Evaluation of RBC status cont'd

4. Red Blood Cell (RBC) counts

- There are about 5.2 million RBCs per ml. of blood in a normal man and 4.7 million in woman.
- Female RBCs are larger in size than male's, but contain less Hb and more water in their stroma.
- This is because androgens promote erythropoiesis, especially Hb synthesis,

- while estrogens promote water retention due to increased Na^+ and Cl^- retention.

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Anaemia and Polycythemia

- Polycythemia is the increase in the PCV, RBC counts or Hb content well above the upper normal limit. It is brought about by:
  - Dehydration: in which the polycythemia is relative
  - Stimulation of erythropoiesis leading to actual increase in RBC in which polycythemia is absolute

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Anaemia and Polycythemia cont'd

- Anaemia is defined as the shortage of RBCs or Hb in blood.
- It is usually diagnosed when the PCV is below the lower limit,

- or when the Hb is below the normal, or when the RBC counts are less that the lower normal limit.

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Anaemia cont'd

- Anaemia can be caused by a number of conditions:
  - Iron deficiency: due to inadequate dietary intake, or more commonly to impaired absorption.
  - Vitamin deficiency, e.g., B_{12}, B_6, folic acid, etc.
  - Inhibition of cell function in the bone marrow by poisonous agents, etc.
  - Inhibition of hemoglobin synthesis

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Causes of anaemia cont'd

- Hemorrhage: excessive loss of blood such that the normal rate of erythropoiesis cannot balance the loss
- Hemolysis, i.e. excessive rate of RBC destruction
- Genetically determined abnormal red cells: these can occur in a number of conditions such as:
  - Sickle cell anemia
  - Thalassemia
  - Hereditary spherocytosis

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Behavior of RBCs in solutions with different osmotic pressure (OP)

- In order to keep the size and integrity of RBCs, they have to remain in an environment with the

- same tonicity (effective osmotic pressure, OP) as plasma of blood (the membrane is flexible but not elastic).

- When effective OP of plasma is lowered sufficiently, hemolysis occurs, leading to release of Hb from RBCs into the surrounding solution.
- The burst cell membranes that remain are known as ghost or shadow cells.
- Such solutions are known as hypotonic.

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Behavior of RBCs in solutions with different osmotic pressure cont'd

- Hemolysis may also be caused by:
  - freezing and thawing (melting)
  - high temperatures
  - substances such as soaps, saponins and bile salts which lower the surface tension on the RBC membranes & thus weaken them.
  - Alcohol, chloroform, ether and acetone which dissolve the cell membrane, leading to hemolysis.

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Behavior of RBCs in solutions with different osmotic pressure cont'd

- Solutions with effective OP similar to that of RBCs in which they can be put without any changes in volume are known as isotonic.
- The best example of isotonic solution is physiological saline – an aqueous solution of 0.85-0.9% NaCl.
- Solutions that exert higher OP than blood are known as hypertonic.
- They cause RBCs to shrink and the cells are said to be crenated.

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Evaluation of RBC status cont'd

5. Erythrocyte osmotic fragility test (EOFT)

- Beacuse of the flexibility of the membranes, RBCs show a degree of resistance to hemolysis.
- This resistance is determined by subjecting samples of concentrated RBCs (by centrigugation)

- to known serially increasing dilutions in test tubes mounted on a rack.

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Erythrocyte osmotic fragility test cont'd

- After 10 mins., the solutions are examined for evidence of hemolysis.
- Solutions in which hemolysis is complete will show a uniform bright red colour due to Hb in the solution.
- Where the solution remains turbid at the top, and clear red at the bottom, hemolysis is partial.
- Where the solution remains a dull red suspension, hemolysis is absent.

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Erythrocyte osmotic fragility test cont'd

- The dilution at which hemolysis begins is the highest osmotic resistance of the RBCs.
- It is about 0.5% NaCl in normal blood.
- The subsequent lower dilutions show complete hemolysis and this usually occurs at about 0.3% NaCl.
- The erythrocyte osmotic fragility test (EOFT) decreases in certain abnormalities

- associated with reduced resistance to hemolysis, known as hemolytic conditions.

- Many blood parasites cause decrease in EOFT, due to cellular stress caused by the parasites.

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White Blood Cells (WBCs)

- WBCs are nucleated blood cells found in less concentrations than RBCs (hundred to thousand RBCs per WBC).
- Their functions in defending the body against invading micro-organisms and toxins is primarily carried out in the body tissues.
- There are about 9000 (4000-11000) WBCs per µL of blood
- WBCs are classified based on two criteria:

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Classification of WBCs

1. Histological classification: based on presence or absence of cytoplasmic granules, the WBCs are grouped into:

(a) Agranulocytes: lack cytoplasmic granules, have large nuclei:

(i) Lymphocytes: are the largest WBCs, have large rounded nuclei, the bulky cytoplasm stains blue-gray and contains many lysosomal enzymes.

(ii) Monocytes: are small cells with large, kidney shaped nuclei with dense staining chromatin and a thin rim of featureless cytoplasm.

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Histological classification cont'd

(b) Granulocytes: possess cytoplasmic granules which are specialized lysosomes, with nuclei divided into several lobes.

- The granules take up different dyes when stained:

(i) Neutrophils: have nuclei with 2-5 lobes, their fine cytoplasmic granules stain **pink** with neutral dyes, but other larger granules stain **dark blue** or purple.

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Granulocytes cont'd

(ii) Eosinophils: have bi-lobed nuclei and their cytoplasmic granules stain deep orange or **red** with acidic dyes (eosin).

(iii) Basophils: their nuclei are large and irregular and their cytoplasmic granules stain **deep blue** or purple with basic dyes (hematoxylin).

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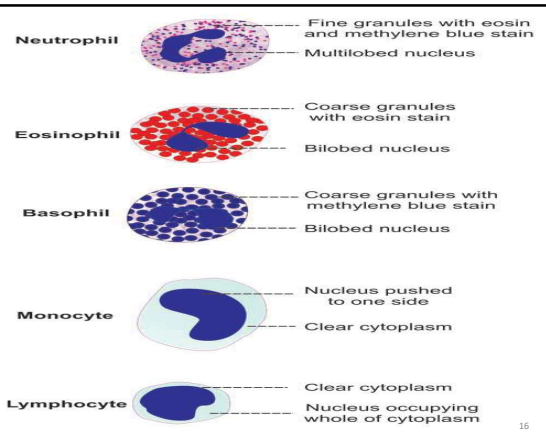

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Classification of WBCs cont'd

2. Functional classification:

Based on the role of the cell types in body defense:

- Phagocytes: includes the Monocytes, neutrophils, basophils and eosinophils.
- Immunocytes: Includes Lymphocytes and Plasma cells.

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Functional classification cont'd

- Immunocytes have 3 functions:
  - providing specificity to the action of phagocytes by selecting the targets of their attack;
  - provide specific immune responses to some diseases;
  - defending the body against foreign tissue transplants and growth of foreign cells (cancers)

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