

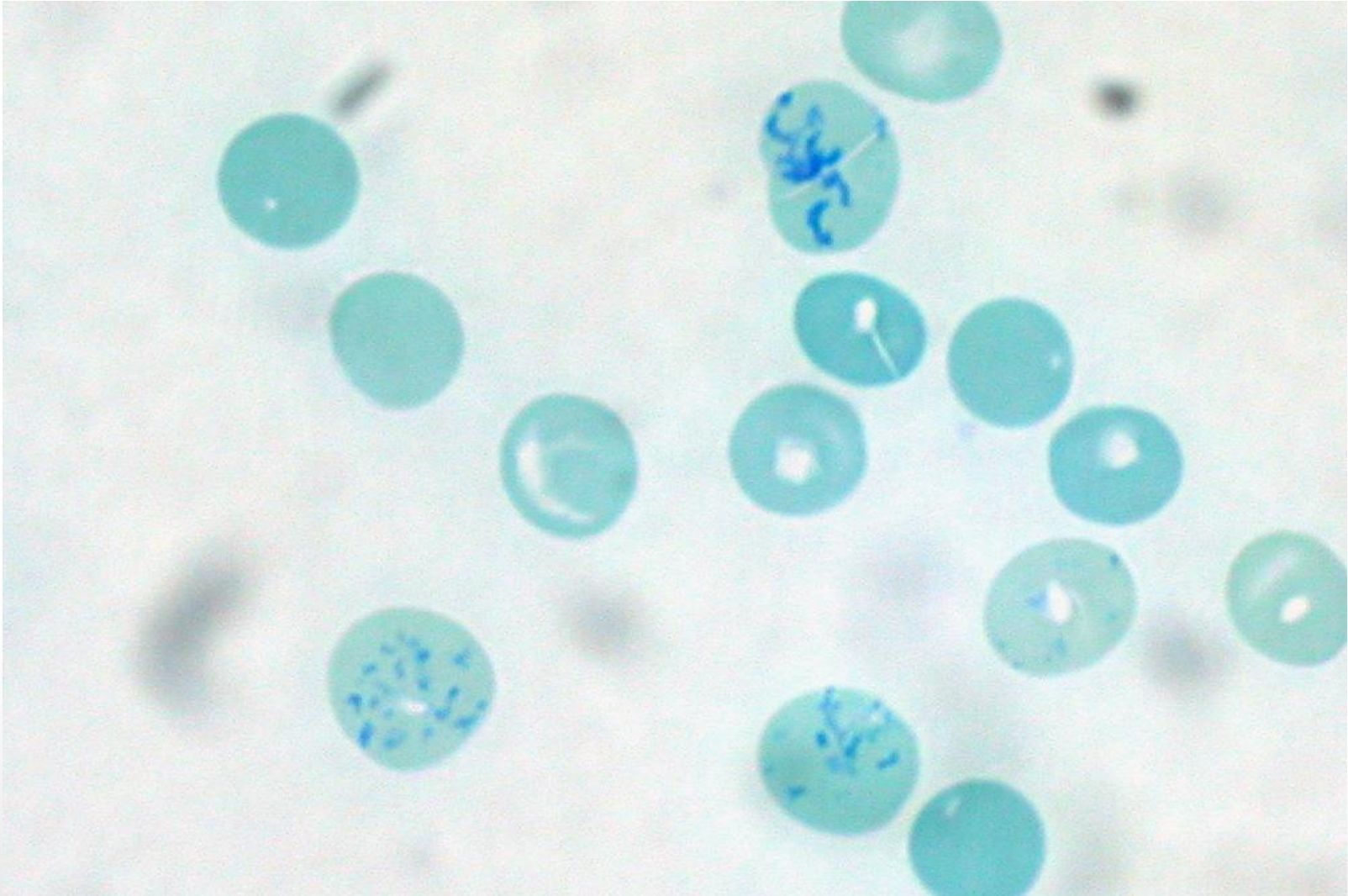
Blood & Lymphoreticular System Physiology Lab

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Reticulocyte Count

- Reticulocytes are the immediate precursor of RBCs.
- Contain a small amount of basophilic material, mainly remnants of the Golgi apparatus & mitochondria
- Following their release into the blood stream they mature within 1-2 days into mature RBCs.
- They normally make 0.5 - 2% of all RBCs
- Reticulocyte count is used to estimate the degree of effective erythropoiesis
- Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure

If supravital staining (new methelene blue) is performed on a blood smear, the reticulocytes appear larger than RBCs and contain dark blue dots and curved linear structures in their cytoplasm which represent remnants of the Golgi apparatus & mitochondria



Performing a Reticulocyte Count

- 500-1000 RBCs should be counted and the number of reticulocytes noted.
- The count is expressed as a percentage
- The percentage can be used to calculate the absolute reticulocyte count (ARC) .
- $ARC = (RBC \text{ count } \times \text{reticulocyte\%})/100$
- The normal absolute reticulocyte count is between 25,000 to 75,000/mm³

Reticulocytosis and Reticulocytopenia

- Condition associated with Reticulocytosis:
 - Hemolytic anemias
 - Recent blood loss
 - Following treatment of nutritional anemias
- Condition associated with Reticulocytopenia:
 - Iron deficiency anemia
 - Aplastic anemia
 - Bone marrow failure

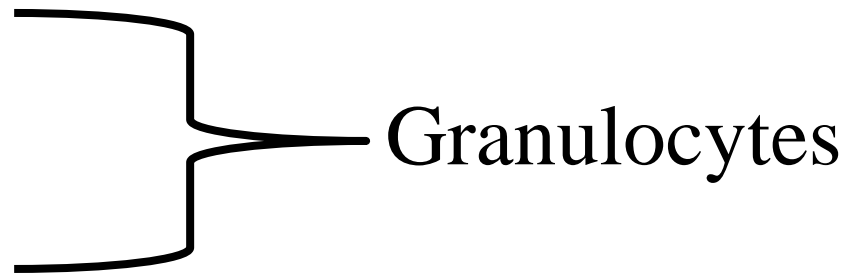
WBC count

- White Blood Cells are part of the immune system
- Move to areas of severe infection or inflammation to provide a rapid and potent defense for the body
- Normal WBC count is 4400 - 11,000 cells/mm³
- Leukocytes (WBC) count is part of the complete blood count (CBC), it is done to get an impression about the immune system, to get more informative results it is often combined with the differential count
- Leukocytosis vs Leukopenia

Differential Leukocyte Count (DLC)

- The blood contains 5 different types of white blood cells which are classified into:
 - ✓ Granulocytes: have cytoplasmic granules which contain enzymes or chemicals, and have a single multi lobed nucleus (segmented)
 - ✓ Agranulocytes: have a single non lobulated nucleus, their cytoplasmic granules are too small to be seen under the light microscope.

1. Neutrophils: 40-80 %
2. Eosinophils: 1-4 %
3. Basophils: < 1%

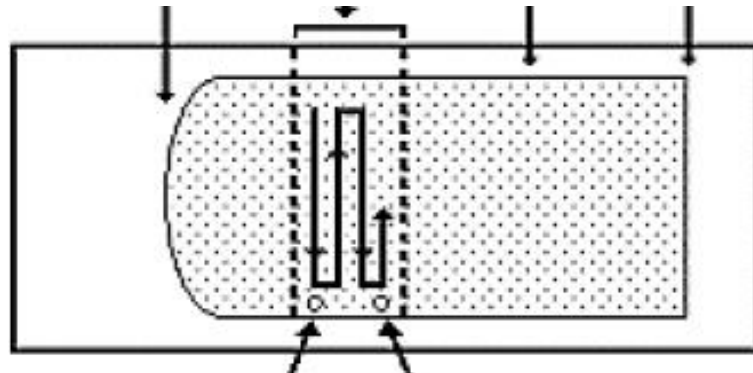


4. Lymphocytes: 20-40%
5. Monocytes: 2-8%



Performing a DLC

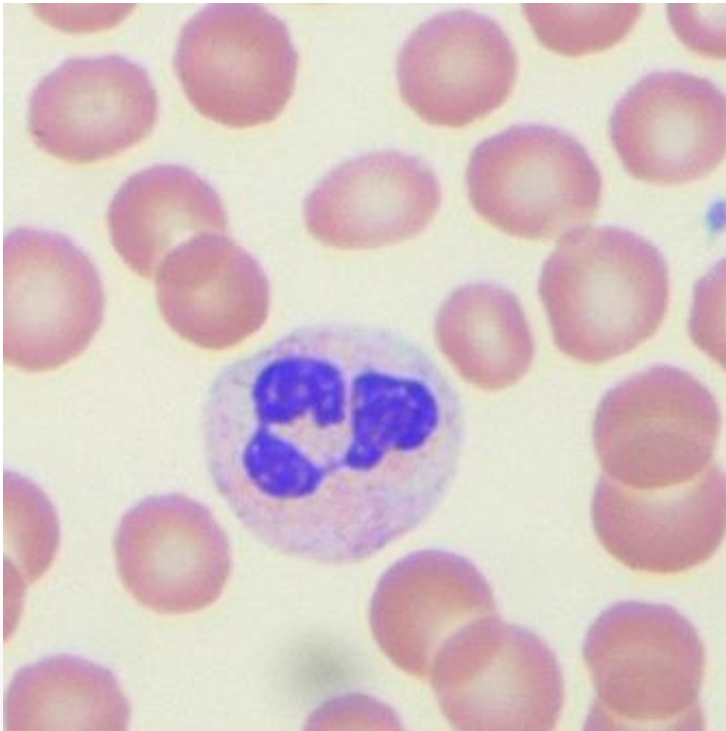
1. A drop of blood is thinly spread over a glass slide, air dried, and stained with an acidic dye (red) and a basic dye (blue-purple).
2. The slide is examined under a microscope using an **oil immersion lens**.
3. Two hundred white cells are then counted and classified.
4. The number of each type of cells is expressed as a percentage.
 - To do this one must be able to distinguish between the 5 types of WBCs



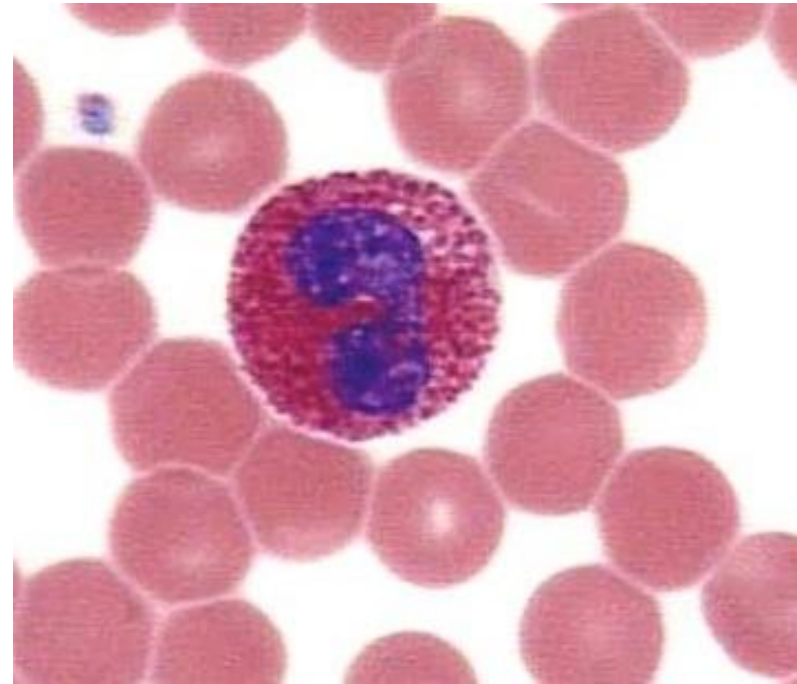


Neutrophils have nuclei with several lobes and fine pink granules in their cytoplasm.

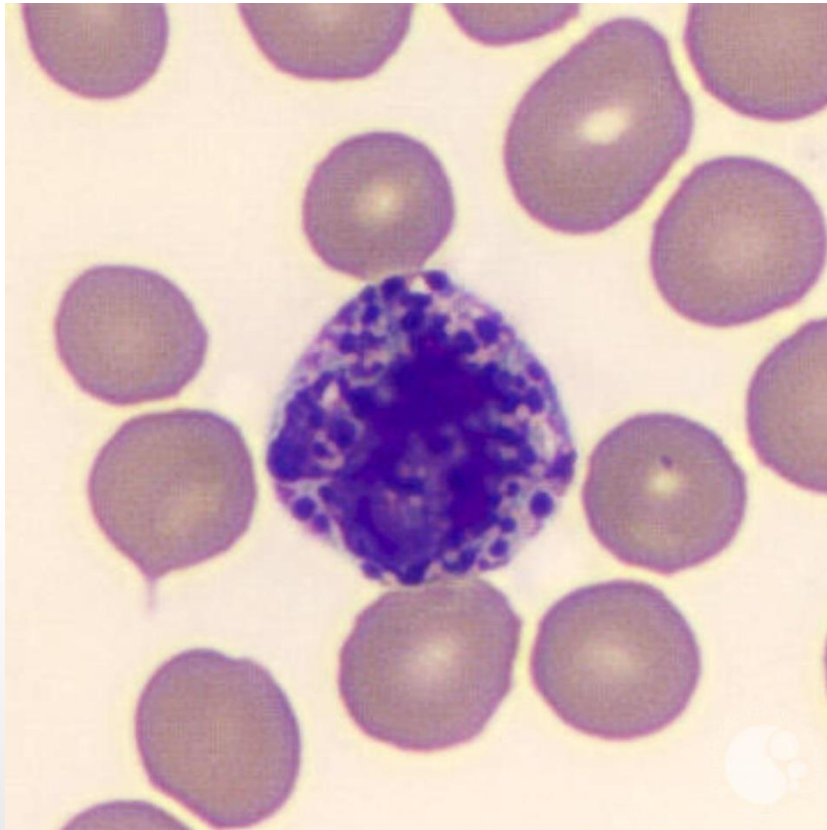
They are called neutrophils, because their granules are not very amenable to staining with either acidic or basic dyes



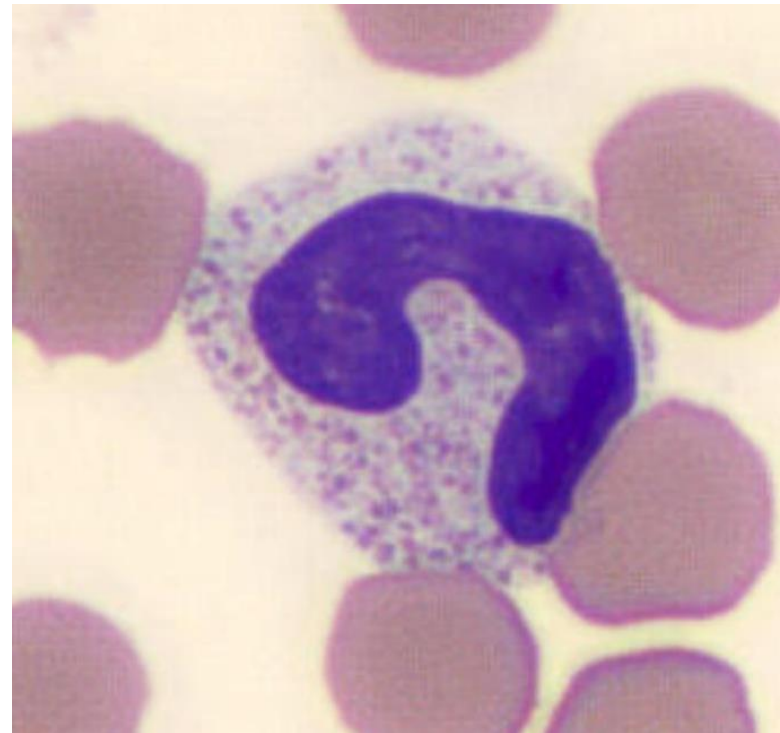
Eosinophils have bi-lobed nuclei and medium-sized granules that can be stained bright red or orange with an acidic dye.



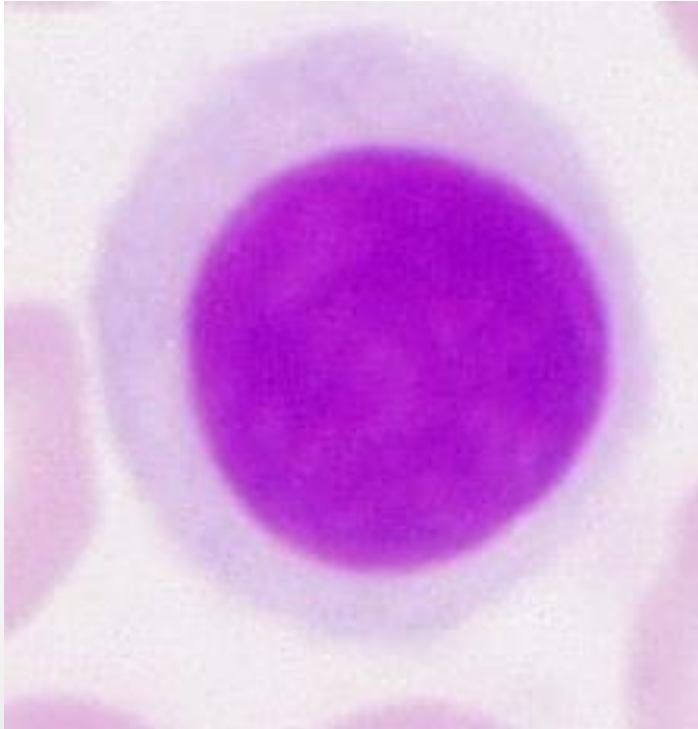
Basophils have bi-lobed or S shaped nuclei and large granules which stain dark blue with basic dyes and completely obscure the nucleus



Neutrophilic Band cells are immature neutrophils, usually make less than 5% of the total WBC count, their nucleus isn't segmented



- **Lymphocytes** have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules. Most cells are small in size.
- We can't differentiate between the B and T lymphocytes under the light microscope.



- **Monocytes** are large cells. They have large indented nuclei, often kidney-shaped. Their cytoplasm has fine purple granules which give it a "ground glass" appearance.



Importance of DLC

- Gives relative percentage of each type of WBC
- Helps reveal the presence of abnormal WBCs like blasts or lymphoma cells.
- Used along with WBC count to generate an **absolute value** for each type of WBCs.
 - Relative percentages can be misleading
 - Absolute values are also useful for monitoring certain conditions.
 - Absolute count = $\text{WBC (cells/ } \mu\text{L)} \times \text{percent of the specific WBC type} \div 100$

Absolute count calculation

- If the WBC count is 6000 cells/mm³ and the lymphocytes make 30% of the DLC, the Absolute lymphocyte count (ALC) will be:

$$\begin{aligned}\text{WBC count} \times (\text{Lymphocyte}\%)/100 &= (6000 \times 30)/100 \\ &= 1800 \text{ cells/mm}^3\end{aligned}$$

- Absolute neutrophil count (ANC)=WBC (cells/ μ L) x percent (neutrophils + neutrophilic band cells) \div 100
- ANC levels have used to assess the risk of opportunistic bacterial infections, in patients receiving chemotherapy.

1. Neutrophilic leukocytosis: is defined as a total WBC above 11,000/ μL along with an absolute neutrophil count (ANC) greater than 7700/ μL
 - Bacterial infections, inflammatory conditions, stress.

2. Lymphocytic leukocytosis : is defined as a total WBC above 11,000/ μL along with an absolute lymphocyte count greater than 4500/ μL
 - Viral infections as infectious mononucleosis, mumps, rubella and pertussis or in acute and chronic lymphocytic leukemias.

3. Monocytic leukocytosis:
 - Acute or chronic bacterial infection and chronic inflammation

4. Eosinophilic leukocytosis :
 - Parasitic infections & allergic conditions

6. Basophilic leukocytosis:

- Allergic conditions

7. Neutropenia : absolute neutrophil count is less than 1,500 cells/ mm³

- Certain infections like typhoid fever, HIV & CMV, chemotherapy, radiotherapy, and autoimmune diseases.

8. Lymphocytopenia:

- May occur in the normal elderly or be associated with chronic infection or malignancy.