THE 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 O2 and CO2.
- 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%).
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Plasma</th>
<th>Red blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>91-95%</td>
<td>65%</td>
</tr>
<tr>
<td>Solid</td>
<td>8-9 %</td>
<td>35%</td>
</tr>
<tr>
<td>Protein</td>
<td>6-8 gm %</td>
<td>31-33%</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.026</td>
<td></td>
</tr>
</tbody>
</table>
Haematocrit or Packed Cell Volume (PCV)

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

Plasma

PCV = 0.45 L/L
= 0.41 L/L

PCV ↓ is anaemia
↑ is polycythaemia
SERUM

Whole blood → Clot formation

Centrifuge

Clot + Clear yellowish fluid (serum)

No anticoagulant
ERYTHROCYTE SEDIMENTATION RATE (ESR)

- Rate of Sedimentation of Erythrocytes.
- ESR at $20 \pm 3 \, ^\circ C$ (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.

Erythropoisis
Formation of Erythrocytes

Leucopoisis
Formation of Leucocytes

Thrombopoisis
Formation of Thrombocytes
- Erythropoiesis → RBC
- Leucopoiesis → WBC
- Granulopoiesis → Granulocytes
- Lymphopoiesis → Lymphocytes
- Megakaryocytes → Platelets
Site of Haemopoiosis

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
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
Polychromatoblasts 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
Erythropoiesis

- Stem cell
- Pronormoblast
- Basophilic normoblast
- Polychromatophilic normoblast
- Orthochromatnic normoblast
- Reticulocytes
- Mature red cell
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):

- Bone marrow
- Stem cell
- Erythroid tissue

- DNA, mRNA

- Erythropoietin

- Red cell mass
- Atm.O2
- Cardiopulmonary function
- Blood volume
- Hb concentration
- $O_2$ affinity

- Renal vacc
- Renal $O_2$ Consumption

- Erythropoietin $O_2$ Sensor
- Produce Epo

- Kidney
  (Liver, Macrophages)
Erythropoiesis

• The proliferation and differentiation of cells from pluripotent noncommitted stem cells of the bone marrow.

• Two main compartments:
Erythropoiesis compartments

- The erythroid progenitor cell compartment
- The erythroid precursor cell (erythron)
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 $\alpha$- and $\beta$- 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.
Intrauterine 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.
Intrauterine 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 4 yrs of life, nearly all the marrow cavities contain red haemopoietic marrow with very few fat cells. By the age of 25 yrs 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

- 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 O₂ and CO₂.

- Normal Range:
  - 5.5 ± 1.0 x 10^{12}/L
  - 4.8 ± 1.0 x 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 $2.3 \times 10^6$ red cell/sec.
ERYTHROPOIETIN

- A polypeptide hormone.
- Glycoprotein of 166 a.a. (mol. li 34 Kdl).
- Major regulator of human erythropoiesis.
- Synthesized mainly in kidney, released in response to hypoxia and acts 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 cytosol 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
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

- 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

- $\alpha$ Globin: 141 a.a.
- $\beta$-like globin chains: 146 a.a.

Structure of globin chains

- Globular, compact structure
- $\sim$75% $\alpha$-helices
- Have a hydrophobic cavity for binding heme.
Haemoglobin

Globin Chains

Heam 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

[Chemical structure image of heme (Fe-protoporphyrin IX)]
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.....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 $\beta$-globin chain → 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

Acid, warm (tissues), high CO₂, low O₂

Hb(O₂)₄
Oxyhemoglobin
**The Bohr Effect**

**In Lungs:**
- High $\text{PO}_2$, $\downarrow \text{H}^+$, $\downarrow \text{CO}_2 \Rightarrow$ high affinity of $\text{Hb}$ for $\text{O}_2$ ($\text{O}_2$ dissociation curve shifts to left).

**In Tissues**
- Low $\text{P O}_2$, $\uparrow \text{H}^+$, $\uparrow \text{CO}_2$, $\uparrow 2,3 \text{DPG} \Rightarrow$ Low affinity of $\text{Hb}$ for $\text{O}_2$ ($\text{O}_2$ dissociation curve shifts to right)
THE BOHR EFFECT

Lungs

\[ 2\text{CO}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{CO}_3 \]

\[ \text{HCO}_3^- + 2\text{H}^+ \rightarrow \text{Hb} + 4\text{O}_2 \]

\[ 4\text{O}_2 \rightarrow \text{Hb} + 2\text{H}^+ \]

\[ 2\text{H}^+ + 2\text{HCO}_3^- \rightarrow 2\text{H}_2\text{CO}_3^- \]

\[ 2\text{H}_2\text{CO}_3^- \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O} \]

Generated by TCA Cycle

Peripheral Tissues

Carbonic Anhydrase

\[ 2\text{CO}_2 \text{ Exhaled} \]
O2 Dissociation curve of Haemoglobin

Saturation %

pO2 (Torr)

26
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 O₂.
- HbF cannot bind 2,3 DPG and has higher affinity for O₂.
  - O₂ can be transported from mother to fetal blood
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

\[
\begin{align*}
2\text{GSH} & \rightarrow \text{G-S-S-G} \\
\text{H}_2\text{O}_2 & \rightarrow 2\text{H}_2\text{O}
\end{align*}
\]

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

- 95% Oxidised by glycolysis
- 5% Oxidized by pentose-phosphate pathways
The role of glycolysis in the functional requirements of mature red cells:

<table>
<thead>
<tr>
<th>Function</th>
<th>EMP</th>
<th>PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Maintenance of shape</td>
<td>ATP</td>
<td></td>
</tr>
<tr>
<td>- Membrane structure and Function</td>
<td></td>
<td>GSH</td>
</tr>
<tr>
<td>- Regulation of O₂ transport</td>
<td>2,3-DPG</td>
<td></td>
</tr>
<tr>
<td>- Reducing potential</td>
<td>NADP</td>
<td>GSH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NADPH</td>
</tr>
</tbody>
</table>
During metabolism, there is production of:
- Superoxides (O$_2$): O$_2$ + e$^-$ → O$_2$
- Hydrogen peroxide (H$_2$O$_2$)
  O$_2$ + O$_2$ + 2H → H$_2$O$_2$ + 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
  \[ \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

- Catalase:
  \[ \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O} \]

- Glutathione
  \[ 2\text{GSH} + \text{RO} – \text{OH} \rightarrow \text{GSSG} + \text{H}_2\text{O} + \text{ROH} \]

  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:
  \[ \text{G-6-P} + \text{NADP}^+ \rightarrow \text{6-Phosphogluconolactone} + \text{NADPH} + \text{H}^+ \]
- 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.

• $250 \times 10^9$ 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
   a- Granulocytes: Play a role in protecting the body against infection by phagocytosis.
      - Neutrophils
      - 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.
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Phagocytose bacteria and play a major role in accurate information.</td>
</tr>
<tr>
<td>Basophils</td>
<td>Resemble mast cells and contain histamine and heparin – play a major role in immunologic hypersensitivity reaction.</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Involved in certain allergic reactions and parasitic infection.</td>
</tr>
</tbody>
</table>
NEUTROPHILS

Responsible for acute inflammatory response

- Increase vascular permeability
- Cause entry of activated neutrophils into tissues
- Cause Activation of Platelets
- Spontaneous subsidence (resolution) of invading organism that have been dealt with successfully

By:
- Platelet, activating factor (PAF)
- Eicosanoids (various prostaglandins and leukotriens)
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.
• Involved in coagulation of blood
Haemolysis of Erythrocyte
Haemolysis

Haemolysed red cells

>120 days

Free Haemoglobin

In Reticuloendothelial cells
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

Haem

Globins
Haemolyzed RBC

RE System

Haemoglobin

Met haemoglobin

Globin + Heme + Fe^{++}

Amino acids

Blood

Biliverdin

Bilirubin

Bilirubin - Albumin complex

Liver

Bilirubin - Albumin

Bilirubin

Transferrin - Fe^{++}

Apotransferrin

Bone marrow

Fe^{++} reutilization
In R.E.S.

**Heme Oxygenase**

Heme → Biliverdin + CO + Fe^{++}

NADPH - Cytochrome Reductase

3O₂ + 3NADPH + H⁺ → 3 NADP⁺

2H⁺

NADPH-Bilirubin Reductase

BILIRUBIN
In Liver

Bilirubin + UDP - Glucuronic acid

\[ \text{Glucuronyl Transferase} \]
\[ \text{Bilirubin Monoglucuronide} + \text{UDP} \]

\[ \text{UDP-glucuronic acid} \]

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.

Examples:
- 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.....
Dubin-Johnsons Syndrome

- Defective excretion of conjugated bilirubin.
- Mildly raised conjugated bilirubin.
- Bilirubin in urine.
- Harmless.
### Types of Bilirubin present in different Jaundice

<table>
<thead>
<tr>
<th>Defect</th>
<th>Types of Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Increased production</td>
<td>Unconjugated bilirubin</td>
</tr>
<tr>
<td>- Haemolytic disease</td>
<td></td>
</tr>
<tr>
<td>- Reduced liver uptake of bilirubin</td>
<td>Unconjugated bilirubin</td>
</tr>
<tr>
<td>- Drug competition</td>
<td></td>
</tr>
<tr>
<td>- Reduced conjugation of bilirubin</td>
<td>Unconjugated bilirubin</td>
</tr>
<tr>
<td>- Developmental defect</td>
<td></td>
</tr>
<tr>
<td>- Drug competition</td>
<td></td>
</tr>
<tr>
<td>- Inherited enzyme defects</td>
<td></td>
</tr>
<tr>
<td>- Gilbert’s disease,</td>
<td>Contd....</td>
</tr>
<tr>
<td>- Criglar Najjar disease</td>
<td></td>
</tr>
<tr>
<td>Defect</td>
<td>Types of Bilirubin</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Decrease secretion of conjugated bilirubin</td>
<td>Mainly conjugated bilirubin</td>
</tr>
<tr>
<td>- Drug competition</td>
<td></td>
</tr>
<tr>
<td>- Inherited defects</td>
<td></td>
</tr>
<tr>
<td>Obstruction of biliary tree (cholestasis)</td>
<td>Mainly conjugated bilirubin</td>
</tr>
<tr>
<td>- Within the liver</td>
<td></td>
</tr>
<tr>
<td>(liver cirrhosis, drugs side-effects)</td>
<td></td>
</tr>
<tr>
<td>- Outside the liver</td>
<td></td>
</tr>
<tr>
<td>(gallstone, neoplasm)</td>
<td></td>
</tr>
</tbody>
</table>
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.
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).

\[
\text{Hb (g/dl blood)} = \text{MCH} \\
\text{RBC Count} \times 10^{12}/l
\]

- Normal range 27-32 pg
Mean Corpuscular Haemoglobin Concentration (MCHC)

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

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

- Normal Range 30-35 g/dl
(a) Normocytic Normochromic Anaemia  
\[(MCV = 85-100 \text{ fl}) \ (MCH = 31-35 \text{ pg})\]

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

1. Iron deficiency.
2. Thalassaemias.
3. Sideroblastic anaemia:
   (a) Refractory.
   (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. 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.
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.
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.
- 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.
- 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.
Causes:

- Infections:
  - Sepsis and septicaemia:
    - Streptococcus, Clostridium, Welchi (gas gangrene)
    - Typhoid fever
    - Viral infection
  - Poisons:
    - Chronic lead poisoning
    - Acute lead poisoning
    - Chemicals (phenylhydrazine, saponins)
    - Snake venoms
- Allergic haemolytic anaemia:
  - Pollens or vegetables
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
- 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 Meagloblastic 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  \[\downarrow\]  Vit. B12 absorption  \[\rightarrow\]  Ineffective erythropoiesis  \[\rightarrow\]  Megaloblastic red cells
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

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

*These concentrations are in micrograms per 100 ml.
# Principal Non-Protein Organic Constituents of Human Blood Plasma

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Protein N :</td>
<td>25 - 40</td>
</tr>
<tr>
<td>Amino acid N</td>
<td>4 - 8</td>
</tr>
<tr>
<td>Amino acids</td>
<td>36 - 65</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.2 - 1.4</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.2 - 0.9</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2 - 6</td>
</tr>
<tr>
<td>Carbohydrates:</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>65 - 90</td>
</tr>
<tr>
<td>Fructose</td>
<td>6 - 8</td>
</tr>
</tbody>
</table>

Contd...
### Principal Non-Protein Organic Constituents of Human Blood Plasma

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic acids:</strong></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.4 - 3.0</td>
</tr>
<tr>
<td>α-ketoglutaric acid</td>
<td>0.2 - 1.0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>8 - 17</td>
</tr>
<tr>
<td><strong>Lipids:</strong></td>
<td></td>
</tr>
<tr>
<td>Total lipids</td>
<td>285 - 675</td>
</tr>
<tr>
<td>Neutral fat</td>
<td>80 - 240</td>
</tr>
<tr>
<td>Cholesterol, total</td>
<td>130 - 260</td>
</tr>
<tr>
<td><strong>Phosphoglyceride:</strong></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>150 - 250</td>
</tr>
</tbody>
</table>
Plasma Proteins
The normal serum protein level is 63-83 g/L.

The type of proteins in serum include:

a. Albumin
b. Globulins
   - \( \alpha \)-globulin: \( \alpha_1 \) & \( \alpha_2 \)-globulins
   - \( \beta \)-globulin: \( \beta_1 \) & \( \beta_2 \) globulins
   - \( \gamma \)-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. α₁ 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. α₁ antitrypsin, α₂ 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

<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>PLASMA CONC. (g/L)</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-albumin</td>
<td>0.3</td>
<td>Binds T3 &amp; T4</td>
</tr>
<tr>
<td>Albumin</td>
<td>40.0</td>
<td>Transport, colloid oncotic pressure</td>
</tr>
<tr>
<td>α1- globulin : α1- antitrypsin</td>
<td>3.0</td>
<td>Anti proteinase</td>
</tr>
<tr>
<td>α2- globulins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceruloplasmin</td>
<td>0.4</td>
<td>Copper transport</td>
</tr>
<tr>
<td>haptoglobin</td>
<td>1.2</td>
<td>Binds haemoglobin</td>
</tr>
<tr>
<td>α2-macroglobulin</td>
<td>3.0</td>
<td>Transport, anti-proteinase</td>
</tr>
</tbody>
</table>

Contd.............
Specific Functions of some proteins

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

\[
\begin{align*}
\alpha_1-\text{Globulin} & \quad - \quad \alpha_1-\text{Fetoprotein} \\
\alpha_1-\text{Antitrypsin} & \\
\alpha_2-\text{Globulin} & \quad - \quad \alpha_2-\text{Fetoprotein} \\
\text{Haptoglobin} & \\
\beta-\text{Globulin} & \quad - \quad \text{Transferrin} \\
\text{Ceruloplasmin} & \\
\gamma-\text{Globulin} & \quad - \quad \text{Antibodies (immunoglobulins)}
\end{align*}
\]
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.
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 $\gamma$-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 HYPOALBUMINEMIA

• 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 lymphangiectasis.
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
\( \alpha_1 \)-globulin

- The normal serum level of \( \alpha_1 \)-globulin is 1-3g/L.
- \( \alpha_1 \)-lipoprotein transport cholesterol.
  - In a rare genetic disorder, \( \alpha_1 \)-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.
The normal $\alpha_2$-globulin level is 6-10 g/L of serum. $\alpha_2$-Macroglubulin make up most of $\alpha_2$-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.
Normal level of $\beta$-globulin in serum is 7-11 g/L.

- $\beta$-lipoprotein transport cholesterol in serum.
- Abetalipoproteinaemia is the complete absence of $\beta$-lipoprotein, pre $\beta$-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 $\beta$-globulin are found in pregnancy, biliary obstruction and nephrotic syndrome.
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.
<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>INCREASED IN</th>
<th>DECREASED IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Dehydration</td>
<td>- Acute and chronic liver disease.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Malnutrition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Malabsorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cirrhosis of liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Severe trauma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Nephrotic syndrome</td>
</tr>
<tr>
<td>Transferrin</td>
<td>- Iron deficiency</td>
<td>- Protein losing conditions</td>
</tr>
<tr>
<td></td>
<td>- In woman taking oral contraceptives.</td>
<td>- Infection; and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Neoplastic disease</td>
</tr>
</tbody>
</table>

Contd.............
<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>INCREASED IN</th>
<th>DECREASED IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceruloplasmin</td>
<td>- Chronic liver disease</td>
<td>Wilson disease</td>
</tr>
<tr>
<td></td>
<td>- Some infections.</td>
<td></td>
</tr>
<tr>
<td>Haptoglobinulin</td>
<td></td>
<td>Haemolytic anaemia</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td></td>
<td>Pulmonary emphysema.</td>
</tr>
<tr>
<td>α2-Macroglobulin</td>
<td>Nephrotic syndrome</td>
<td>Liver disease in children leading to cirrhosis.</td>
</tr>
<tr>
<td></td>
<td>collagen disorder</td>
<td></td>
</tr>
<tr>
<td>α-Fetoprotein</td>
<td>Hepatocellular carcinoma</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td>- Congenital fibrinogen def.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Shock.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Complication of pregnancy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Major surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Snake bites.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Disseminated carcinoma</td>
</tr>
</tbody>
</table>
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°C. Aggregate at 30°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

- $\alpha_1$-antitrypsin.
- $\alpha_1$-antichymotrypsin.
- $\alpha_1$-acid glycoprotein.
- Ceruloplasmin.
- Haptoglobin.
- Complement component C3 and C4.
- Antithrombin III.
<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-antitrypsin</td>
<td>- Chronic obstructive pulmonary disease.</td>
</tr>
<tr>
<td></td>
<td>- Neonatal hepatitis syndrome cytogenic cirrhosis.</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Hepatitis or cirrhosis (unexplained)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>In-vivo haemolysis.</td>
</tr>
<tr>
<td></td>
<td>Ineffective erythropoiesis.</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>DEFICIENCY</th>
<th>ASSOCIATED ABNORMALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-Antitrypsin</td>
<td>Obstructive pulmonary disease (Chronic or emphysema) liver disease.</td>
</tr>
<tr>
<td>Anti-thrombin</td>
<td>Thrombosis</td>
</tr>
<tr>
<td></td>
<td>Pulmonary embolism</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>Severe recurrent or chronic infection</td>
</tr>
<tr>
<td>Complement</td>
<td>Severe, recurrent infection.</td>
</tr>
<tr>
<td>C1 esterase inhibitor</td>
<td>Recurrent non-pruritic swelling of skin and mucus membrane (hereditary angioneurotic edema)</td>
</tr>
</tbody>
</table>
# PLASMA PROTEIN CHANGES IN LIVER DISEASES

<table>
<thead>
<tr>
<th>Liver disease</th>
<th>HPT</th>
<th>A1b</th>
<th>C3</th>
<th>LDL</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>TRF</th>
<th>Pre-Alb</th>
<th>α1-AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Pure” Biliary Obstruction</td>
<td>↑↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td></td>
<td>↑↑</td>
<td>(↑↑)</td>
<td>↑↑</td>
<td>↓↑</td>
<td>↓↓↓</td>
<td>↑↓</td>
</tr>
<tr>
<td>Advanced Hepatic Cirrhosis</td>
<td>↓↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>↑↑</td>
<td>(↑↑)</td>
<td>↑↑</td>
<td>↓</td>
<td>↓↓</td>
<td>↑</td>
</tr>
<tr>
<td>Acute Viral Hepatitis</td>
<td>(↓)</td>
<td></td>
<td></td>
<td></td>
<td>(↑)</td>
<td>↑</td>
<td>(↑)</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Infection Mononucleosis</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td>(↑)</td>
<td>↑</td>
<td>(↑)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↓ = Decrease  (↓) = May be decreased  
↑ = Increase  (↑) = 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.
<table>
<thead>
<tr>
<th>TEST</th>
<th>SUSPECTED DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>Liver disorders.</td>
</tr>
<tr>
<td>Glucose</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>Iron and Total Iron Binding capacity</td>
<td>Anaemias</td>
</tr>
<tr>
<td>Urea</td>
<td>Renal function</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Renal function</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Gout</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>Water and electrolyte balance</td>
</tr>
<tr>
<td>Plasma enzymes</td>
<td>Liver, cardiac, muscle, etc.</td>
</tr>
<tr>
<td>Cholesterol/Lipids</td>
<td>Cardiac diseases</td>
</tr>
<tr>
<td>Blood gases</td>
<td>Acid-Base balance</td>
</tr>
</tbody>
</table>
EXAMPLES OF ORGAN-SPECIFIC PROFILES

<table>
<thead>
<tr>
<th>TESTS</th>
<th>EXAMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolyte profile</td>
<td>Na⁺, K⁺, Cl⁻, HCO⁻³</td>
</tr>
<tr>
<td>Liver function tests</td>
<td>Bilirubin, alkaline phosphatase, alanine transaminase (SGPT), Plasma albumin.</td>
</tr>
<tr>
<td>Bone Profile</td>
<td>Ca²⁺, alkaline phosphatase, phosphate.</td>
</tr>
<tr>
<td>Kidney function tests</td>
<td>Creatinine, urea.</td>
</tr>
<tr>
<td>Acid-Base balance</td>
<td>pH, PCO₂, HCO⁻³</td>
</tr>
<tr>
<td>Cardiac profile</td>
<td>Lactate dehydrogenase (LDH), Creatinine phosphokinase (CPK), Aspartate transaminase (SGOT)</td>
</tr>
<tr>
<td>Endocrine profile</td>
<td>T3, T4, TSH, and Thyroid function</td>
</tr>
</tbody>
</table>
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, $\alpha_1$-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 haemoglobinimeter attachment:
     - Widely used.
     - Very accurate.
     - Simple.
     - Estimates total RBC, WBC, MCV, MCH, MCHC.
### Inherited Abnormalities of Plasma Proteins

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Associated Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-Antitrypsin</td>
<td>Obstructive pulmonary disease (Chronic bronchitus or emphysema), liver disease.</td>
</tr>
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<td>Anti-thrombin</td>
<td>Thrombosis.</td>
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<td></td>
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</tbody>
</table>
Several fetal proteins are 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:
- $\alpha$-feto proteins
- $\alpha_2$–ferroprotein.
- $\beta$-fetoprotein.
- Alkaline phosphatase.
- Fetal sulphoglycoprotein antigen.
- $\gamma$-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 A₂ 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..........
Differentiation and confirmation of haemoglobinopathies and thalassaemias:
- Electrophoresis.
- Hb A₂ quantitation.
- Hb F quantitation.
- Hb stability test.
- Determination of \( \alpha/\text{non-}\alpha \) 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

Glucose-6-Phosphate $\xrightarrow{\text{G-6-PD}}$ 6-phospho-gluconolactone

$\text{NADP}^+$ $\xrightarrow{\text{G-6-PD}}$ NADPH$^*$

$\text{NADPH}^*$ 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.
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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Acute Hepatitis</th>
<th>Chronic Hepatitis</th>
<th>Cirrhosis</th>
<th>Cholestasis</th>
<th>Hepatic Infiltration</th>
<th>Hepatocellular Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Bilirubin</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGOT SGPT</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Urinary Bilirubin Urobilirubin</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Hepatitis associated antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma protein electrophoresis</td>
<td>√</td>
<td></td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>5’ Nucleotidase</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Fetoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>γ-Glutamyl Transferase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>√</td>
</tr>
</tbody>
</table>
## TYPES OF BILE PIGMENTS PRESENT IN PLASMA, URINE AND FEACEA IN DIFFERENT TYPES OF JAUNDICE

<table>
<thead>
<tr>
<th>Disease</th>
<th>Plasma</th>
<th>Uric</th>
<th>Feaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Bilirubin</td>
<td>Excess Conjugated Bilirubin (Direct Van der Brough Reaction)</td>
<td>Uro Bilirubin</td>
</tr>
<tr>
<td>Normal</td>
<td>Present</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>Haemolytic Jaundice</td>
<td>+</td>
<td>-</td>
<td>Increased</td>
</tr>
<tr>
<td>Hepatic (Infective hepatitis)</td>
<td>II</td>
<td>+</td>
<td>Variable</td>
</tr>
<tr>
<td>Post Hepatic</td>
<td>III</td>
<td>II</td>
<td>Absent</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>TESTS</th>
<th>CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination of urine</td>
<td>Suspected renal damage.</td>
</tr>
<tr>
<td>The water deprivation or vasopressin test</td>
<td>Most useful single test to confirm renal tubular impairment.</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>Quantitative test for glomerular impairment.</td>
</tr>
<tr>
<td>Estimation of plasma urea and creatinine</td>
<td>Guide to progress and prognosis if there is severe renal damage or obstruction.</td>
</tr>
</tbody>
</table>
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**

<table>
<thead>
<tr>
<th>Glomerulal Proteinuria*</th>
<th>Overflow Proteinura</th>
<th>Revert Tubular Disease Proteinuria**</th>
<th>Nephrogenic Proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Albumin</td>
<td>↑ Bench flow protein</td>
<td>↑ α2-Globular</td>
<td>↑ IgG, IgM</td>
</tr>
<tr>
<td>↑ Transferrin</td>
<td>↑ Myoglobin ad</td>
<td>↑ β-Globular</td>
<td>IgE</td>
</tr>
<tr>
<td>↑ Acid Glycoprotein</td>
<td>Haemoglobin</td>
<td>Slightly ↑ albumin and transferrin</td>
<td></td>
</tr>
<tr>
<td>↑ α1-Antitrypsin</td>
<td>↑ Acid glycoprotein</td>
<td>↑ β2-Microglobulin</td>
<td></td>
</tr>
<tr>
<td>↑ IgG</td>
<td>↑ α1-Antitrypsin</td>
<td>↑ Lysozone</td>
<td></td>
</tr>
</tbody>
</table>

* Ratio of albumin to low mol-wt. protein 20:1
** Ratio of albumin to low mol-wt. protein 1:1
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).
Creatine phosphokinase (CPK).
• Creatine and Creatinine in Serum.
• Calcium.
• Na⁺ in Serum.
• K⁺ in Serum.
• Mg⁺² in Serum