

# WBC Count



# WBC(leukocyte)

They are cells of the immune system.

- Nucleated
- Big in size

They are 5 types:

- **Neutrophils**
- **Eosinophils**
- **Basophils**
- **Lymphocytes**
- **Monocytes**



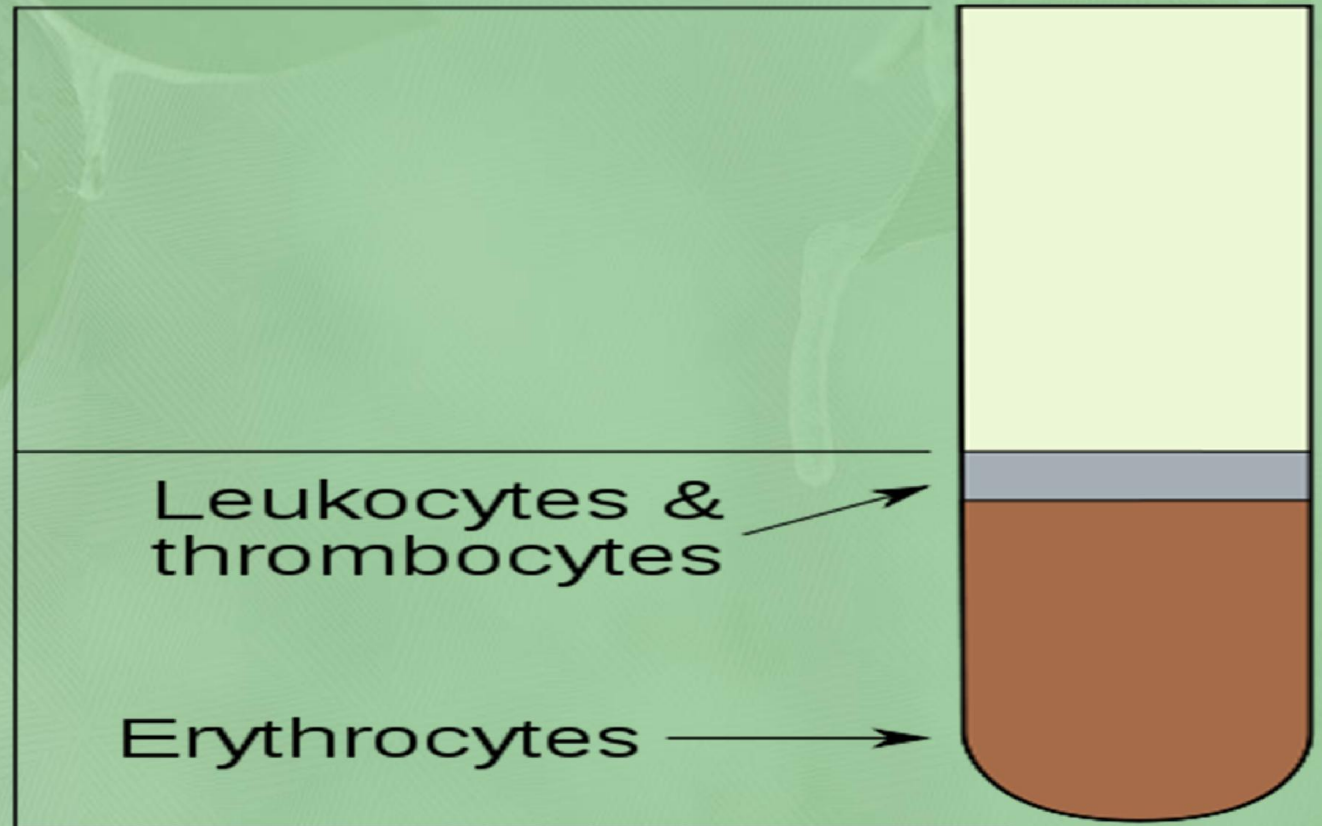
WBC are separated into a thin white layer when whole blood is centrifuged

Plasma

Formed  
elements

Leukocytes &  
thrombocytes

Erythrocytes



# WBC functions

1-Defence and protection against bacteria, viruses, fungi and parasites.

2-Allergy.

3-Production of immunoglobulins

Normal range:

4-10  $\times 10^3$  cells/ $\mu\text{L}$  or cells/ $\text{mm}^3$

4-10  $\times 10^9$  cells/L

**leukocytosis** : high number of white blood cells.

Due to:

chronic infections, inflammation, leukemia and allergy.

**Leukopenia**: decreased WBC count

Due to:

chemotherapy, radiation therapy, some types of cancer ,malaria and TB.



## WBC Count

This is a blood test to measure the total number of white blood cells (WBCs).

It is almost part of the CBC (complete blood count).

There are several methods that can be used to determine the number of leukocytes per microliter of blood :

1- Automatic blood cell counter:

**Advantages:**

1-The most accurate method

2-the error in results is approximately(1- 2%)

# 2-Manual WBC count

## Advantages:

- 1-less expensive
- 2-automated analyzers are not reliable in counting abnormal cells.



To make the counting of WBC easier ,we use **WBC diluent** (2% acetic acid+ gentian violet)

This fluid lyse the red cells and the WBC remain intact and stain the nucleus deep violet by the gentian violet.

**Method:**

50 $\mu$ L( EDTA blood)+ 0.95 ml of WBC diluent

The cells are counted on a  
**hemocytometer**

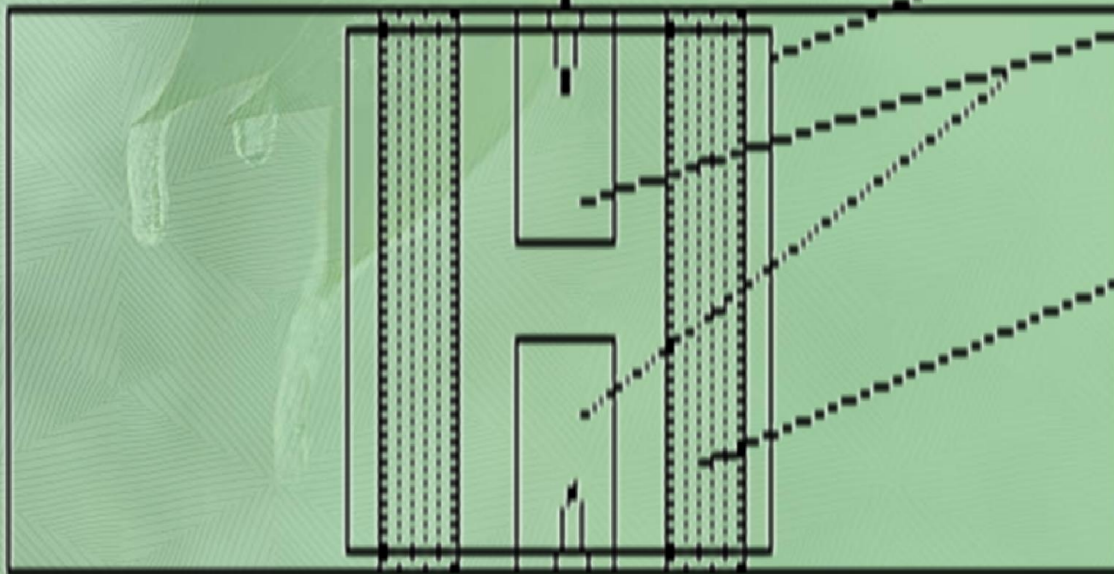
by using the microscope ( 10x 0r 40x)

Sample introduction point

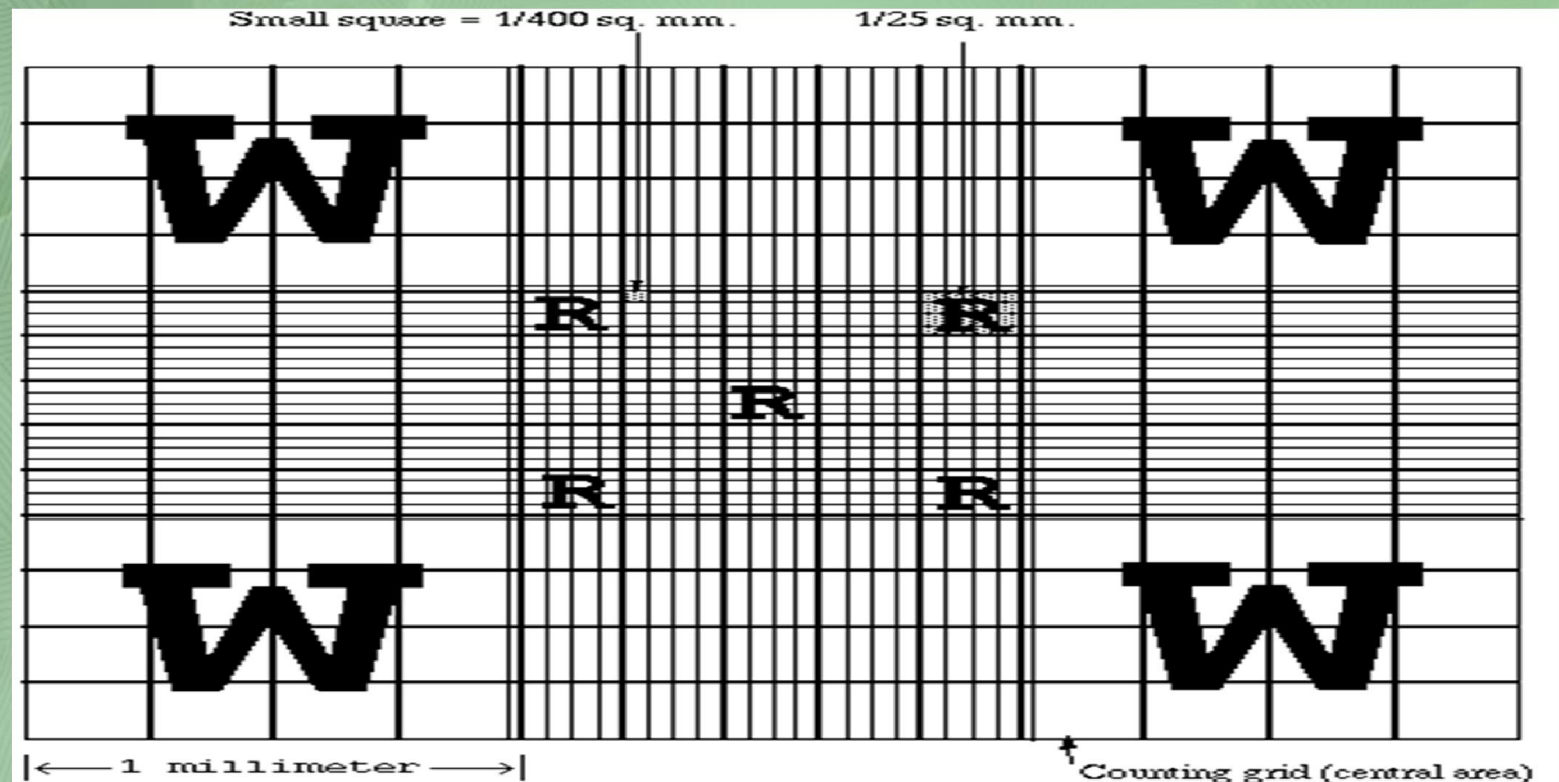
Cover glass

Counting  
chambers

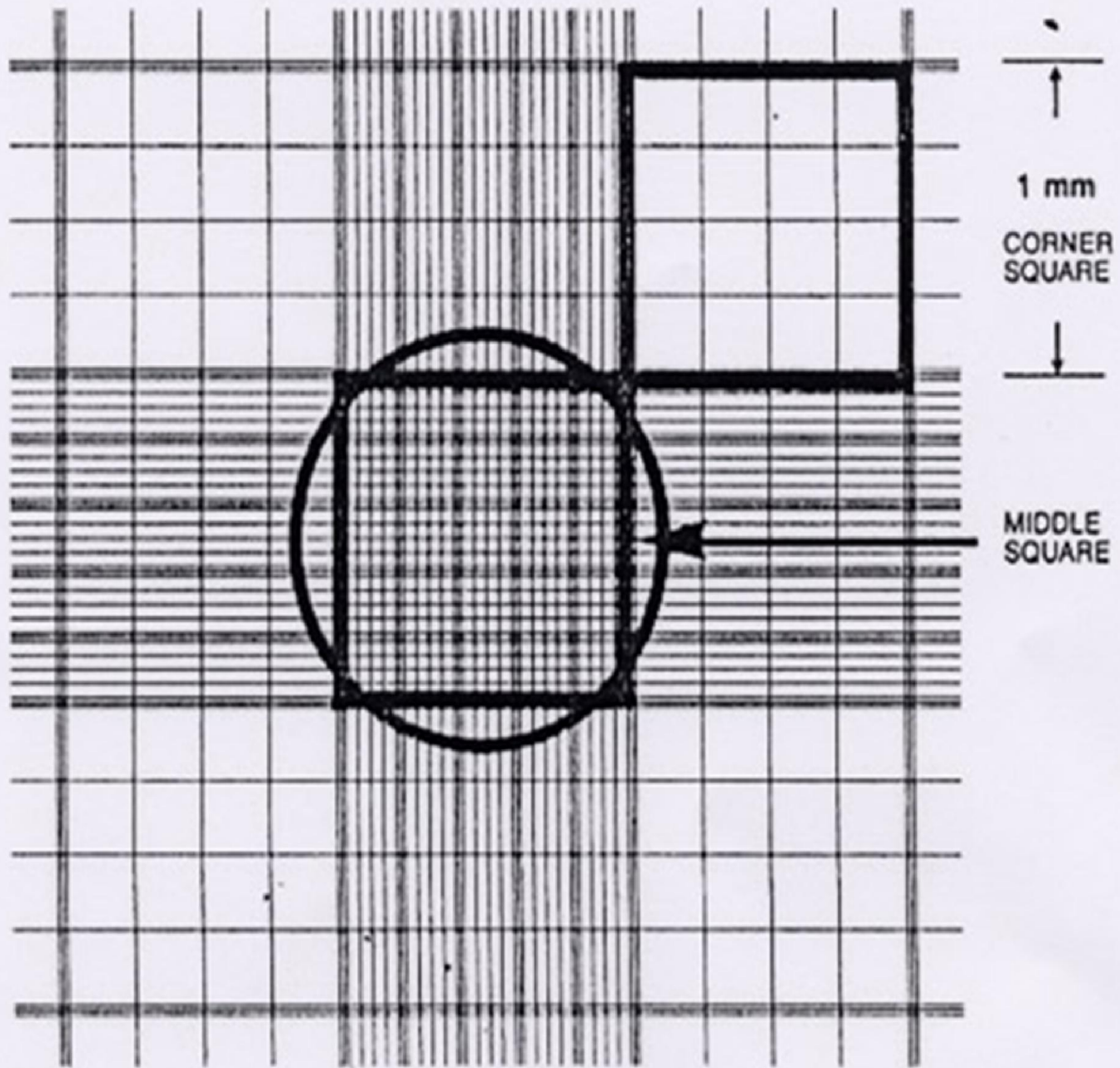
Cover glass  
mounting support



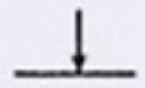
Count any square from the four large corner squares indicated by "W". Each of those squares contains 16 smaller squares







CORNER  
SQUARE

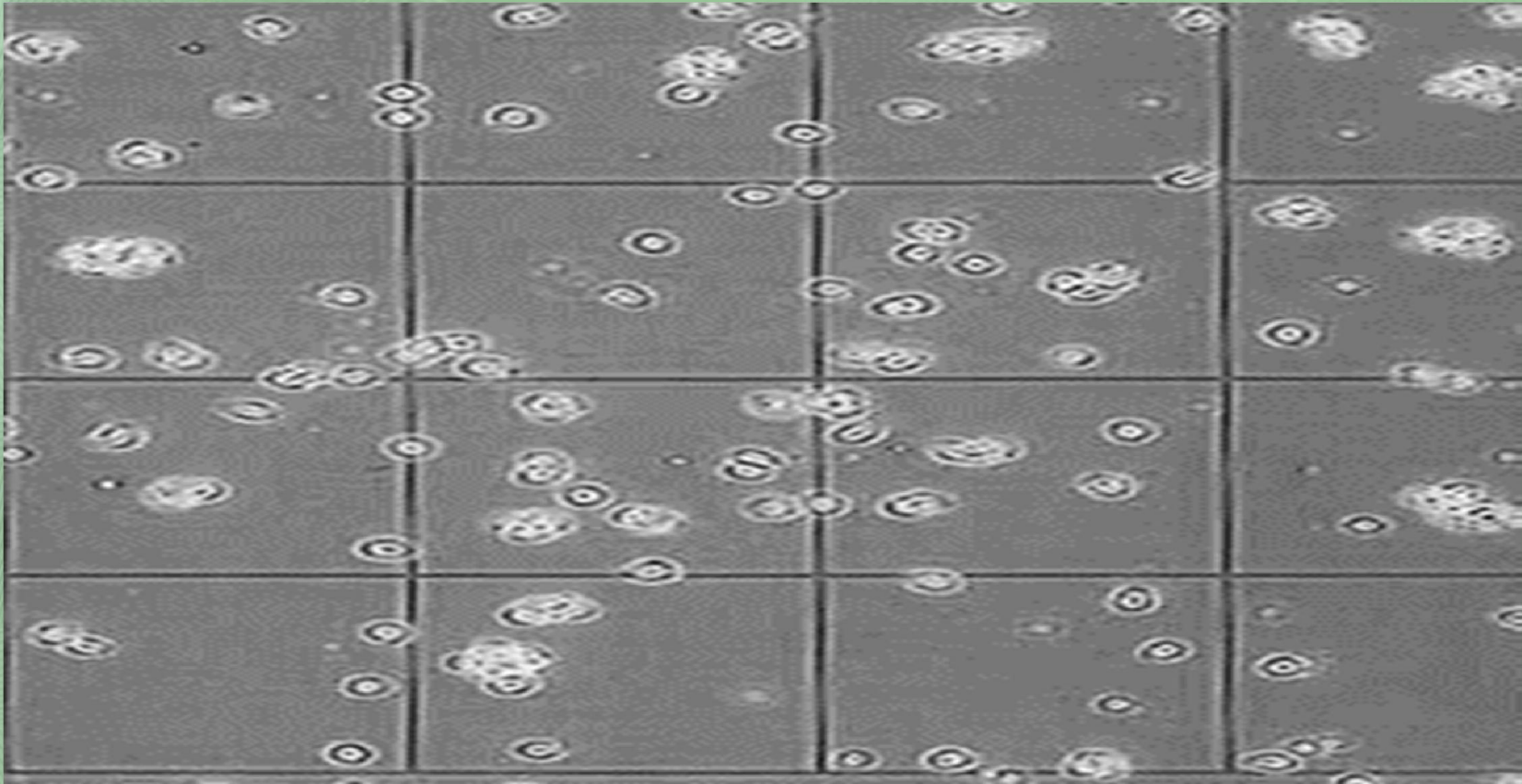


MIDDLE  
SQUARE





Light is low  
the condenser is down





## Dilution

50  $\mu\text{L}$  blood  $\div$  50 = 1 volume of blood

0.95ml = 950  $\mu\text{L}$  diluent  $\div$  50 = 19 volume of diluent

$$\frac{1}{19+1} = \frac{1}{20}$$

Dilution = 20 times

The volume of 1 corner square is:

1mm (width) X 1mm (height) X 0.1  
mm (depth)

= 0.1 mm<sup>3</sup> = 0.1 μL

# Calculation:

N (cells) found in 0.1  $\mu\text{L}$   
size of 1 square

?  $\rightarrow$  1  $\mu\text{L}$   
normal range cells/1  $\mu\text{L}$

Number of cells in 1ML in diluted blood

$$= \frac{N \times 1}{0.1} = \frac{N \times 1}{\frac{1}{10}} = N \times 10$$

WBC in whole blood =  $N \times 10 \times 20$  (dilution)

**WBC in whole blood =  $N \times 200$**



# disadvantages of manual WBC count:

- 1-Require the lyses of erythrocytes prior to counting white blood cells.
- 2-This method is less accurate (with an error of up to 10%)

## **WBC (leukocytes)**

WBCs divided into 2 broad groups according to function :

**1-Phagocyte**

**2-Immunocyte**

Phagocytosis + immune function help in protect body against infection

# **Phagocytes:**

granulocytes + non granulocyte  
e.g:monocyte

# **immunocytes:**

lymphocytes

# **Granulocytes:**

neutrophils  
eosinophils  
basophils



# There are 5 types of mature WBC in peripheral blood

- **Neutrophil:**

mature, band (stab), hypersegmented neutrophil and toxic granulation

- **Basophils**

- **Eosinophils**

- **Monocytes**

- **lymphocytes:**

T –lymphocyte, B –lymphocyte and NK(natural killer).

## Differences in WBC shapes are in:

- Size of cells
- Nucleus(lobes )
- Granules(size & color)

# WBC functions

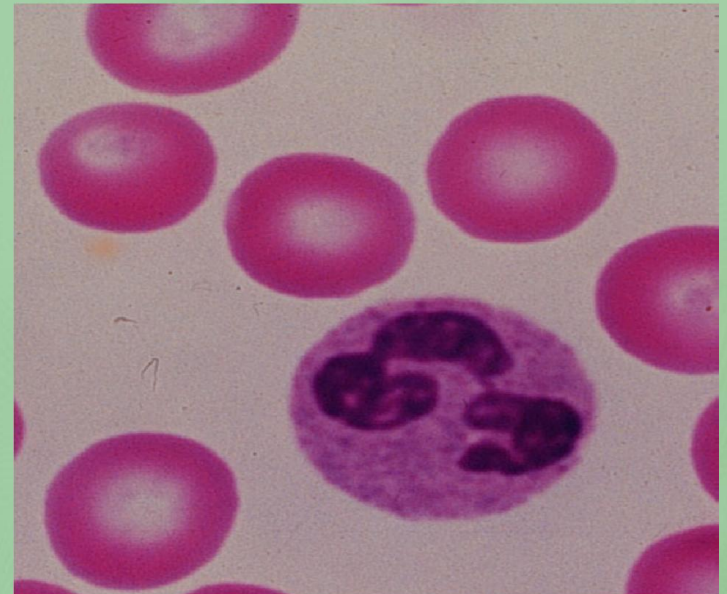
- **Defence and protection against infection**  
(neutrophils&monocytes)
- **Allergy** (eosinophils)
- **Production of immunoglobulins Ab**  
(B lymphocytes)



# Neutrophil

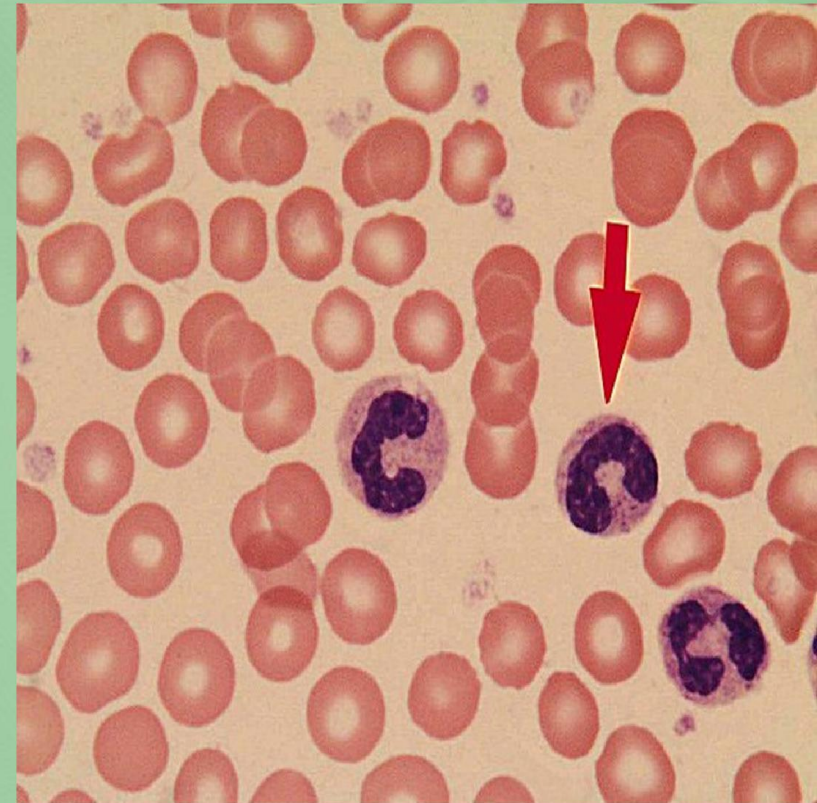
## 1- mature neutrophil

- **Size:** 13M( 3 times bigger than RBC)
- **Nucleus:** has deep violet nucleus containing 3-5 lobes.
- **Cytoplasm:** pale cytoplasm with fine pink granules.
- **Normal range** :40-75%
- **Function:**  
Defense against bacteria.



## 2-Stab form or band type( young form) neutrophil

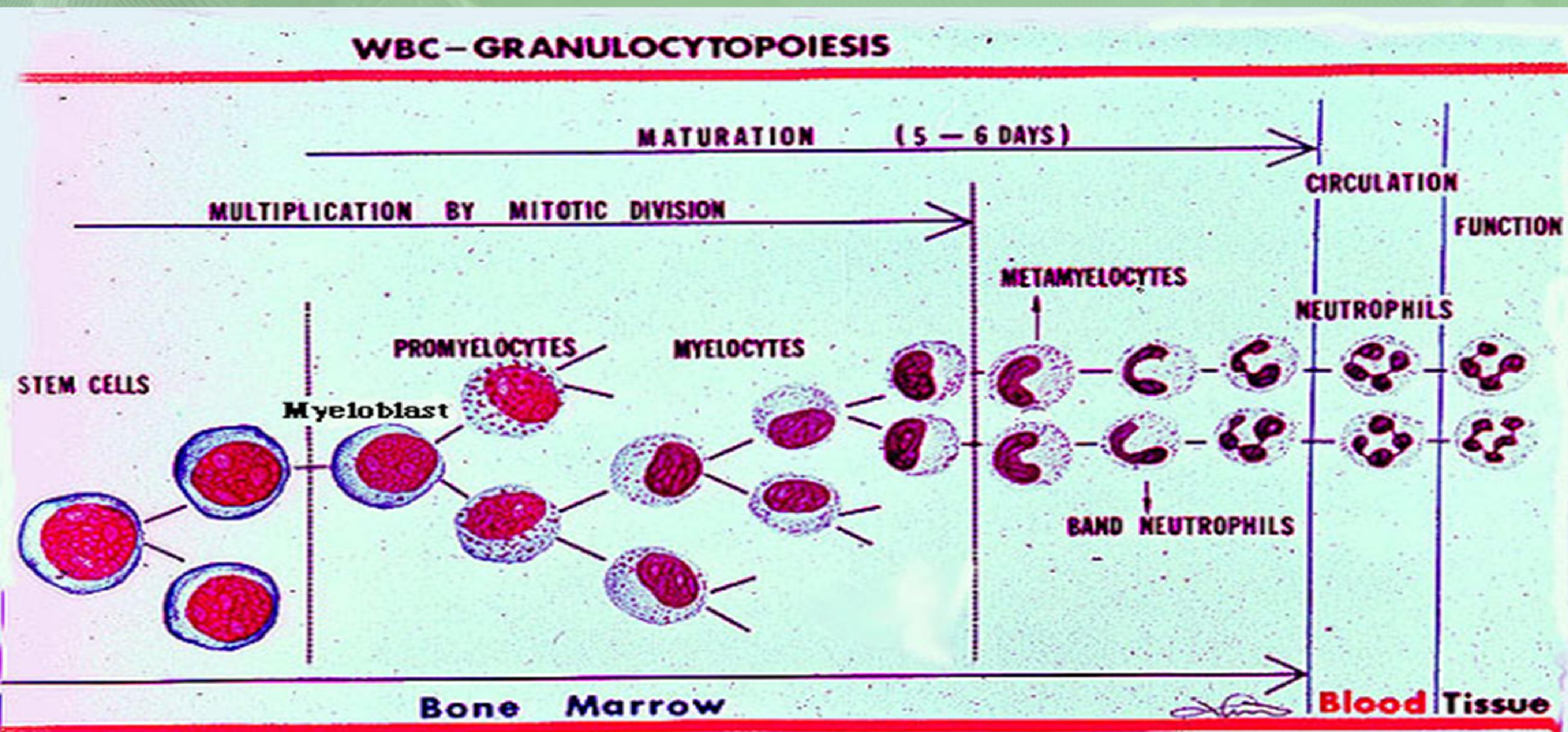
nonsegmented,  
long and narrow  
nucleus. The band-  
like nucleus can  
take any shape but  
it is never be  
straight.





# Shift to the left

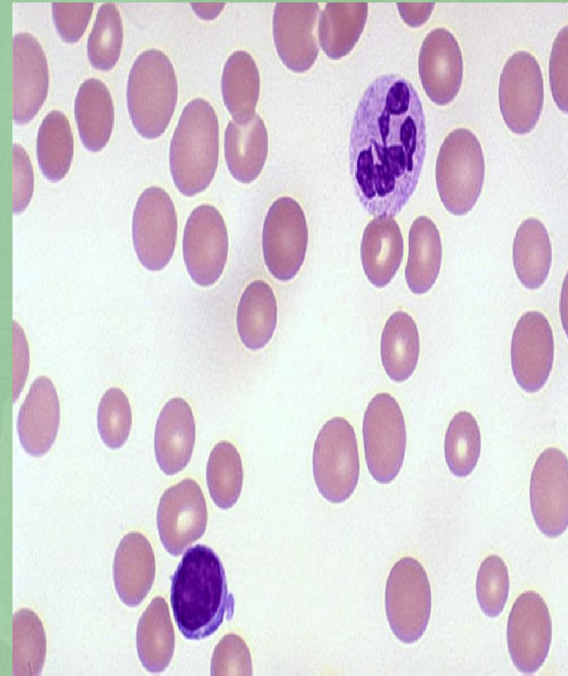
- increase in the numbers of immature neutrophils, primarily band forms and metamyelocytes or more immature cells may also be present; usually in response to an infection.



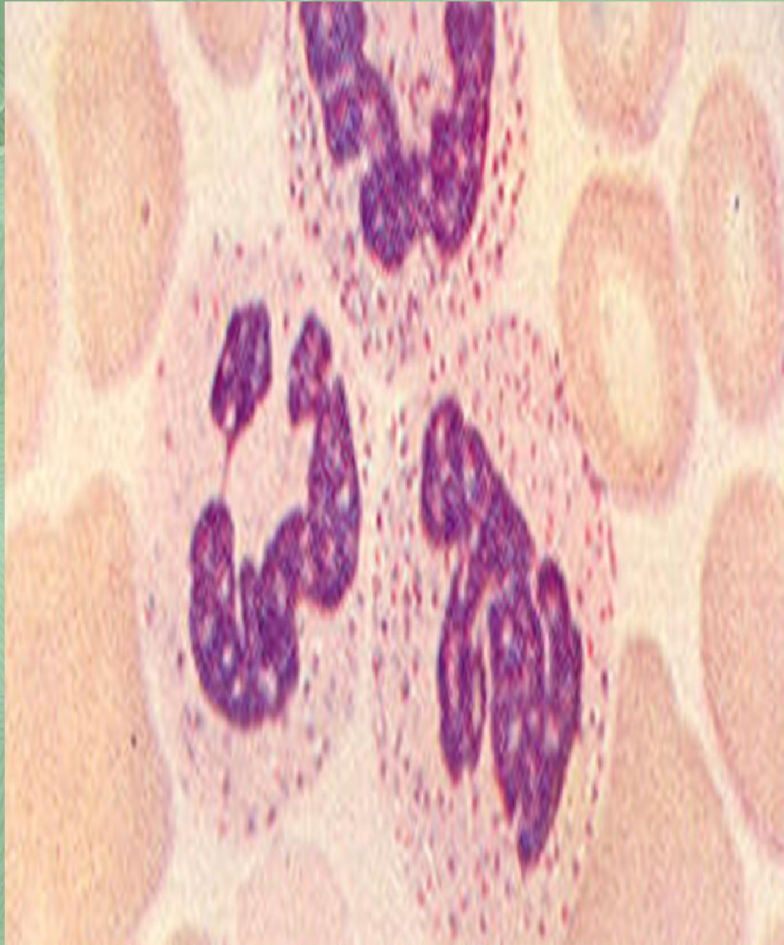


## 3-hypersegmented neutrophil

- Larger than mature neutrophil
- Has more than 5 lobes
- **Found in :**
- Megaloblastic anaemia
- Chronic infections
- **high no of hypersegmented neutrophil=Shift to the right**



## 4- toxic granulation



- the accumulation of big, dark granules in segmented neutrophils



- ↑ no of neutrophils (neutrophilia) in case of:

1- acute bacterial infection

2- acute hemorrhage

3- inflammation

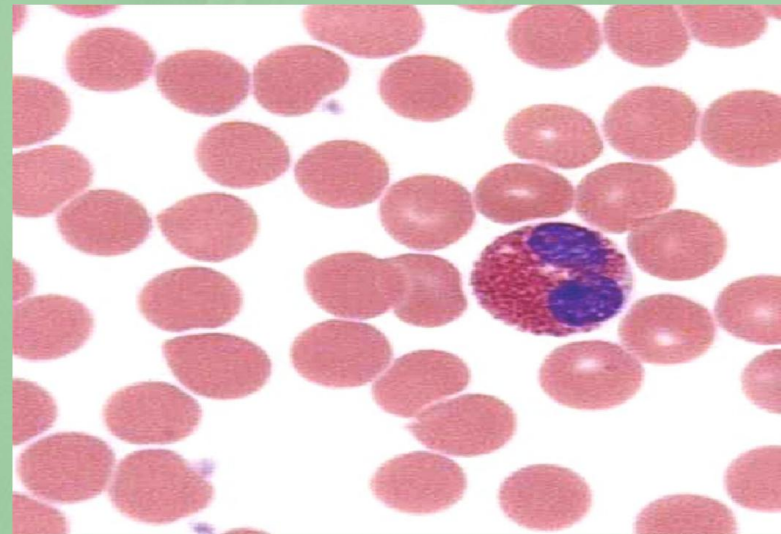
4-tissue necrosis

5-corticosteroid therapy



# Eosinophil:

- **Cytoplasm:** (red orange granules)  
Similar to neutrophil except in the cytoplasm, there are coarse and more deeply red staining granules
- **Nucleus:** usually have 2 lobes (sunglasses).
- **Normal range:** 1-6%
- **Function:**
  - 1-Allergy response
  - 2-Defense against parasite



- **Eosinophilia:**

1-Allergy

2-Parasitic infection

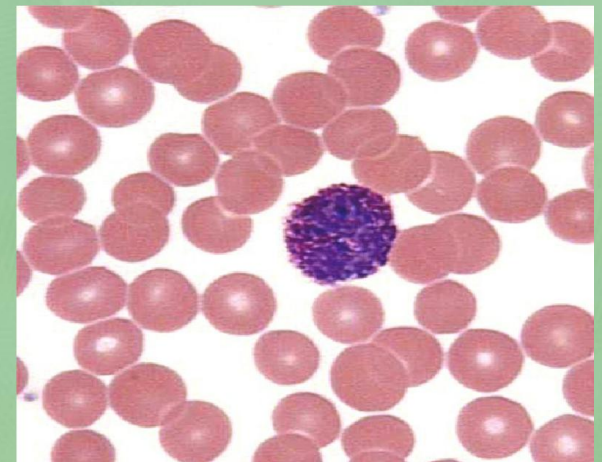
3-Drug sensitivity

4-Skin disease



# Basophils

- in tissue ,they become mast cells.
- only occasionally seen in normal Peripheral blood
- **Cytoplasm:** have dark blue large cytoplasm granules which mask the nucleus
- **Nucleus:** irregular broken nucleus and contain heparin + histamin
- Smaller than eosinophils
- Normal range: < 1-2%.



- **Basophilia:**

1-chronic myeloid leukemia

2-Polycythemia

3-CGL (chronic granulocytic leukemia)

4-Hypothyroidism

5-Ulcerative colitis

6-Nephrosis

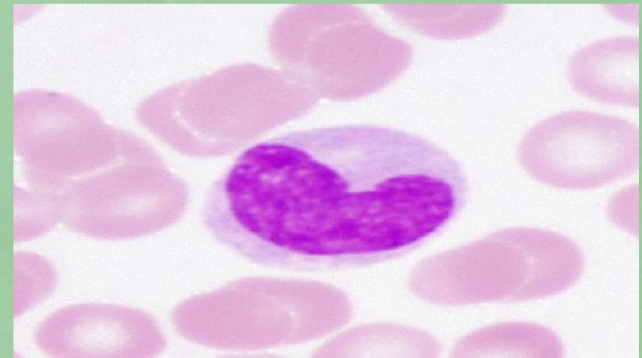
7-Delayed hypersensitivity reaction



# Monocyte:

- Largest WBC(non granulocyte)
- Multifunction:
  - in tissue → phagocytosis
  - in immune system →
    - (1) IFN interferon activation
    - (2) chemotaxis
    - (3) phagocytosis
    - (4) Ag presentation

- Size 15-18 M
- Irregular out lines,
- **Cytoplasm:** transparent, gray blue cytoplasm giving a ground glass appearance .
- vaculoes are seen sometimes in the cytoplasm.
- **Nucleus:** irregular shape usually found as kidney shape or folded.
- Monocytes spend only short time in marrow and after circulation for 20-40 hrs leave blood to enter tissues.
- Normal range 2-10%





- **Monocytosis:**

1-TB

2-Brucellosis

3-Subacute bacterial endocarditis

4-syphilis

5- mainly all types of chronic infection

6-CML (chronic myelomonocytic leukemia)

# Lymphocyte:

- Normal range: 20-40%
- Immunocyte
- two unique features characteristic of the immune system:
  - 1-the ability to generate Ag specificity
  - 2 -immunological memory
- 3 types:
  - T cells** → derived from BM stem cells then pass to thymus to mature as T-cells ( T helper , T cytotoxic)
  - B cells** → derived from BM stem cells to blood as B-cells give Abs.
  - NK** → Large cells with cytoplasmic granules, cytotoxic cell



# Small lymphocyte

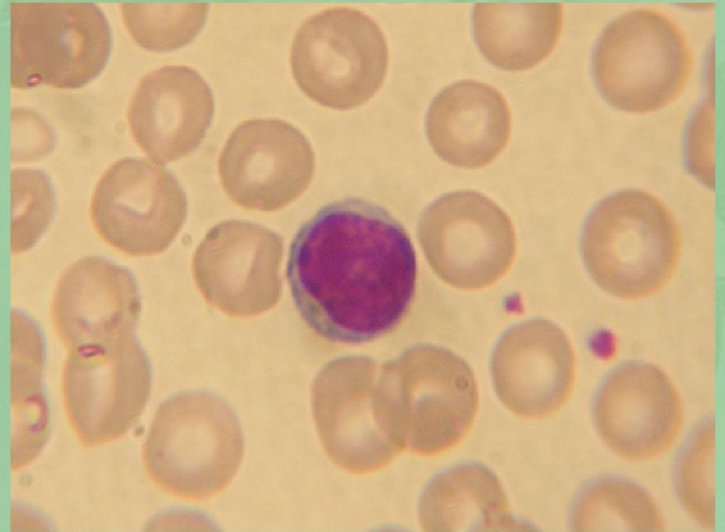
- Small lymphocyte:
- Size 8 M
- Small regular cells
- **Nucleus** :regular staining with deep dark violet.
- Condensed chromatin and the nucleus is not segmented filling most of the cytoplasm
- **Cytoplasm** :stains deep blue

# Large lymphocyte

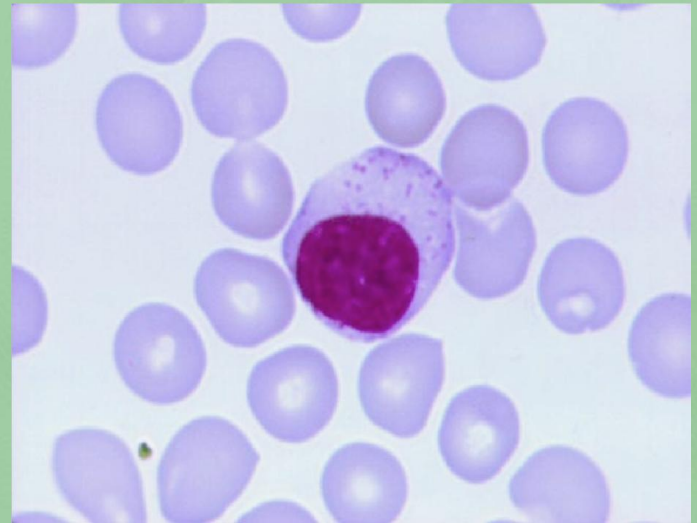
- Large cell about 22 M
- **Nucleus:** less condensed than small lymphocyte and more condensed than monocyte and it is round in shape.
- Nucleus color is deep violet.
- **Cytoplasm :** stained blue and it is abundant and some times few fine granules are seen
- regular outline.



Small lymphocyte



Large lymphocyte



- **Lymphocytosis:**
  - viral infection
    - hepatitis
    - CMV( cytomegalovirus)
    - infectious mononucleosis
  - Lymphoproliferative disorders:
    - lymphoma
    - CLL ( chronic lymphocytic leukemia)



## Absolute values of WBC

- Absolute counts of neutrophil =  $(70 \div 100) \times 10000 = 7000 / \mu\text{L}$
- Neutrophil =  $2 - 7 \times 10^3 / \mu\text{L}$
- Lymphocyte =  $1 - 3 \times 10^3 / \mu\text{L}$
- Eosinophil =  $0.02 - 0.5 \times 10^3 / \mu\text{L}$
- Monocyte =  $0.2 - 1 \times 10^3 / \mu\text{L}$
- Basophil =  $0.02 - 0.1 \times 10^3 / \mu\text{L}$