

# Histology - HLS

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# Blood Cells

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	RBCs (Erythrocytes )	WBCs (Leukocytes )
Types	1 type	5 types
	Not true cells ( <b>lack nuclei and organelles</b> )	True cells ( <b>nucleated with few organelles within cytoplasm</b> )
Number	Male: 4.5-5.5 million/mm <sup>3</sup> female:4-5 million/mm <sup>3</sup>	4500-11000/mm <sup>3</sup>
Diameter	6-9um (7.5um)	6-20um
Life span	120 days	Few days-years
Origin and maturation	Bone marrow	Bone marrow and lymphoid tissue ( <b>maturation completed in the thymus</b> )
Shape	Biconcave discs	Spherical inside the blood ( <b>in the connective tissue they become irregular in shape</b> )
Function	Gas exchange	Defense ( <b>part of the immune system</b> )
Motility	Non motile (they are flowing with the blood but are unable to move by themselves)	Motile (they have to enter the interstitial fluid and connective tissue in order to perform their functions)
	Function exclusively within vascular system (if they leave blood vessels, it is called bleeding or hemorrhage)	Function mainly OUTSIDE blood vessels in the tissues in the site of infection (in the ECM of connective tissue )

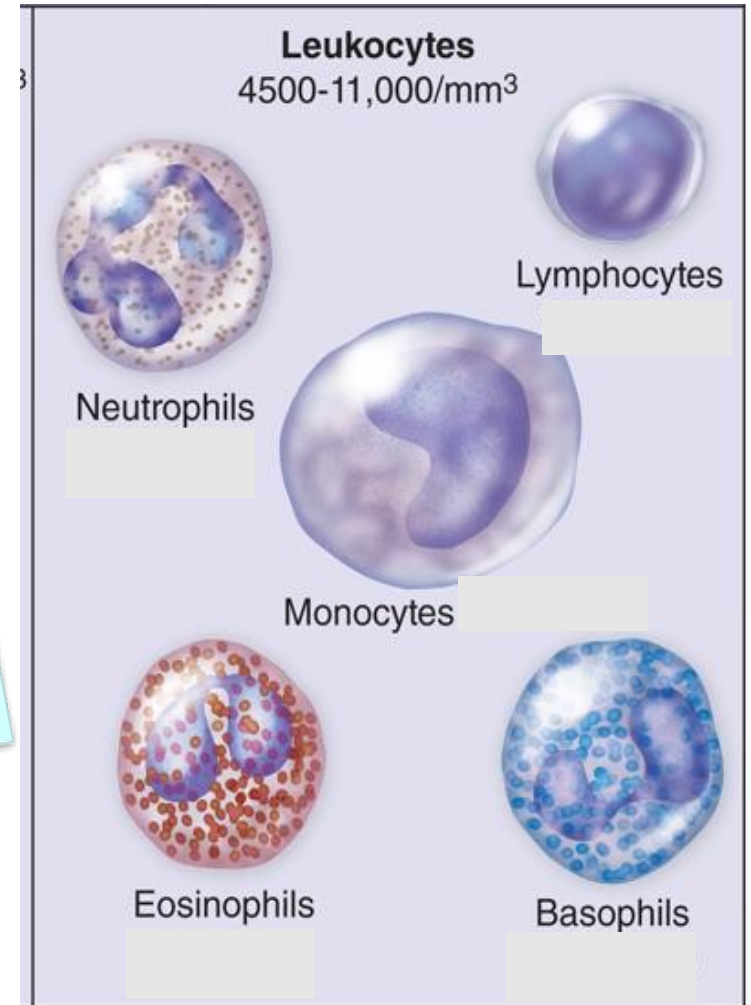
- ❖ WBCs perform their functions by marginating and rolling in order to squeeze in between endothelial cells to reach the site of infection [**process called diapedesis**] in the C.T attracted by chemotactic factors ( **toxins released from microbes when they proliferate** )
- ❖ Toxins activate the endothelium, so it becomes sticky, the endothelium expresses certain receptors for WBCs to recognize them ,stick into the endothelium and squeeze into endothelial cells.
- ❖ **Diapedesis : migration of the WBCs into the surrounding tissue**
- ❖ Marginating ->Rolling ->squeezing-> reaching the site of infection

# Leukocytes

- Originate in the bone marrow and released continuously into the blood
- Travel in bloodstream but function mainly **outside** blood vessels (in loose CT)
- Leukocytes form a mobile army that helps protect the body from damage by bacteria, viruses, parasites, toxins and tumor cells
- 5 types organized into 2 groups **according to presence or absence of specific granules in cytoplasm**
  - **Granulocytes**
    - Neutrophils
    - Eosinophils
    - Basophils
  - **Agranulocytes**
    - Lymphocytes
    - Monocytes

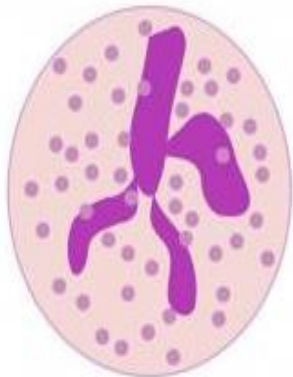
*Dr. Heba Kalbouneh*

Leukocytes, or WBCs, are nucleated and subdivided into granulocytes and agranulocytes, depending on the presence or absence of **specific** granules in their cytoplasm.

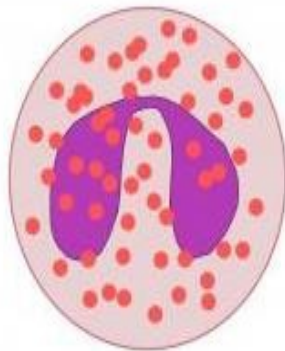


# Leukocytes (White Blood Cells)

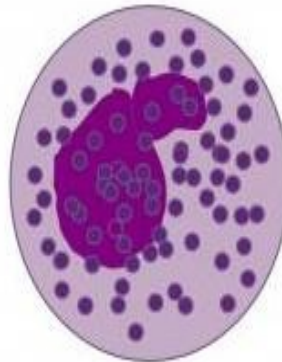
## Granulocytes



**Neutrophil**  
Specific granules have the same affinity for the acidic and basic dyes



**Eosinophil**  
Specific granules stained with eosin, they are acidophil



**Basophil**  
Specific granules stained with basic dye

## Agranulocytes



**Monocyte**  
**Largest cell of WBC**



**Lymphocyte**

# Cytoplasmic granules

**Specific granules**  
Secondary granules

We use them to classify WBCs

**Non-specific granules**

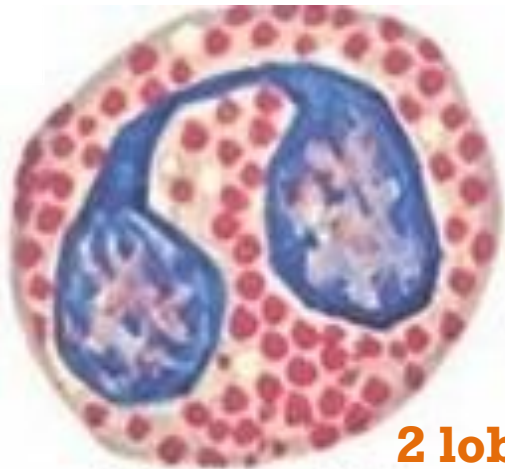
Azurophilic granules  
Lysosomes  
Primary granules

**Non-specific granules, present in all types of WBCs such as, Lysosomes (contain hydrolytic enzymes) in the cytoplasm are stained by azure dye ( appear blue in color )**

## Granulocytes

- Cytoplasmic granules (containing enzymes or chemicals) -> makes cytoplasm look grainy
- Single multi-lobed nucleus (segmented)
- All are phagocytic; they engulf and consume foreign cells and material
- **All WBCs are single nucleated cells but for granulocytes the nucleus is segmented**
- 3 main types:

**Large granules, Red**



**Eosinophil**

**2 lobes**

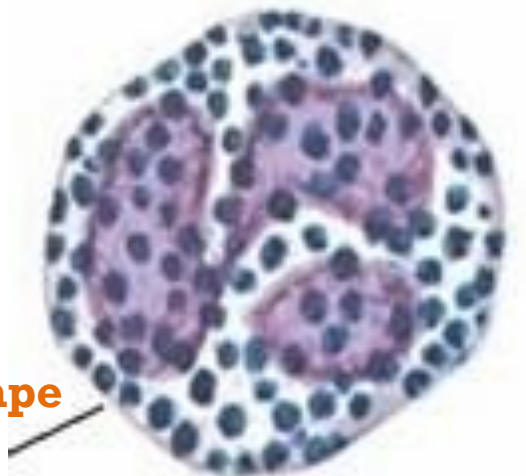
**Small granules, pale pink/ salmon pink**



**Neutrophil**

**4 lobes**

**Large granules, Blue**

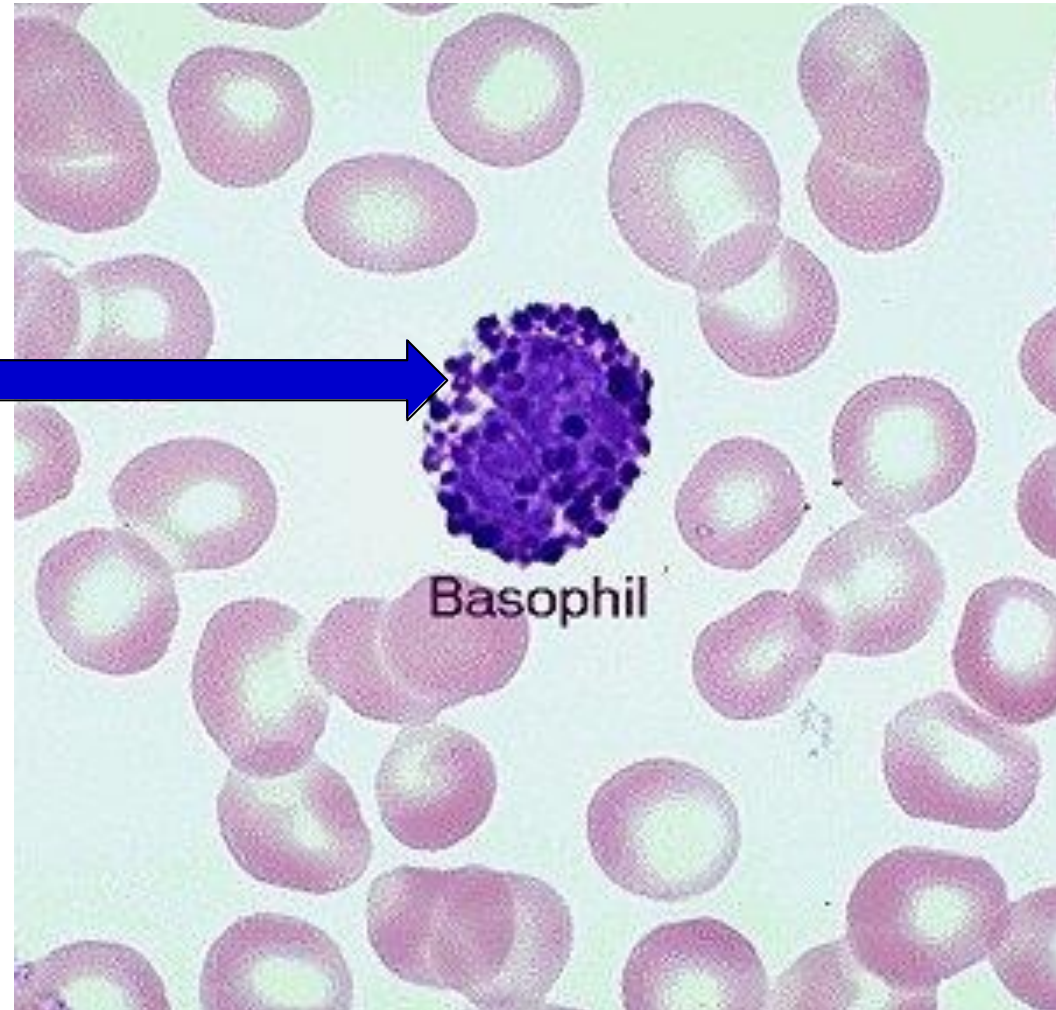


**Basophil**

**Irregular in shape ( s- shaped )**

# Basophil

*These specific granules stain with basic stains*

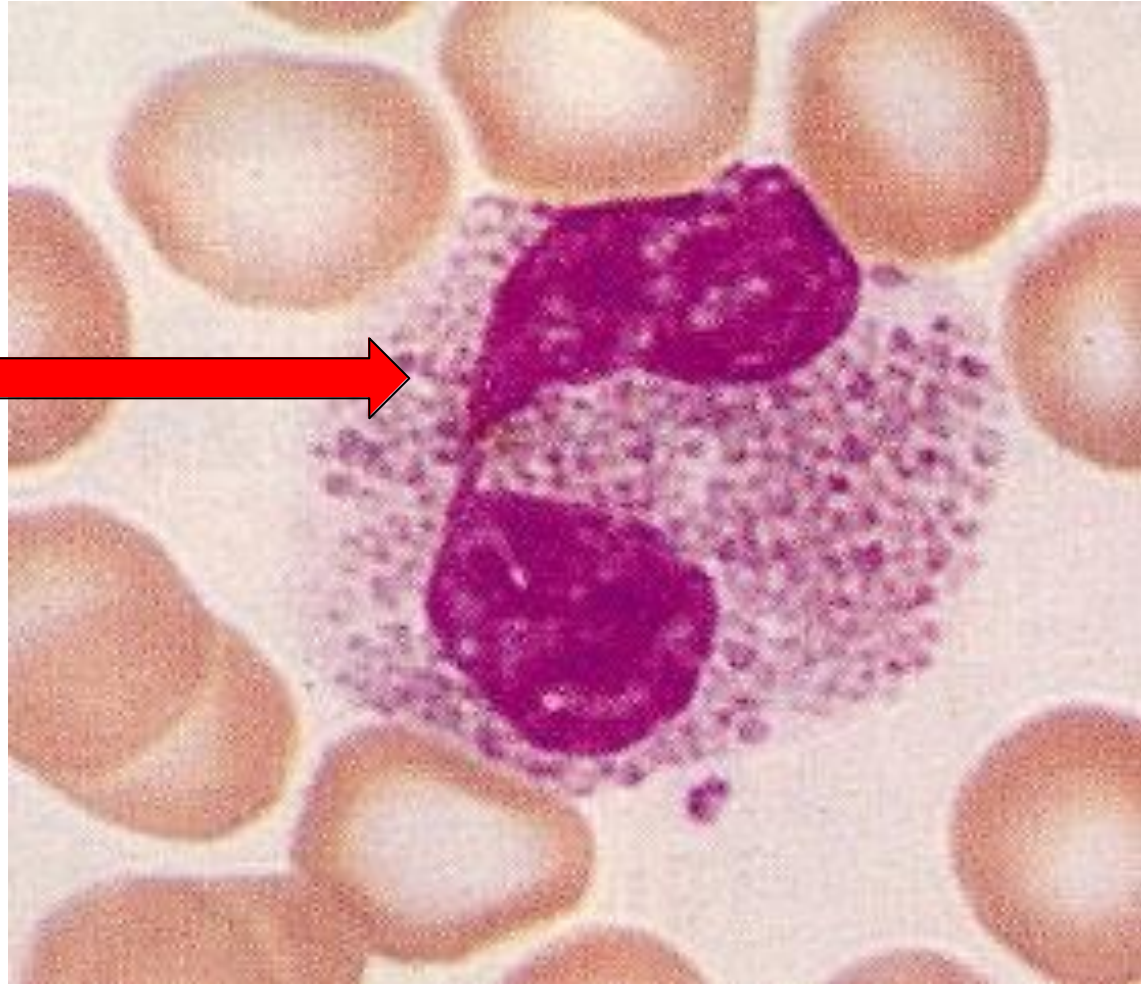


Basophil



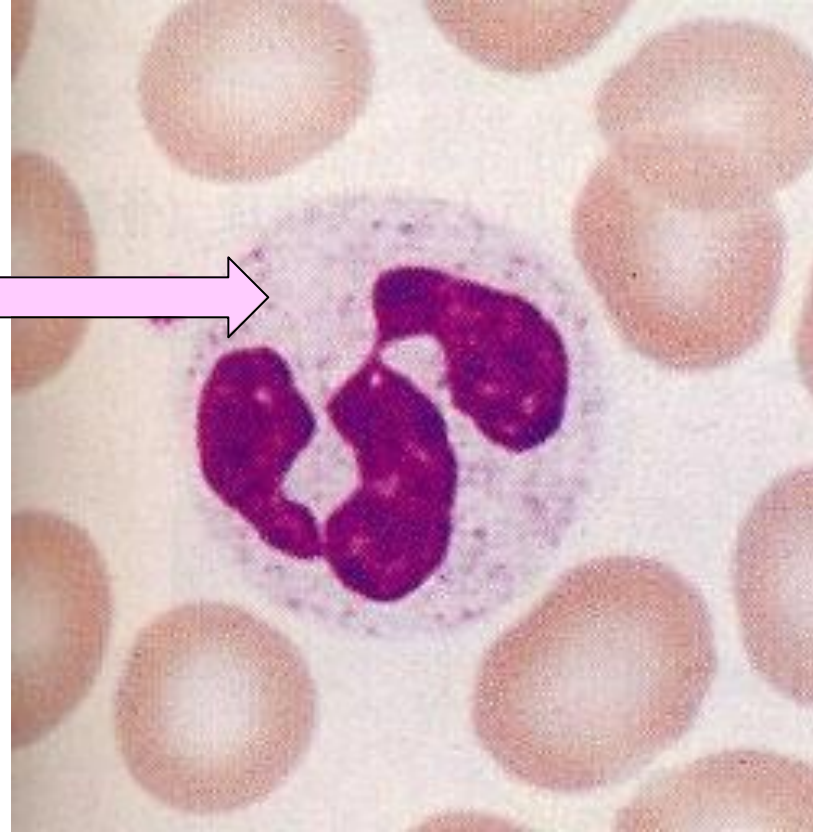
# EOSINOPHIL

*These specific granules  
stain red with acidic  
stains such as eosin*



# Neutrophil

**These granules are neutrophilic, meaning they show no special affinity for either acidic or basic stains but are stained mildly by both**



# Neutrophils

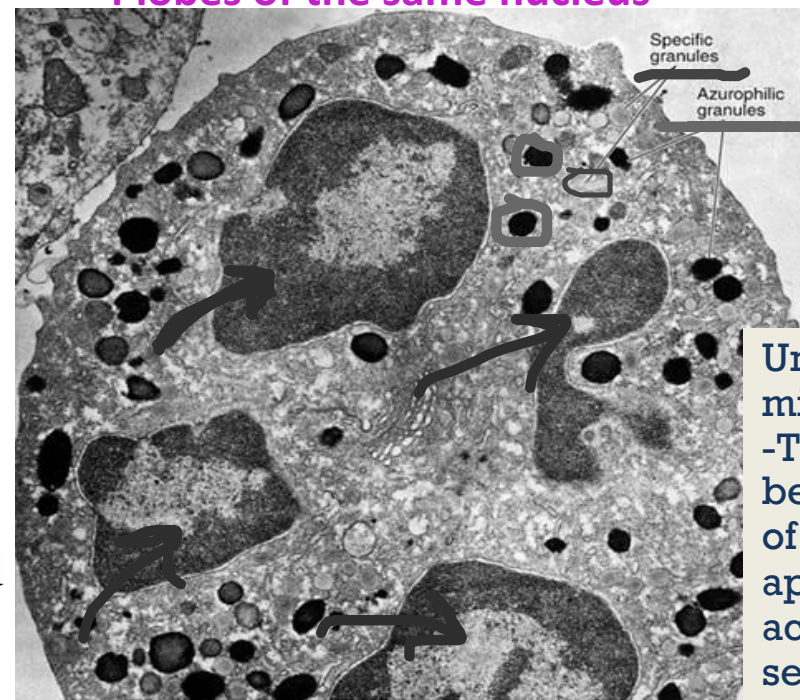
- The most common leukocyte
- 2-5 lobes in nucleus connected by “threads” of nuclear material (**polymorphs**)
- **Neutrophils are formed in the bone marrow by hematopoiesis, once this cell is ready to be released in the blood stream it has two lobes, it circulates for a few hours inside the blood then it has three lobes, about to leave the blood and enter the connective tissue it has four lobes then inside the connective tissue about to die by apoptosis it has five lobes.**
- **[called polymorphs because we have a variable number of lobes inside the cell, the number of lobes indicate the maturity state]**

- Light pink cytoplasm
- Called neutrophils because cytoplasm takes up red (acidic) and blue (basic) stains equally
- Specialized for responding to Bacterial invasions- Acute infections- Acute pyrogenic infections
- Neutrophils are short-lived cells with a half-life of 6-8 hours in blood and a life span of 1- 4 days in connective tissues before dying by apoptosis.



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4 lobes of the same nucleus

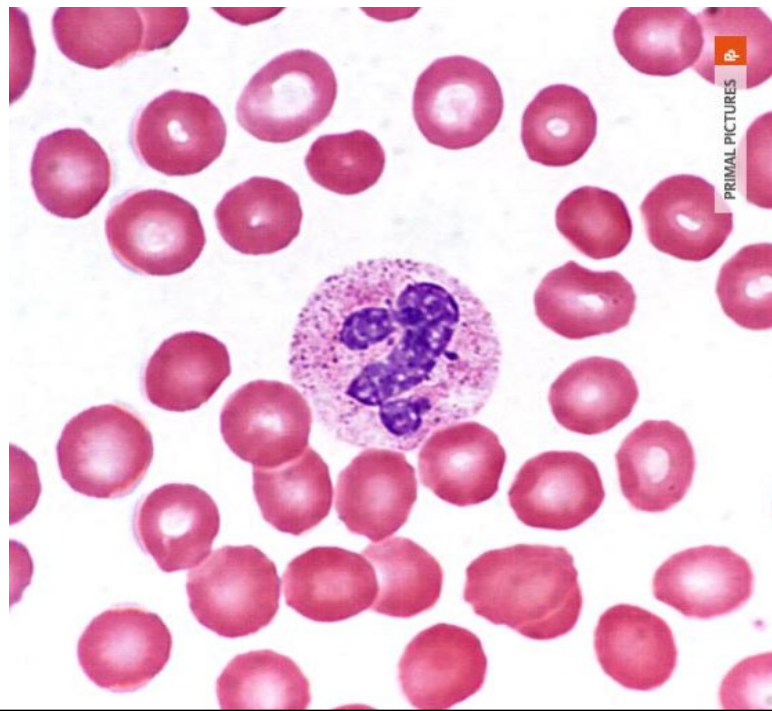


Under the electron microscope  
-The connections between the lobes of the nucleus appear separated according to the section



In females, the inactive X chromosome (**Barr body**) may appear as a drumstick-like appendage on one of the lobes of the nucleus (*about 3% of neutrophils in peripheral blood*)

*in females inside the nucleus we have XX chromosome one is for survival of the cells and other X chromosome is usually clumped at the inner aspect of nuclear membrane*



Neutrophils are the first WBCs that leave the blood in large numbers to reach the site of inflammation

**Why???**

*Cells of acute infection*

- 1- The most abundant*
- 2- The most motile*
- 3- Neutrophil chemotactic factors are the first released*

- **Specific granules (secondary)**

- **Lysozyme (destroys the cell wall of bacteria)**

- **Phagocytin (bactericidal)**

**It kills bacteria directly**

- **Lactoferrin (bacteriostatic)**

**Stops the proliferation of bacteria**

- **iron-binding protein draws iron from the site of infection, iron is necessary for bacteria to proliferate.**

- **Collagenase (destroys collagen fibers in ECM of connective tissue)**

- **Also we have elastase which destroys the elastic fibers**

- **And protease destroys the proteoglycan and glycoproteins**

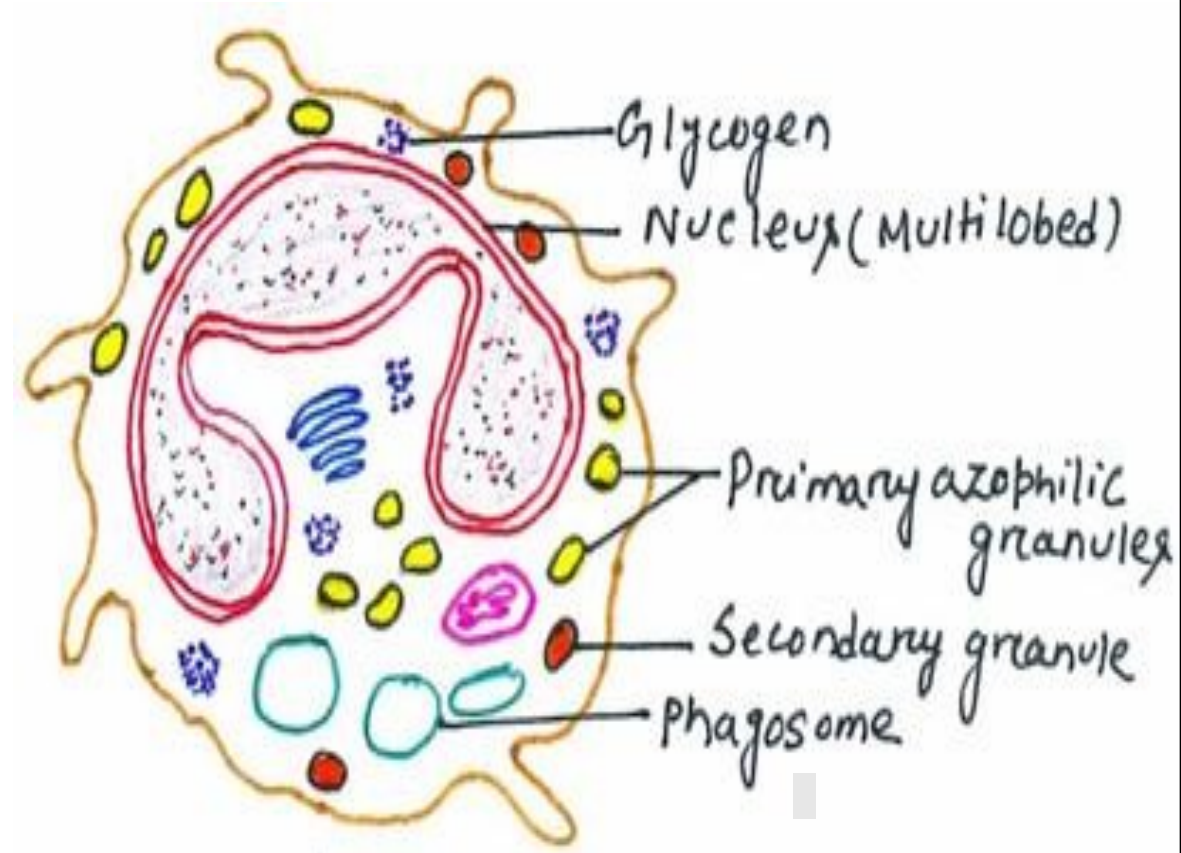
- **Azurophilic granules (primary)**

- **Myeloperoxidase**

Form  $H_2O_2$ , HOCL:  
powerful cytotoxin

- **Acid hydrolase**

- **Defensins Simler to Antibodies (cytotoxic to microbes)**



- ❖ **The epithelium is the first barrier in the body that rests on the basement membrane and exactly under the epithelium lays the connective tissue which contains fixed cells like fibroblast, resident cells -macrophages- and a scattered number of mast cells that have many granules containing histamine , heparin and inflammatory mediators.**
- ❖ **Connective tissue is vascularized (contains blood vessels and capillaries)**
- ❖ **ECM contains collagen and other types of fibers and ground substances- proteoglycans and glycoproteins-**
- ❖ **Breakage in our skin by bacteria into the underlying connective tissue and producing toxins to damage the host cells will stimulate the macrophages to phagocytose foreign material and stimulate the mast cells to secret their granular content .**
- ❖ **Inflammatory mediators released by macrophages, damaged host tissue and toxins all of them act as chemotactic agents.**
- ❖ **Neutrophils kill bacteria by phagocytosis and form phagosome inside it, the ingested material. The phagosome unites with lysosomes forming phagolysosomes containing bacterial and lysosomal enzymes and after that ,they fuse with specific granules to completely kill foreign materials. We call these cells microphages because they are able to eat up small-sized microbes. For large-sized bacteria they secret their granular content to the extracellular matrix by exocytosis)**

- ❖ **collagenases and elastases are secreted from neutrophils to facilitate the movement of the cells within the connective tissue .**
- ❖ **These cells die after performing their function at the site of infection which would be swelling because of the increased blood flow caused by releasing histamine. At the end, dead neutrophils and bacteria, lysed ECM and damaged host cells and fluid from the plasma [all together would form what is called pus] would appear at the site of infection. We can call neutrophils pus cells because there're the most common cells found in pus.**
- ❖ **After eliminating the bacteria from the site of infection, the last WBC to be recruited to enter the infection site is monocyte.**
- ❖ **Monocyte is a large cell when it leaves the blood and enter the connective tissue it differentiates into macrophages which would start to eat up the dead cells and clean up the mess**
- ❖ **So, neutrophils either phagocytose the foreign material or they release their granular content into the ECM of connective tissue to fight the foreign material**



Dead neutrophils, bacteria, lysed ECM, and tissue-fluid form a viscous, usually yellow collection of fluid called **pus**.

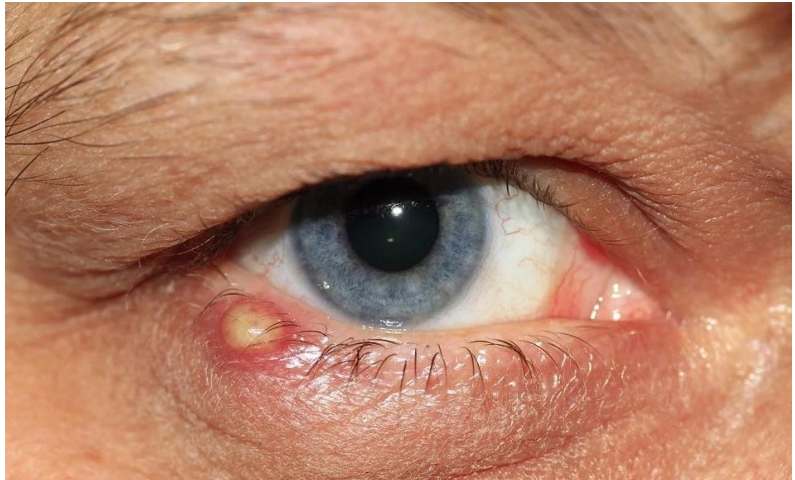
**Pus is a viscous, usually yellow collection of fluid.**

**Pyrogenic** is referring to bacterial infections that make pus while **pyrogenic** is producing heat  
Because the pus activate the hypothalamus to elevate the body temperature



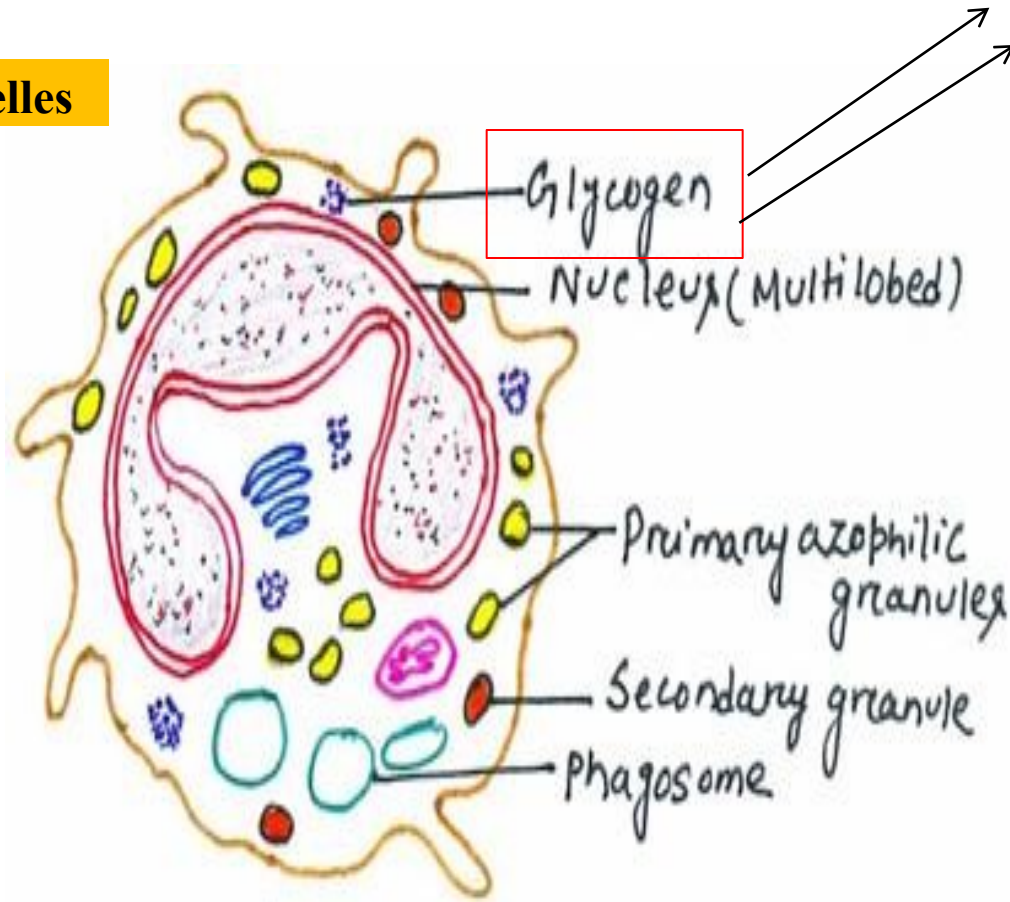
**Pus appears slightly yellowish in color or green, [some types of bacteria have green pigments and myeloperoxidase enzyme inside lysosome of neutrophils has green pigment ]**

**Pus is pyrogenic**





Few organelles



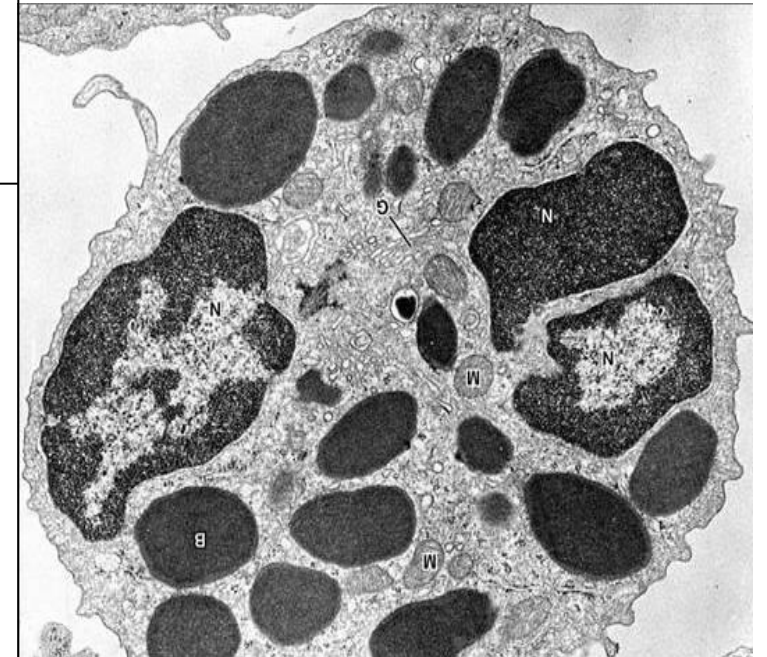
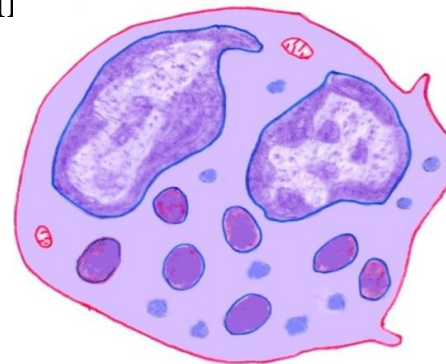
The ability of neutrophils to survive in an anaerobic environment is highly advantageous, because they can kill bacteria and help clean up debris in poorly oxygenated regions, for example, damaged or necrotic tissue lacking normal microvasculature.

**Neutrophilia:** is an increase in the number of neutrophils in peripheral blood. (in case of acute infections and in the peripheral blood of a patient with pus) [pyogenic or pyrogenic infections]

**Neutropenia:** is the decrease in the number of neutrophils

# Basophils

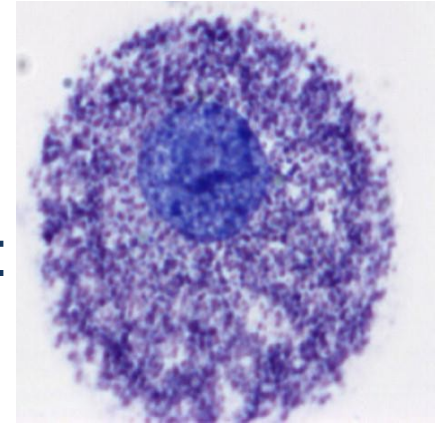
- Rarest leukocyte , **the normal range is between 0-1% – might not see these under the microscopes( it is normal not to find any single basophils in the peripheral blood)**
- Usually have bi-lobed, S-shaped nuclei obscured by the large basophilic granules (**reason behind not seeing the nuclues under LM is the density of these large granules**)
- **Under EM these granules have dense-like appearance.**
- Has large granules that stain dark purple/ blue in basic dyes (*basophil* = basic loving)
- Granules contain histamine, heparin and eosinophilic chemotactic factor that mediate inflammation in all reactions and parasitic infections



# Mast cells

**Mast cells have round nucleus and their filled with basophilic granules contain histamine, heparin and chemotactic factor.**

**They are found inside the connective tissue specially in the mucosa of the GI tract and respiratory tract and in cutaneous membranes because these cells are involved in allergic reactions.**



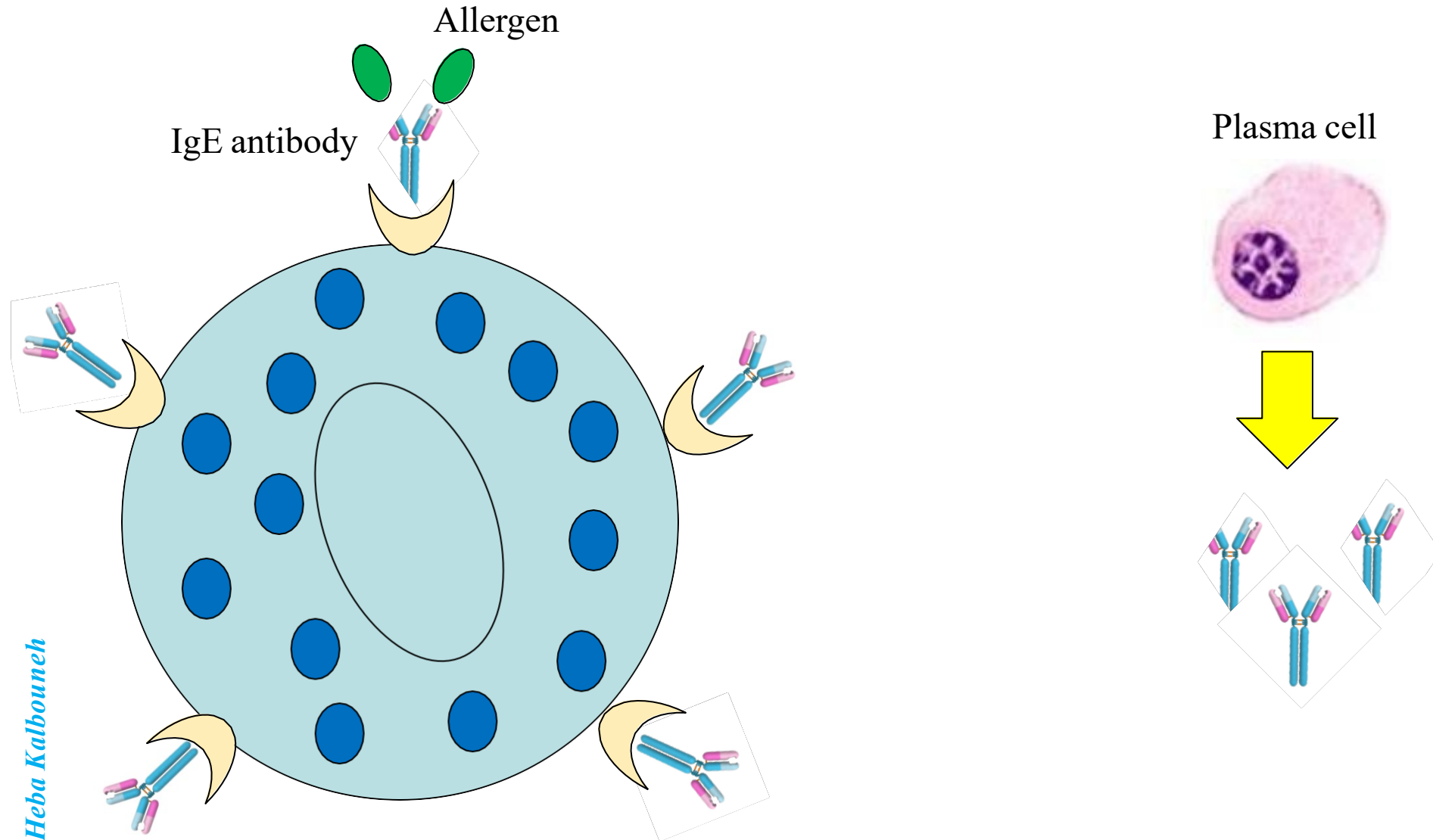
**Once these cells are stimulated by certain allergens, they degranulate and release their content to the interstitial fluid**

**What are the symptoms of allergy ?**

**1- swelling 2- redness 3- itch**

- **Swelling because of the dilation of the blood vessels and the increased permeability of the blood vessels so more fluid is leaving the plasma at the site of allergy**
- **Itchy because inflammatory mediators irritate the nerve endings**
- **We treat these symptoms by anti-histamines**
- **Basophils have the same functions of mast cells, so it is normal not to find basophils in the peripheral blood**

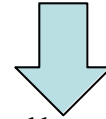
Both basophils and mast cells have surface receptors for immunoglobulin E (IgE), and secrete their granular components in response to certain antigens and allergens.



First exposure

*Exposure may be by ingestion, inhalation, injection, or direct contact*

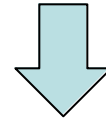
In some individuals substances such as certain pollen proteins or specific proteins in food are allergenic, that is, elicit production of specific IgE antibodies, which then bind to receptors on mast cells and immigrating basophils.



Second exposure

Upon subsequent exposure, the allergen combines with the receptor-bound IgE molecules, triggering rapid exocytosis of the cytoplasmic granules.

Release of the inflammatory mediators in this manner can result in bronchial asthma, cutaneous hives, rhinitis, conjunctivitis, or allergic gastroenteritis.



**Immediate or type 1 hypersensitivity**

**Some of the inflammatory mediators are leukotriene which cause spasm in the smooth muscles of the bronchi**

In some individuals a second exposure to a strong allergen, such as that delivered in a bee sting, may produce an intense, adverse systemic response. Basophils and mast cells may rapidly degranulate, producing vasodilation in many organs, a sudden drop in blood pressure, and other effects comprising a potentially lethal condition called

**Anaphylaxis or anaphylactic shock.**



**Basophils** account for up to 15% of infiltrating cells in allergic dermatitis and skin allograft rejection

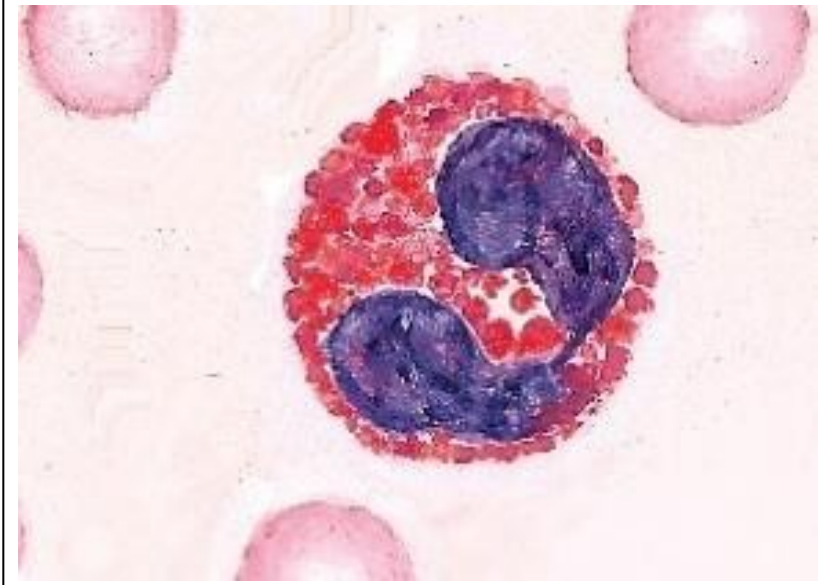


**Allergic** dermatitis (eczema) is an itchy skin rash that develops when you come into contact with an **allergen**



# Eosinophils

- Usually have bi-lobed nuclei connected by a short “thread” of nuclear material
- Large cytoplasmic granules, which stain red with the acidic eosin dye (*eosinophil* = eosin loving)
- Help in ending allergic reactions and in fighting parasitic infections



**Under electron microscope they appear crystalloid in shape**

## Specific granules (Crystalloid granules):

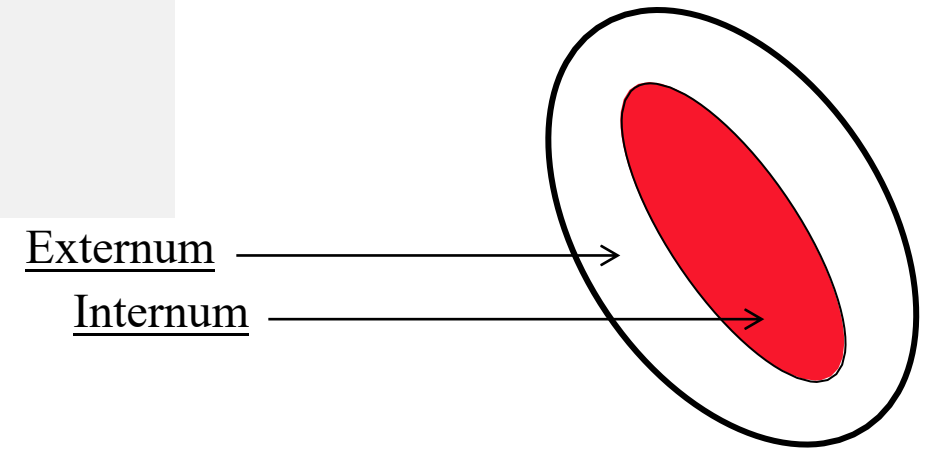
- Oval in shape, with flattened crystalloid cores
- Two parts:

Externum (pale): contains histaminase (stops the action of histamine) and sulfatase (stops the action of heparin)

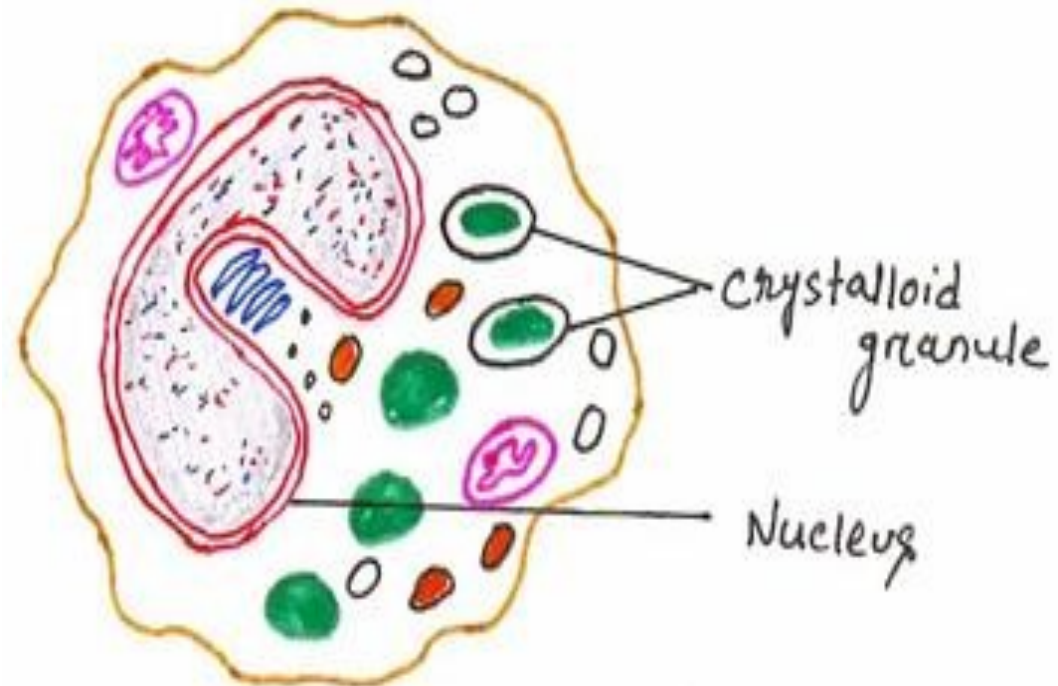
Internum (dark): contains basic protein to kill parasites

Eosinophilia: Increased numbers of eosinophils may indicate parasitic infection

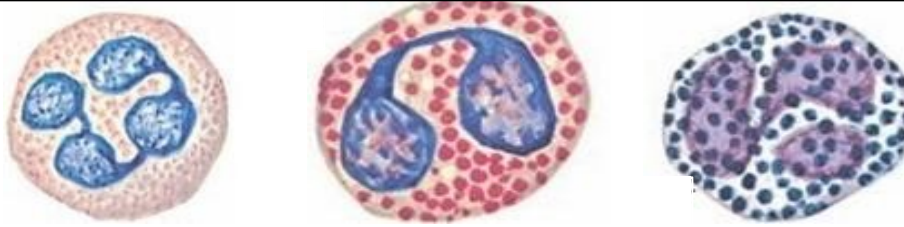
Crystalloid granule



Eosinophils have a particular phagocytic affinity for **antigen-antibody complex**







	Neutrophil	Eosinophil	Basophil
Percentage (WBCs)	Most —————> Least		
Size	12-15um	12-15um	12-15um
Life span	Few days	Few days	Few days
Nucleus	2-5 lobes	2 lobes	Irregular (S-shaped)
Phagocytic activity	Most —————> Least		
Motility	Most —————> Least		

**Diapedesis**  
(Gr. dia, through + pedesis, to leap)

General features of granulocytes

- Spherical in blood stream, irregular in connective tissue
- Highly motile with different shapes due to their amoeboid movement
- Leave blood stream by migrating between the endothelial cells by a process called **diapedesis**