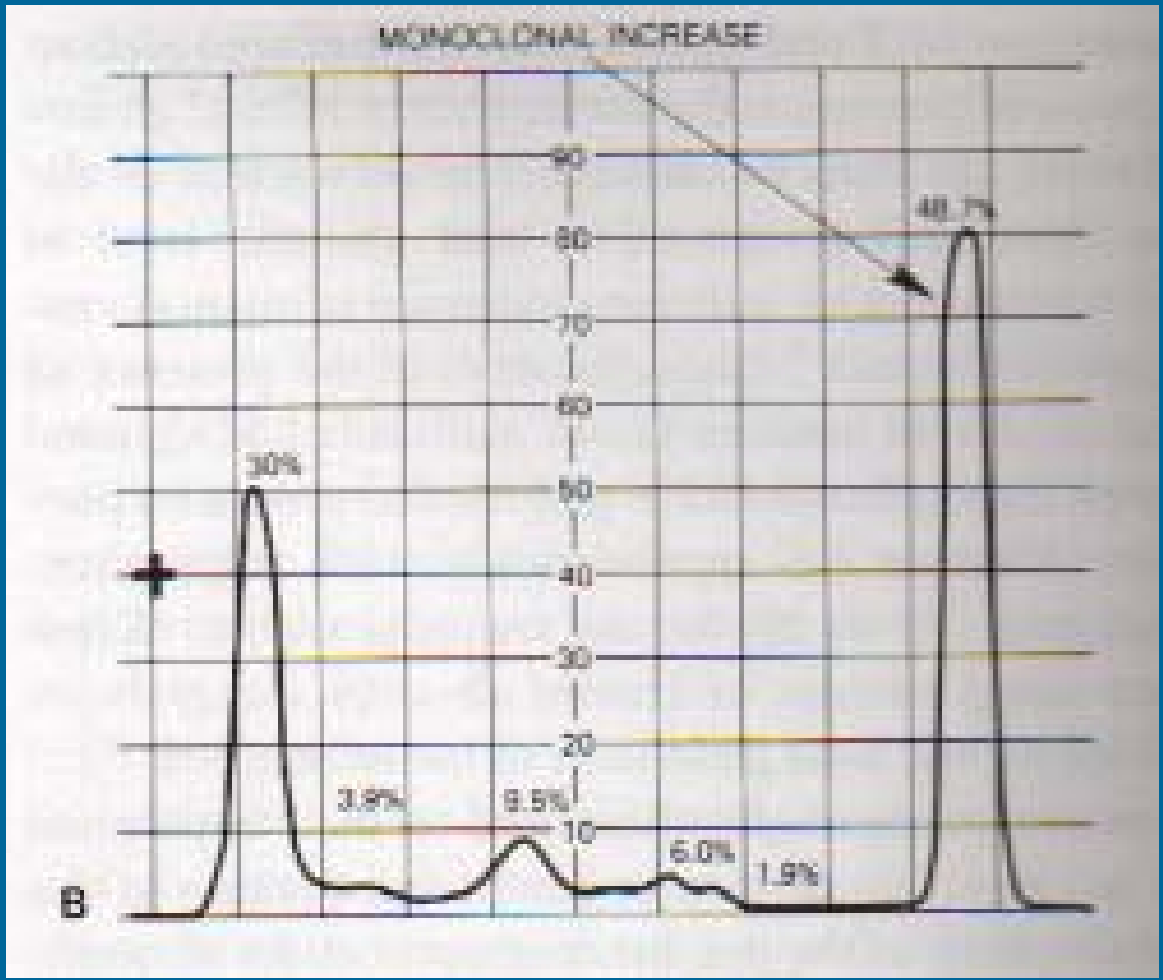
- Boundary electrophoresis: Separation in free liquid media
- Zone electrophoresis - Separation in stabilizing media (e.g. Pager, Cellulose acetate, Starch, Polyacrylamide, Agarose)

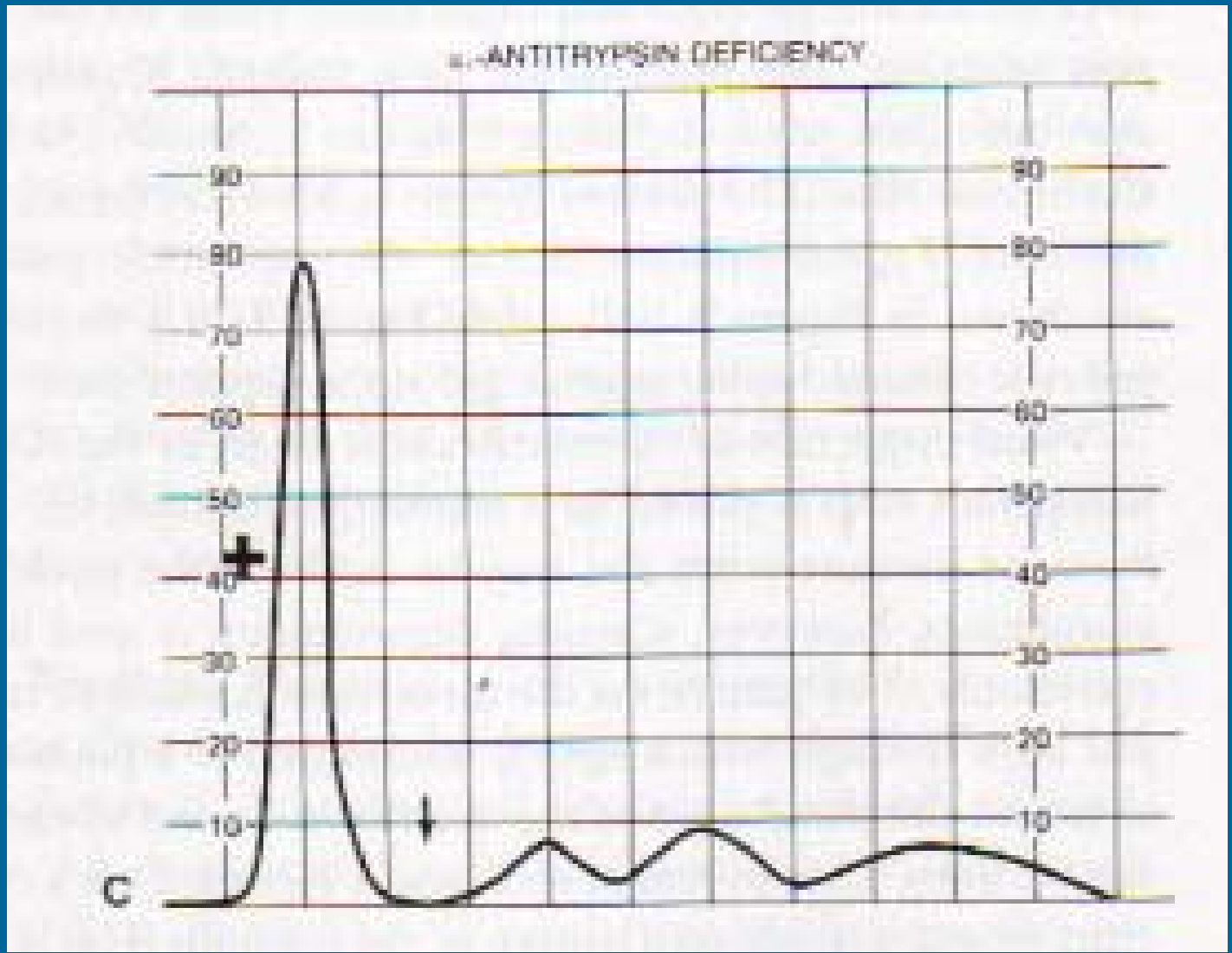
Electrophoresis

- Separates proteins on the basis of their charge.
- Types:
  - Free boundary: separation under an electric field in a fluid media. Separates plasma proteins five bands: albumin(54-58%), α1 globulins (6-7%), α2 globulins(8-9%), β globulins (13-14%), γ globulins (11-12%).
  - Zone electrophoresis: Separation under an electric field in a solid media e.g. paper, starch, cellulose, Acrylamide etc. Separates plasma proteins into: Albumin, α1 globulins, α2 globulins, β globulins, γ globulins and fibrinogen.

NORMAL HUMAN SERUM PROTEIN ELECTROPHORESIS




MONOCLONAL INCREASE
90
80
70
60
50
40
30
20
10
48.7%
30%
3.9%
9.5%
6.0%
1.9%
B


α1-ANTITRYPSIN DEFICIENCY
90
80
70
60
50
40
30
20
10
C

SERUM PROTEIN DEFECTS

- Normal serum protein levels:

  Total serum protein level: 63-83 g/dL.
- Hyperproteinaemia:

  Total serum protein level: > 90 g/dL.
- Hypoproteinaemia:

  Total serum protein level: < 63 g/dL.

INDIVIDUAL PROTEIN FRACTION

ALBUMIN

- A low molecular weight protein (M.Wt= 65,000).
- Functions include:
  - **Transport**
  - **Osmotic pressure regulation**
- Synthesized in the liver.
- Deficiency: in liver disease and kidney disease.

GLOBULINS

- Heterogenous group
- Can be separated into different fractions on the basis of their electrophoretic mobility and sedimentation coefficient:

α1-Globulin	-	α1- Fetoprotein
		α1- Antitrypsin
α2- Globulin	-	α2- Fetoprotein
		Haptoglobin
β- Globulin	-	Transferrin
		Ceruloplasmin
γ- Globulin	-	Antibodies (immunoglobulins)

FIBRINOGEN

- A globulin of very high mol. wt.
- Can be precipitated easily.
- Can be converted to fibrin which causes the blood clot formation.
- Synthesized exclusively in the liver.

BIOCHEMICAL ABNORMALITIES OF PROTEINS

- Total protein abnormalities.
- Abnormalities of individual protein fraction:
  - Serum albumin.
  - Carrier proteins.
  - Protease inhibitors.
  - Immunoglobulins.
  - Embryonic and fetal protein abnormalities. associated with human neoplasia.

TOTAL SERUM PROTEIN ABNORMALITIES

Hypoproteinaemia may result from:

1. Water access caused as a result of:
   a. Overhydration.
   b. Artifactual cause - blood taken from the “drip” arm.
2. Excessive loss of protein (mainly albumin):
   a. Through the kidney in nephrotic syndrome
   b. From the skin after burns
   c. Through the skin in protein losing enteropathy.
3. Decreased synthesis of proteins
   a*. Severe dietary protein deficiency e.g. in Kwashiokar
   b*. Severe liver disease (mainly albumin).
   c. Severe malabsorption.

* There may be no fall in total protein if γ-globulin is raised

HYPOLBUMINAEMIA

- Normal albumin level = 32-52 g/L.
- Hypoalbuminaemia: the level of albumin <32 g/L.
- Frequently encountered.
- Consequence:
  - Oedema
  - Hypocalcaemia
  - Alteration in the levels of protein-bound substance due to loss of carrier protein.

CAUSES OF HYPOALBUMINAEMIA

- Decrease albumin synthesis:
  a. Liver disease (specially chronic diseases).
  b. Malnutrition.
  c. Alcoholism
- Increased albumin loss:
  a. Renal disease (nephrotic syndrome).
     - Loss of albumin in urine (proteinuria).
  b. Extensive burns:
     - Loss of albumin through skin - transdution.

CAUSES OF HYPOALBUMINAEMIA .....Contd

- Defective intake:
  a. Malabsorption due to gastro-intestinal disease
- Protein-losing enteropathy (rare)
  - Excessive loss of protein from the body into the gut.
  - Occurs in a variety of conditions such as :
    a. Ulceration of the bowel.
    b. Lymphatic obstruction.
    c. Intestinal lymphaangiectasis.

CAUSES OF HYPOALBUMINAEMIA .....Contd

- Haemodilution
  a. Over hydration.
  b. Late stage of pregnancy.
- Artefactual
  a. Blood drawn from “drip” arm.
- Non-specific causes (common)
  - In many acute conditions including minor illnesses such as colds and boils.
  - Often in hospitalized patients.
  - Upright position when drawing blood.
  - Newborn babies.
- Increased degradation of albumin. In:
  - Idiopathic
  - Familial idiopathic hypercatabolic hypoproteinemia.
  - Wislcott-Aldrich syndrome

ABNORMALITIES OF CARRIER PROTEINS

α1-globulin

- The normal serum level of α1-globulin is 1-3g/L.
- α1-lipoprotein transport cholesterol.
  - In a rare genetic disorder, α1-lipoprotein deficiency (Tangiers disease), its level is reduced causing the accumulation of cholesterol esters in tissues resulting in:
    - Tonsillar enlargement.
    - Hepatomegaly.
    - Lymphadenopathy

α1-FETOPROTEIN (AFP)

- AFP is synthesized in fetus at 14-40 weeks of gestation.
- AFP levels decline rapidly after 2 weeks of age.
- In adults it is found primarily in:
  - association with hepatocellular cancer of liver and embryonic tumor of the ovary and testes.
  - Cases of gastric and prostatic carcinoma.
  - Viral hepatitis.
  - Cirrhosis.
- AFP detection is very useful in diagnosis of primary liver cancer.

α2-GLOBULIN

The normal α2–globulin level is 6-10 g/L of serum.

α2-Macroglobulin make up most of α2-globulin fraction.

- It is a large molecule
- In nephrotic syndrome, it is retained in serum and levels are found to increase.
- Haptoglobin : binds free haemoglobin. Low levels are found in hemolytic conditions since the haptoglobin/ haemoglobin complex is catabolised better than free haptoglobin.

β-GLOBULIN

Normal level of β-globulin in serum is 7-11 g/L.

- β-lipoprotein transport cholesterol in serum.
- Abetalipoproteinaemia is the complete absence of β-lipoprotein, pre β-lipoprotein and chylomicron. This causes:

  . Inability to transport lipid from intestine or the liver.

  . Plasma cholesterol deficiency.

  – It is clinically characterized by intestinal malabsorption under steatorrhea, progressive atasia, retinitis pigmentation and crenation of erythrocytes.
- High levels of β-globulin are found in pregnancy, biliary obstruction and nephrotic syndrome.

TRANSFERRIN

- Transferrin is a β-globulin.
- It binds free iron in serum.
- Normally it is about one third saturated with iron.
- Transferrin levels are decreased in:
  - *Liver disease (e.g. cirrhosis).*
  - *Chronic infections.*
  - *Nephrosis.*
  - *Congenital atransferrinaemia.*
- Increased serum transferrin levels occur during increased transferrin synthesis caused as a result of iron deficiency anaemia.

ALTERATION OF PLASMA PROTEIN CONCENTRATION

PROTEIN	INCREASED IN	DECREASED IN
Albumin	Dehydration	- Acute and chronic liver disease. - Malnutrition - Malabsorption - Cirrhosis of liver - Burns - Severe trauma - Nephrotic syndrome
Transferrin	- Iron deficiency - In woman taking oral contraceptives.	- Protein losing conditions - Infection; and - Neoplastic disease

Contd.....

ALTERATION OF PLASMA PROTEIN CONCENTRATION .....Contd

PROTEIN	INCREASED IN	DECREASED IN
Ceruloplasmin	- Chronic liver disease - Some infections.	Wilson disease
Haptoglobulin		Haemolytic anaemia
$\alpha 1$-Antitrypsin		Pulmonary emphysema.
$\alpha 2$-Macroglobulin	Nephrotic syndrome collagen disorder	Liver disease in children leading to cirrhosis.
α-Fetoprotein	Hepatocellular carcinoma	
Fibrinogen		- Congenital fibrinogen def. - Shock. - Complication of pregnancy. - Major surgery - Snake bites. - Disseminated carcinoma

INFLAMMATORY RESPONSE

- Assessment of the presence and degree of inflammation can be obtained from the levels of “acute phase protein”
  - Positive acute phase proteins: Increase during inflammation.
  - Negative acute phase proteins: decrease during inflammation.

Cryoglobulins

- Pure monoclonal
  - IgG
  - IgM
  - IgA
- Mixed.
- Consist of complexes of immunoglobulins or altered immunoglobulins.
- Insoluble at 4^{o}C. Aggregate at 30^{o}C

ACUTE PHASE PROTEINS

- Indicators of inflammatory disease with:
  - ESR
  - Leukocytosis
  - Fever
- Indicate active state of inflammation.
- Constitute: $\alpha 1$-antitrypsin
- Carrier proteins:
  - Haptoglobin.
  - Ceruloplasmin.
  - Fibrinogen.
  - C-reactive proteins
  - $\alpha 1$-acid glycoprotein

CLINICAL INDICATIONS FOR ASSESSMENT OF ACUTE PHASE PROTEINS

- Presence of inflammatory disease.
- Differential diagnosis of inflammatory disease.
- Estimation of the endpoint of therapy.
- Monitoring therapeutic effectiveness.
- Postsurgical follow-up in patients at risk of postoperative infections.
- Follow-up of patient with malignancy.

POSITIVE ACUTE PHASE PROTEINS

- α1-antitrypsin.
- α1-antichymotrypsin.
- α1-acid glycoprotein.
- Ceruloplasmin.
- Haptoglobin.
- Complement component C3 and C4.
- Antithrombin III.

SPECIFIC INDICATIONS FOR QUATIFICATION OF SOME ACUTE PHASE PROTEINS

PROTEIN	DISEASE
$\alpha 1$-antitrypsin	- Chronic obstructive pulmonary disease. - Neonatal hepatitis syndrome cytogenic cirrhosis.
Ceruloplasmin	Hepatitis or cirrhosis (unexplained)
Haptoglobin	In-vivo haemolysis. Ineffective erythropoiesis

EMBRYONIC AND FETAL PROTEIN ASSOCIATED WITH HUMAN NEOPLASIA

- Several fetal proteins and synthesized in human tumors.
- They are released in biological fluid.
- Useful in - diagnosis of malignancy
  - monitoring of therapy for cancer
  - evaluation of prognosis:
- The protein often found associated with tumors are:
  - – α1-fetoproteins
  - – α2-H fetoprotein
  - – β2-S fetoprotein
    - regain alkaline phosphatase
    - fetal sulphoglycoprotein antigen
  - – γ-fetoproteins
    - Carcinoembryonic antigen of the gastrointestinal tract.

INHERITED ABNORMALITIES OF THE Plasma Proteins

DEFICIENCY	ASSOCIATED ABNORMALITY
$\alpha 1$-Antitrypsin	Obstructive pulmonary disease (Chronic or emphysema) liver disease.
Anti-thrombin	Thrombosis Pulmonary embolism
Immunoglobulin	Severe recurrent or chronic infection
Complement	Severe, recurrent infection.
C1 esterase inhibitor	Recurrent non-pruritic swelling of skin and mucus membrane (hereditary angioneurotic edema)

PLASMA PROTEIN CHANGES IN LIVER DISEASES

Liver disease	HPT	A1b	C3	LDL	IgG	IgM	IgA	TRF	Pre-Alb	α1-AT
"Pure" Biliary Obstruction	↑↑		↑↑	↑↑						
Advanced Hepatic Cirrhosis	↓↓	↓	↓		↑↑	(↑↑)	↑↑	↓	↓↓	↑
Acute Viral Hepatitis	(↓)				(↑)	↑	(↑)			↑
Infection Mononucleosis	↓				(↑)	↑	(↑)			

↓ = Decrease
↑ = Increase

(↓) = May be decreased
(↑) = May be increased

NEGATIVE ACUTE PHASE PROTEINS

- Albumin.
- Transferrin.
- Pre-albumin.

BIOCHEMICAL INVESTIGATIONS IN THE DIAGNOSIS OF DISEASE STATES

TYPES OF BIOCHEMICAL TESTS

1. Discretionary tests.
2. Profile and screening investigations.
   a. On patients.
   b. On apparently healthy individuals.

COMMONLY REQUESTED DISCRETIONARY BIOCHEMICAL TESTS

TEST	SUSPECTED DISEASE
- Bilirubin	Liver disorders.
- Glucose	Diabetes Mellitus
- Iron and Total Iron Binding capacity	Anaemias
- Urea	Renal function
- Creatinine	Renal function
- Uric acid	Gout
- Electrolytes	Water and electrolyte balance
- Plasma enzymes	Liver, cardiac, muscle, etc.
- Cholesterol/Lipids	Cardiac diseases
- Blood gases	Acid-Base balance

EXAMPLES OF ORGAN-SPECIFIC PROFILES

	TESTS
- Electrolyte profile	Na^+, K^+, Cl^-, HCO^-_3
- Liver function tests	Bilirubin, alkaline phosphatase, alanine transaminase (SGPT), Plasma albumin.
- Bone Profile	Ca^{x2}, alkaline phosphatase, phosphate.
- Kidney function tests	Creatinine, urea.
- Acid-Base balance	pH, PCO_2, HCO^-_3
- Cardiac profile	Lactate dehydrogenase (LDH), Creatinine phosphokinase (CPK), Aspartate transaminase (SGOT)
- Endocrine profile	T3, T4, TSH, and Thyroid function

METHODS USED IN IDENTIFICATION AND QUANTITATION OF NORMAL AND ABNORMAL BLOOD PROTEINS

a. Plasma Proteins
b. Haemoglobin

METHODS FOR PLASMA PROTEIN ESTIMATION

- Quantitation:
  - *Total Protein*
  - *Albumin*
  - *Globulin*
- Manually - Biuret method. Colour development with Cu^{+2} reagent.
- Autoanalyser - SMAC
  - American monitor
- Method for specific protein:
  - *Immunodiffusion e.g. transferrin, immunoglobulins*
  - *Nephelometric method e.g. Albumin, α1-antitrypsin, immunoglobulins.*
  - *RIA method e.g. Ferritin, Immunoglobulin, Protein Hormones.*

IDENTIFICATION

- Electrophoresis:
  - Widely used method.
  - Simple.
  - Proteins are separated on the basis of the charges under an electric field.
  - Useful investigation of disease states e.g. liver, renal diseases, infections.

IMMUNODIFFUSION

- Used for specific protein identification.
- Simple procedure.
- Proteins are identified on the basis of precipitation reaction with respective antibodies.

IMMUNOELECTROPHORESIS

- Complex procedure.
- Accurate.
- Proteins are identified on the basis of their change and precipitation reaction with respective antibody.

METHODS USED FOR ESTIMATION OF HAEMOGLOBIN

- Estimation of total haemoglobin:
  a. Manually: Cyanomethaemoglobin method
     - Not used commonly.
     - Not very accurate.
  b. Autoanalyzer: Coulter Counter with haemoglobinometer attachment:
     - Widely used.
     - Very accurate.
     - Simple.
     - Estimates total RBC, WBC, MCV, MCH, MCHC.

INHERITED ABNORMALITIES OF PLASMA PROTEINS

DEFICIENCY	ASSOCIATED ABNORMALITY
$\alpha 1$-Antitrypsin	Obstructive pulmonary disease (Chronic bronchitus or emphysema), liver disease.
Anti-thrombin	Thrombosis. Pulmonary embolism.
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Complement	Severe, recurrent infection.
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  - diagnosis of malignancy.
  - monitoring of therapy for cancer.
  - evaluation of prognosis.
- The protein often found associated with tumors are:
  - α-feto proteins
  - $\alpha 2$–ferroprotein.
  - β-fetoprotein.
  - Alkaline phosphatase.
  - Fetal sulphoglycoprotein antigen.
  - γ-fetoproteins.
  - Carcinoembryonic antigen of the gastrointestinal tract.

IDENTIFICATION OF HAEMOGLOBIN TYPES

a. Electrophoresis at alkaline acid pH

- Simple procedure
- Accurate
- Useful for identification of several Hb variants (not all).
- Proteins can be quantitated by using a densitometer.

IDENTIFICATION OF HAEMOGLOBIN TYPES

b. Isoelectric Focussing:

- Separation on the basis of isoelectric pH of haemoglobin variants.
- Simple method.
- Does not separate all variants.

LABORATORY INVESTIGATIONS OF ANAEMIA

- Haemoglobin, RBC and PCV.
- Red cell indices.
- Red cell morphology.
- Iron and TIBC estimation.
- Hb A_2 and F estimation.
- Haemoglobin electrophoresis at acid and alkaline pH.

METHODS USED FOR INVESTIGATION OF HAEMOGLOBINOPATHIES

- Detection of haemoglobinopathies and thalassaemias - Haematological Tests.
  - Hb
  - RBC count
  - PCV
  - MCH
  - MCHC
  - Red cell morphology

Contd.....

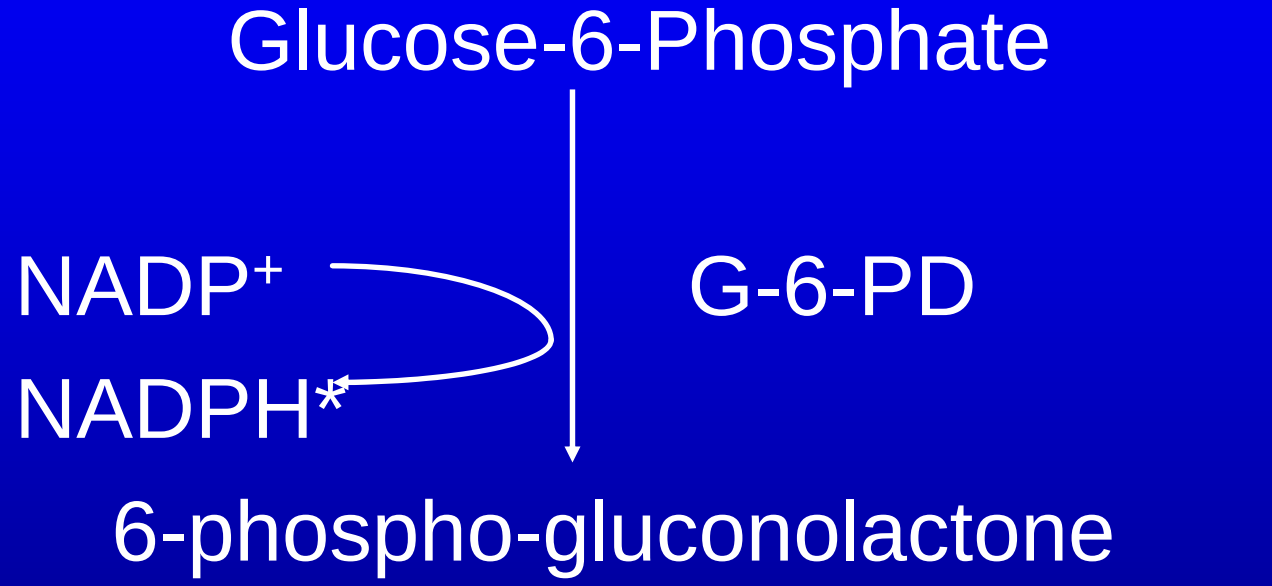
METHODS USED FOR INVESTIGATION OF HAEMOGLOBINOPATHIES ...Contd

- Differentiation and confirmation of haemoglobinopathies and thalassaemias:
  - Electrophoresis.
  - Hb A_2 quantitation.
  - Hb F quantitation.
  - Hb stability test.
  - Determination of α/non-α globin chain ratio.
  - Studies at gene level.

BIOCHEMICAL TEST IN THE INVESTIGATION OF G-6-PD PD DEFICIENCY

1. Estimation of red cell G-6-PD activity
   - Spot tests
   - Spectrophotometric method.
2. Phenotyping by electrophoresis.

ESTIMATION OF G-6-PD ACTIVITY REACTION



* NADPH is estimated by measuring

a. Fluorescence (under UV Lamp)

b. Absorbance at 340 nm

PATHOLOGICAL CHANGES IN LIVER DISEASE

a. Liver cell damage

(acute hepatitis, toxins, chronic hepatitis, prolonged biliary obstruction, cirrhosis, hepatic congestion).

b. Cholestasis

- Intrahepatic cholestasis:

(Viral hepatitis, biliary cirrhosis, infiltration of the liver).

- Extra hepatic cholestasis

(Gallstone in the common bile duct, fibrosis of the bile duct, carcinoma of head of pancreas, external presence of tumour).

CONSEQUENCES OF LIVER DISEASES

- ↓ Synthesis of plasma proteins.
- ↑ Release of hepatic proteins and enzymes.
- ↓ Excretion of some metabolites.

LIVER FUNCTION TESTS

The principal function of the liver include:

- Conjugation and excretion of bilirubin.
- Metabolism of carbohydrates, proteins and lipids.
- Detoxication of drugs, metabolite and hormone.
- Excretion of various natural and foreign substances into the biliary tract.
- Storage.

LIVER FUNCTION TESTS

- Total bilirubin.
- Transaminase (SGPT & SGOT)
- Alkaline phosphatase.
- Albumin
- Total protein

TESTS PERFORMED IN SUSPECTED CASES OF DIFFERENT LIVER DISEASES

Tests	Acute Hepatitis	Chronic Hepatitis	Cirrhosis	Choles-tosis	Hepatic Infiltration	Hepato-cellular Carcinoma
Plasma Bilirubin	√		√	√		
SGOT SGPT	√ √	√	√ √	√	√	√
Urinary Bilirubin Urobilirubin	√ √					
Hepatitis associated antigen		√				
Plasma protein electrophoresis		√	√			
Alkaline phosphatase			√	√	√	√
5’ Nucleotidase			√	√	√	√
α-Fetoprotein						√
γ-Glutamyl Transfe-rase				√		

TYPES OF BILE PIGMENTS PRESENT IN PLASMA, URINE AND FEACEA IN DIFFERENT TYPES OF JAUNDICE

Disease	Plasma		Uric		Feaces
	Total Bilirubin	Excess Conju-gated Bilirubin (Direct Van der Brough Reaction)	Uro Bilirubin	Bilirubin	Urobilirogen
Normal	Present	-	Present	Absent	Present
Haemolytic Jaundice	+	-	Increased	Absent	ⅡI
Hepatic (Infective hepatitis)	ⅡI	+	Variable	+	Low
Post Hepatic	III	ⅡI	Absent	ⅡI	Absent

FUNCTIONS OF THE KIDNEY

- To excrete water and ions from the body.
- To maintain the composition of plasma normal by excreting or reabsorbing substances.
- To maintain acid-base balance.
- To excrete metabolic end products, hormones, drugs.
- To control blood pressure.

CHOICE OF RENAL FUNCTION TESTS

TESTS	CONDITION
- Examination of urine	Suspected renal damage.
- The water deprivation or vasopressin test	Most useful single test to confirm renal tubular impairment.
- Creatinine clearance	Quantitative test for glomerular impairment.
- Estimation of plasma urea and creatinine	Guide to progress and prognosis if there is severe renal damage or obstruction.

PLASMA PROTEIN CHANGES IN RENAL DISEASE

↓ Albumin

↑ α2-globulins

↓ γ-globulins (often)

↓ C3 and C4 in acute glomerulonephritis

↓ C3, normal C4 in membrane proliferative glomerulonephritis.

URINARY PROTEIN CHANGES IN RENAL DISEASES

Glomerulal Proteinura*	Overflow Proteinura	Revert Tubular Disease Proteinuria**	Nephrogenic Proteinuria
↑ Albumin	↑ Bench flow protein	↑ α2-Globular	↑ IgG, IgM
↑ Transferrin	↑ Myoglobin ad	↑ β-Globular	IgE
↑ Acid Glycoprotein	Haemoglobin	Slightly ↑ albumin and transferrin	
↑ α1-Antitrypsin	↑ Acid glyco-protein	↑ β2-Microglobulin	
↑ IgG	↑ α1-Antitrypsin	↑ Lysozone	

* Ratio of albumin to low mol-wt. protein 20:1

** Ratio of albumin to low mol-wt. protein 1:1

BLOOD TESTS FOR BONE DISEASE

Blood Ca^{+2} level.

- Blood phosphate level.
- Alkaline phosphatase level.
- Parathyroid hormone level.

BLOOD TESTS FOR DIAGNOSIS OF DIABETES MELLITUS

- Random blood glucose estimation.
- Fasting blood glucose estimation.
- Testing for glucose in urine.
- Two-hour post-glucose blood glucose level.
- Glucose Tolerance Test (GTT).

MUSCLE DISEASE TESTS

Creatine phosphokinase (CPK).

- Creatine and Creatinine in Serum.
- Calcium.
- Na^{+} in Serum.
- K^{+} in Serum.
- Mg^{+2} in Serum