BLOOD

Blood is fluid CT that circulates through cardio-vascular system. It consists of fluid matrix and many cells. A fibrous component (fibrin) is also present during blood clotting. The fluid phase (matrix) is called plasma. Various cells are suspended within it. Upon removal of fibrinogen and fibrin, remaining fluid is serum. In adult animal, blood volume is about 8 – 10% of body weight. Plasma component represents 55% of blood volume, with formed or cellular elements making up remaining 45%.

Functions of blood:
- Transport of nutrients and oxygen to cell
- Transport of waste products and CO₂ away from cell
- Delivery of hormones
- Maintain homeostasis by acting as buffer
- Transport of cells of immune system
- Heat regulation
- Platelets play role in hemostasis

PLASMA

It is liquid, straw-coloured extra-cellular material that imparts fluid properties to blood

Blood + Anti-coagulant

Centrifuge

Plasma at top

Buffy coat in middle

(Platelets & leukocytes)

Red cell mass at bottom

90 % water

10 % Dissolved substance and solids

Plasma proteins: 7 % of total plasma
Plasma proteins: Albumin, Globulin & Fibrinogen

Albumin:
- Main plasma protein (approx. half of total protein) synthesized in liver
- Responsible for exerting concentration gradient between blood and extra-cellular fluid. It is called colloid osmotic pressure. If albumin leaks out of blood vessel into loose CT, osmotic pressure falls and fluid accumulates in tissue leading to edema.
- Also act as carrier protein and transport hormones (thyroxine), metabolites (bilirubin), drugs (barbiturates)

Globulin:
- Includes immunoglobulin (γ-globulin) and non-immunoglobulin (α- and β-)
- Immunoglobulins are antibodies secreted by plasma cells. Non-immunoglobulins are secreted by liver
**Fibrinogen:**
These are largest plasma proteins (340 kDa). They are synthesized in liver.

![Fibrinogen Diagram]

**ERYTHROCYTES**
These are anucleated cells devoid of typical organelles. They function only within blood stream to bind oxygen for delivery to tissue and in exchange bind CO\(_2\) for removal from tissue. Mammalian erythrocyes are unique among the vertebrates as they are non-nucleated cells in their mature form. These cells have nuclei during early phases of erythropoiesis, but extrude them during development as they mature in order to provide more space for hemoglobin. Their shape is biconcave disc and **oval in camel/reptiles/birds. Nucleated RBC seen in birds.**

Size varies from 3.5 – 7.5 µ
- RBC of dog: 7 µ
- RBC of sheep: 4.5 µ
- RBC of goat: 3.2 µ
- RBC of ox: 6 µ
- RBC of horse: 5.7 µ

**Howel-Jolly bodies** are nuclear remnants normally seen in horse and cat.

**Life-span of RBC:**
- Cattle: 145 days
- Horse: 160 days
- Goat: 125 days
- Dog: 110 days
- Cat: 68 days+
- Birds: 28-35 days

RBCs are rich in hemoglobin. Lack of nucleus allows more room for hemoglobin and biconcave shape helps increase its oxygen carrying capacity. Disc shape facilitates gas exchange because more Hb molecules are closer to plasma membrane than they would be in spherical shape. Thus, gases have less distance to diffuse within cell to reach binding site on Hb.

An important property of RBC is to adhere to each other along their concave surfaces, thus forming rows or **rouleaux** like piled up coins. It is due to surface tension. **Rouleaux formation is common in horse.**

**RBCs in male are more in number as compared to females** because androgens increases RBC formation and estrogen decreases RBC formation.
Erythropoiesis is RBC formation. Kidney hormone that controls erythropoiesis is erythropoietin (glycoprotein)

**LEUCOCYTES**

a. Granulocytes
   1. Neutrophils
   2. Eosinophils
   3. Basophils

b. Agranulocytes
   1. Lymphocytes
   2. Monocytes

<table>
<thead>
<tr>
<th>Blood cell</th>
<th>% age Distribution</th>
<th>Size (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>40 – 60</td>
<td>9 – 12</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>20 – 40 (50 – 60 in cow/pigs)</td>
<td>5 – 10 (small), 10 – 18 (large)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3 – 8</td>
<td>16 – 25</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 – 8</td>
<td>12 – 14</td>
</tr>
<tr>
<td>Basophils</td>
<td>0 – 1.5</td>
<td>9 – 12</td>
</tr>
<tr>
<td>RBC</td>
<td></td>
<td>3.5 – 7.5</td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td>2 – 4</td>
</tr>
</tbody>
</table>

Overview of peripheral blood cell types (mature blood cells), blood smear. Wright stain

**NEUTROPHILS**

- Most numerous WBC. They are larger than RBC
- *Multi-lobed nucleus.* So, called polymorphonuclear neutrophils or polymorphs. Possess 2 – 4 lobes joined by thinner nuclear strands
- *In females, presence of Barr bodies (drum-stick shaped)*
- Contains granules:
  1. Primary granules: Also known as *Azurophilic granules.* These are larger and less numerous. They are peroxidase positive. They mainly contain myeloperoxidase and defensins. Myeloperoxidase generate bactericidal hypochlorite and chloramines.
  2. Secondary granules: These are *specific granules.* These are smaller and twice as numerous as primary granules. They lack peroxidase activity. They contain collagenase.
Cytoplasm of neutrophils is transparent as granules are neutral staining. In birds/reptiles/amphibians, cytoplasmic granules are large and red and are called HETEROPHILS.

**EOSINOPHILS**

- Bilobed nucleus
- Presence of two types of granules: Azurophilic and Specific granules
- **Azurophilic granules** are lysosomes, contain hydrolase. They cause destruction of parasites and hydrolysis of Ag-Ab complex. They are larger than primary granules of neutrophils
- **Specific granules** contain following proteins. These are:
  - Major Basic Protein: It is arginine-rich and is responsible for intense acidophilia of granule
  - Eosinophil Cationic Protein
  - Eosinophil derived neurotoxin
- MBP, ECP have cytotoxic effect on protozoans and helminthic parasites
- EDN cause nervous dysfunction in parasites

Eosinophils are associated with allergy, parasitic infection and chronic inflammation.

**BASOPHILS**

- Least numerous with bi-lobed nucleus
- Plasma membrane possess F\(_c\) receptors for IgE
- Contains 2 types of granules
  a. Azurophilic granules
  b. Specific granules: Contains heparin, histamine, heparin sulphate and Leukotrienes
  - Heparin is anticoagulant and promotes lipolysis whereas Histamine and Heparan sulphate causes vasodilation of blood vessels. Leukotrienes are modified lipids that triggers prolonged constriction of smooth muscles in pulmonary airways
  - **Granules have deep purple staining (intense basophilia) due to increase sulphate concentration**
  - Granules are rod-shaped in feline and stain dull purple due to lack of sulphated GAGs

**LYMPHOCYTES**

- Main cells of lymphatic system with round nucleus
- Most common agranulocyte
- High nuclear : cytoplasmic ratio

**MONOCYTES**

- **Largest WBC with indented/horse-shoe shaped nucleus. They become macrophages once they enter extra-vascular space**
- Circulating monocytes and tissue macrophages comprises mononuclear phagocyte system (MPS). It includes stellate macrophages (Kuffer cells) in liver, alveolar macrophages in lungs, microglia in brain
PLATELETS (Thrombocytes in poultry)

These are small, membrane bounded, anucleated cytoplasmic fragments derived from "megakaryocytes". Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells.

The cytoplasm of platelets can be divided into two areas: the chromomere and the hyalomere. The chromomere is located centrally where the granules tend to aggregate. The hyalomere surrounds the chromomere and is a clear, blue, non-granular zone. Ultra-structural studies revealed that structurally there are 4 zones. These are:

a. Peripheral zone: Consist of plasma membrane covered by glycocalyx
b. Structural zone: Consist of microtubules, actin filaments, actin-binding proteins that form network supporting plasma membrane. About 8 – 24 microtubules reside as bundle immediately below actin filament network. They are responsible for maintaining platelet’s shape

c. Organelle zone: It occupies centre of platelet and consist of mitochondria, peroxisomes, glycogen and granules. 3 types of granules are present.
   1. α – granule: Release Platelet derived growth factors which stimulate smooth muscle cells and fibroblasts to divide and thus allow tissue repair
   2. Delta – granule: Contain nucleotides, thromboxane A₂, and serotonin. They facilitate platelet adhesion in area of injury
   3. λ- granule: Similar to lysosome and contain hydrolytic enzymes
d. Membrane zone have 2 channels
   1. Open canalicular system (OCS)
      They are invaginations from plasma membrane into cytoplasm. Bovine platelets are devoid of OCS
   2. Dense tubular system (DTS)
      They serve as storage site of calcium. They generally do not connect with surface of platelet

Platelets are involved in hemostasis. They continuously survey endothelial lining of blood vessels for gaps and breaks.

- When there is injury, exposed CT at site promotes platelet adhesion.
- Adhesion triggers platelet de-granulation
- There is release of serotonin, and thromboxane A₂
- Serotonin is potent vasoconstriction and decrease local blood flow to injury site
- Thromboxane A₂ causes platelet aggregation to form primary hemostatic platelet plug to stop bleeding
- Glycocalyx provides surface for conversion of fibrinogen into fibrin. Fibrin form loose mesh over initial plug and form dense aggregation of fibrins and form secondary hemostatic platelet plug
- Finally clot is lysed by plasmin, a fibrinolytic enzyme that circulates in plasma in inactive form called plasminogen. Enzymes released from λ- granule also help in this process.
• Endothelial cells release tissue plasminogen activator (TPA) which causes plasminogen conversion

<table>
<thead>
<tr>
<th>Mast cells</th>
<th>Basophils</th>
</tr>
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<tbody>
<tr>
<td>Can divide</td>
<td>Cannot divide</td>
</tr>
<tr>
<td>Differentiate into mature form after reaching CT</td>
<td>Differentiate before reaching blood</td>
</tr>
<tr>
<td>Larger</td>
<td>Smaller</td>
</tr>
<tr>
<td>Life span: weeks to month</td>
<td>Few days</td>
</tr>
<tr>
<td>Spherical nucleus</td>
<td>Bi-lobed nucleus</td>
</tr>
<tr>
<td>Abundant purplish granules</td>
<td>Less granule than in mast cells</td>
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</tbody>
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