Volgograd state medical university

Department of histology, embryology, cytology

BLOOD.
HISTOLOGY OF THE FORMED ELEMENTS

Lecture for the 1st year students

Volgograd

The objectives:

1. Learn the structure of function of different blood formed elements: red blood cells, white blood cells and platelets.

2. Reveal structural and functional correlations in the subclasses of the white blood cells: granulocytes and agranulocytes.

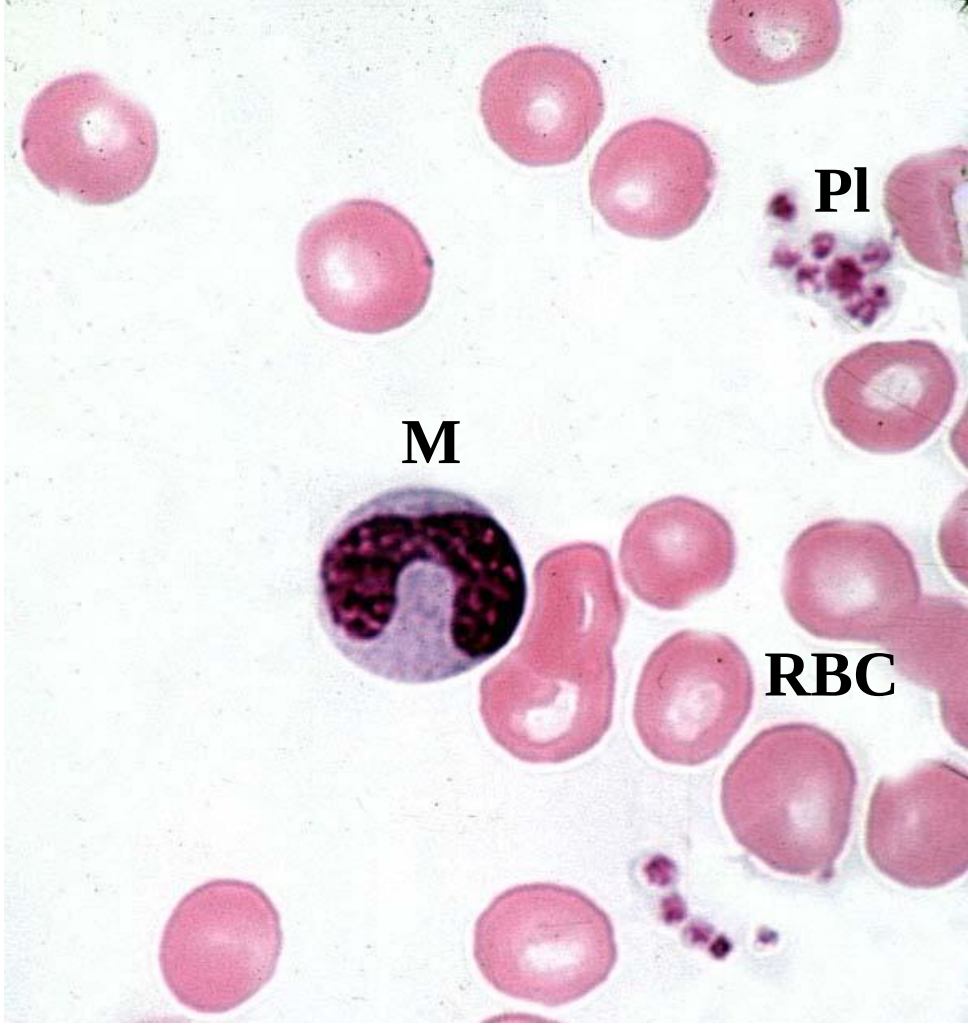
3. Compare the structure and ultrastructure of granulocytes: eosinophuils, basophils, neutrophils, and to reveal functional correlations.

4. Distinguish the types of agranulocytes: monocytes and lymphocytes and their subtypes. Define their role in the immune responce.

5. Evaluate red and white blood cell count. Assess normal range of eosinophils, basophils, neutrophils, lymphocytes and monocytes in the blood smear.

Blood is a bright to dark red, viscous, slightly alkaline fluid (pH 7.4) that accounts for approximately 7% of body weight.

Blood is a specialized connective tissue composed of formed cellular elements, the red (RBC) and white (M) blood cells, and the platelets (Pl) suspended in a clear slightly yellow fluid called plasma. In normal adults blood volume is in the range 4.5-6 l of which 55% by volume is plasma, about 45% is erythrocytes (i.e. blood fraction or hematocrit) and 1% or less contains leukocytes (white cells) and platelets. Normal values are 40-50% in men and 35-45% in women. The fluid component of blood leaves the capillaries and small venules to enter connective tissue spaces as extracellular fluid (with electrolytees and small molecules like in plasma but with lower concentration of proteins.



Peripheral blood smear.
Human. x 1325.
M – monocyte.

FUNCTIONS OF BLOOD

The functions of blood are numerous and complex, and involve not only the formed elements, but also the very many substances dissolved in plasma that reflect the metabolic activity of the tissues, connected via the blood circulation. Some main functions include:

- **distribution of oxygen to all tisssues, and waste carbon dioxide and nitrogenous products, respectively, to the lungs and kidneys;**
- **transportation of nutrients processed by the gut and liver; transportation of other metabolites (hormones, signal molecules) to the target organs and cells;**
- **regulation of body temperature, pH, electrolytes, glucose and cholesterol levels; maintenance of vascular fluid volume;**
- **protection against infection (migration of the leukocytes) and prevention of blood loss following injuryб**

-hemocoagulation.

Properties of the main formed elements in blood

	erythro-cytes	plate-lets	neutro-phils	eosi-no-phils	baso-phils	mono-cytes	lympho-cytes
size, mcm	7.1-7.9	2-4	9-12	10-14	8-10	15-20	7-16
lifespan in circu-lation	4 months	10 days	1-2 days	1-2 days	hours-days	3 days	3 days-20 years
differen-tial leuko-cyte count	99% of all elements	-	**Leukocyte formula:** 40-75%	1-5%	0-1%	3-9%	20-35%
cells per liter	**Hemogram (blood formula, complete blood count)**						
Male/ Female	$3.9\text{-}5.5\times10^{12}$ / $3.7\text{-}4.9\times10^{12}$	250-300 $\times10^{9}$	all WBC: $3.9\text{-}9\times10^{9}$				

DATE 24/10/08 TIME 11:57:00
SEQ. # 6
ID 8
Patient Name:_____
DOB:__/__/__ Age:_____ OPR ID:_____

WBC	7.5		10^9/L
RBC	4.01		10^{12}/L
HGB	106	L	g/L
HCT	0.301	L	L/L
MCV	75	L	fL
MCH	26.4		pg
MCHC	352	H	g/L
RDW	13.6		%
PLT	454	H	10^9/L
MPV	7.3		fL

NE	51.2	%
LY	37.5	%
MO	9.3	%
EO	1.5	%
BA	0.5	%
NE#	3.82	10^9/L
LY#	2.79	10^9/L
MO#	0.69	10^9/L
EO#	0.11	10^9/L
BA#	0.04	10^9/L

FLAGS
WBC:*WBC ATL
RBC:
PLT:

DATE 23/10/08 TIME 12:03:00
SEQ. # 15
ID 0
Patient Name:_____
DOB:__/__/__ Age:_____ OPR ID:_____

WBC	11.5	H	10^9/L
RBC	4.68		10^{12}/L
HGB	132		g/L
HCT	0.379		L/L
MCV	81		fL
MCH	28.3		pg
MCHC	349		g/L
RDW	12.7		%
PLT	328		10^9/L
MPV	7.3		fL

NE	63.8	%
LY	26.1	%
MO	7.4	%
EO	2.0	%
BA	0.7	%
NE#	7.31	10^9/L
LY#	2.99	10^9/L
MO#	0.85	10^9/L
EO#	0.23	10^9/L
BA#	0.08	10^9/L

FLAGS
WBC: IMM
RBC:
PLT:

DATE 27/10/08
SEQ. # 19
ID 19

TIME 11:17:13

Patient Name:_____
DOB:__/__/__ Age:_____ OPR ID:_____

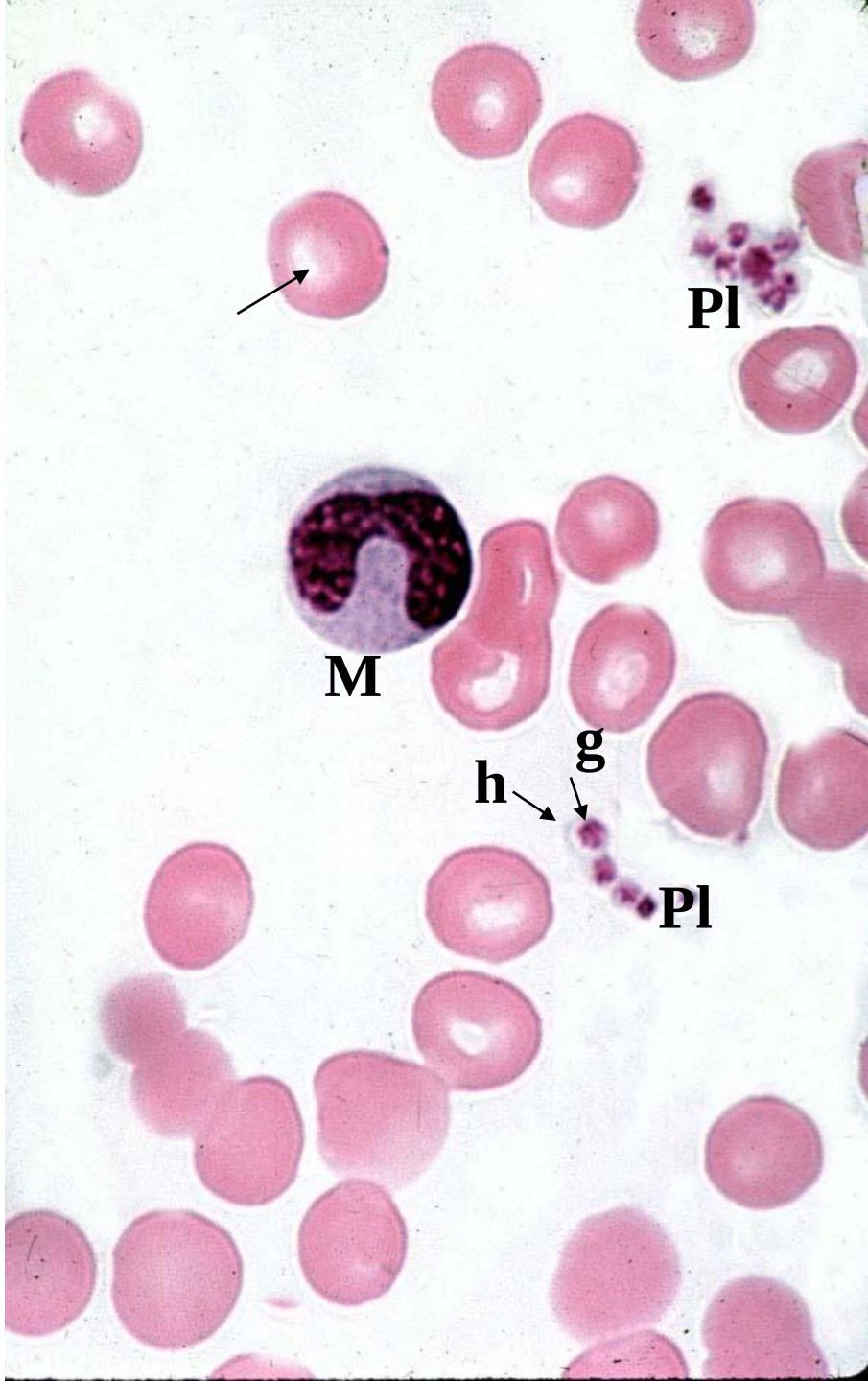
WBC	5.4	10^9/L
RBC	5.15	10^{12}/L
HGB	145	g/L
HCT	0.419	L/L
MCV	81	fL
MCH	28.2	pg
MCHC	346	g/L
RDW	12.8	%
PLT	248	10^9/L
MPV	7.9	fL

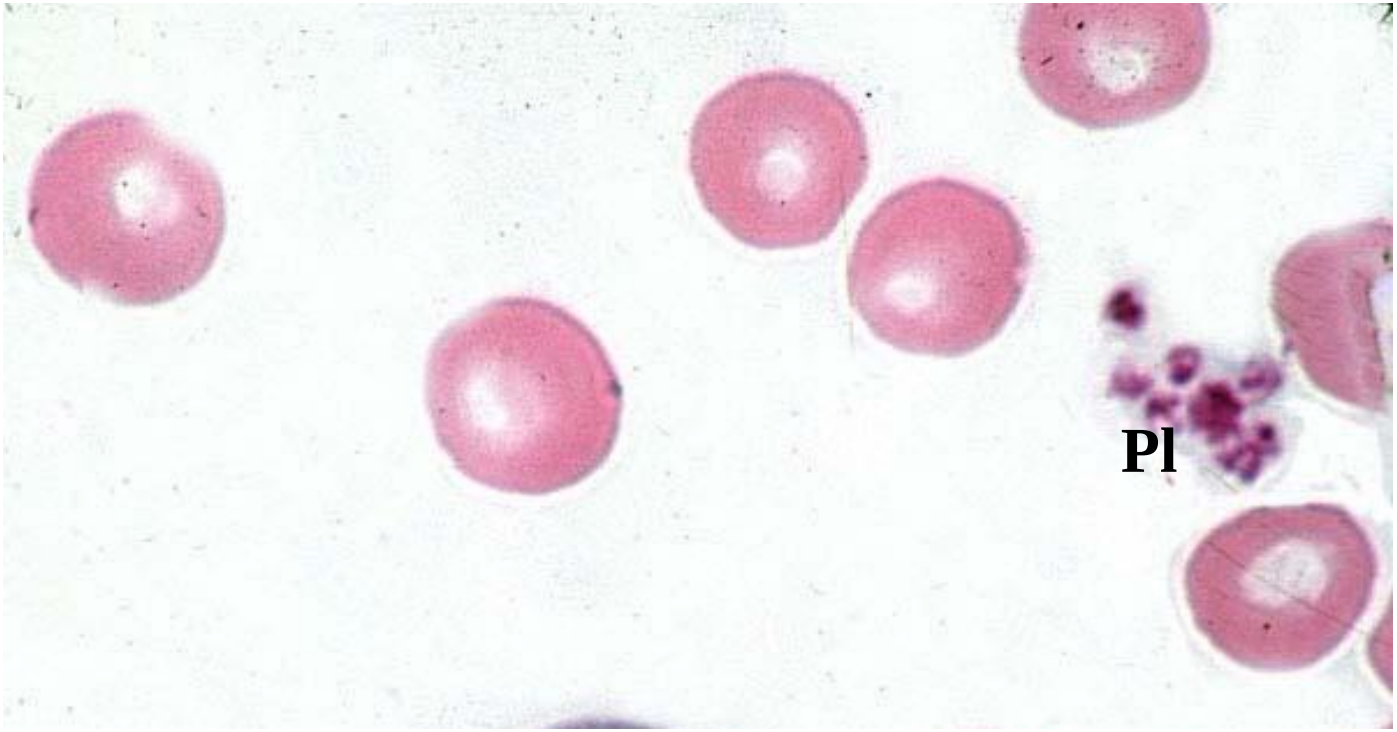
NE	50.0		%
LY	36.0		%
MO	10.9	H	%
EO	2.8		%
BA	0.3		%
NE#	2.68		10^9/L
LY#	1.93		10^9/L
MO#	0.58		10^9/L
EO#	0.15		10^9/L
BA#	0.02		10^9/L

FLAGS
WBC: ATL IMM
RBC:
PLT:

Red blood cells. Peripheral blood smear. Human. x 1325.

RBC are salmon pink biconcave discs 1.8 mcm thick (2.0 mcm at its widest region and 1.0 mcm at the center) and ranges from 6.5-8.5 mcm in diameter, the size decreasing slightly with age. These cells display a central clear region (arrows) that represents the thinnest area of the biconcave disc. Due to its biconcave shape which the center of the cell appears pale. Its main cytoplasmic constituent is the protein complex hemoglobin, which results in its characteristic acidophilic staining property. Oxygen-carrying hemoglobin which binds the acidic eosin dye used in staining hence acidophilia of the cytoplasm. The biconcave shape maximizes their surface area/volume ratio and thereby facilitates oxygen exchange.





Mature red blood cells

Pl – platelets.

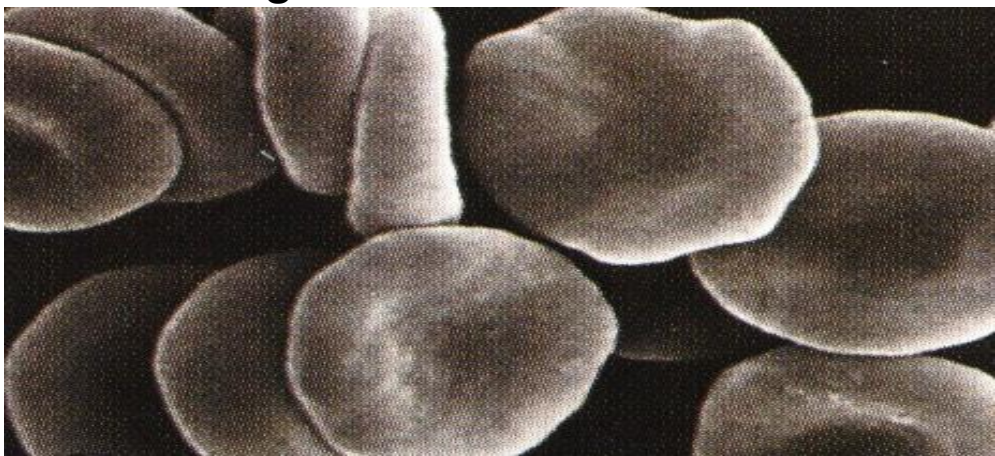
Red blood cells are packed with hemoglobin, a large tetrameric protein composed of the four polypeptide chains, each of which is covalently bound to an iron-containing heme. The globin moiety of hemoglobin releases CO_2 and the iron binds to O_2 in the regions of high oxygen tension (lung). In the oxygen-poor regions hemoglobin releases O_2 and binds CO_2. This property of Hb makes it ideal for the conveyance of respiratory gases.

Typical appearance of a mature red cell in a stained smear of the peripheral blood. Red cells do not have a nucleus as this is lost during formation.

Ultrastructurally red blood cells have a cell membrane which surrounds an electron-dense cytoplasm. There are no discernable organelles as these have been degraded during differentiation. They contain soluble enzymes in the cytosol like carbonic anhydrase facilitating the formation of the carbonic acid from the carbon dioxide. This acid dissociates forming bicarbonate (HCO_3^-) and hydrogen (H^+). It is bicarbonate The ability of bicarbonate to cross the erythrocyte cell membrane is mediated by the intergarl membrane protein band 3, It is a couples anion transporter that exchanges intracellular bicarbonate for extracellular Cl^-. Despite a lack of organelles red cells are metabolically active and derive energy by anaerobic metabolism of glucose and through ATP generation by the hexose monophosphate shunt which produces high-energy molecule of NADPH. Glycolyic enzymes are also dissolved in the cytoplasm of the erythrocytes.

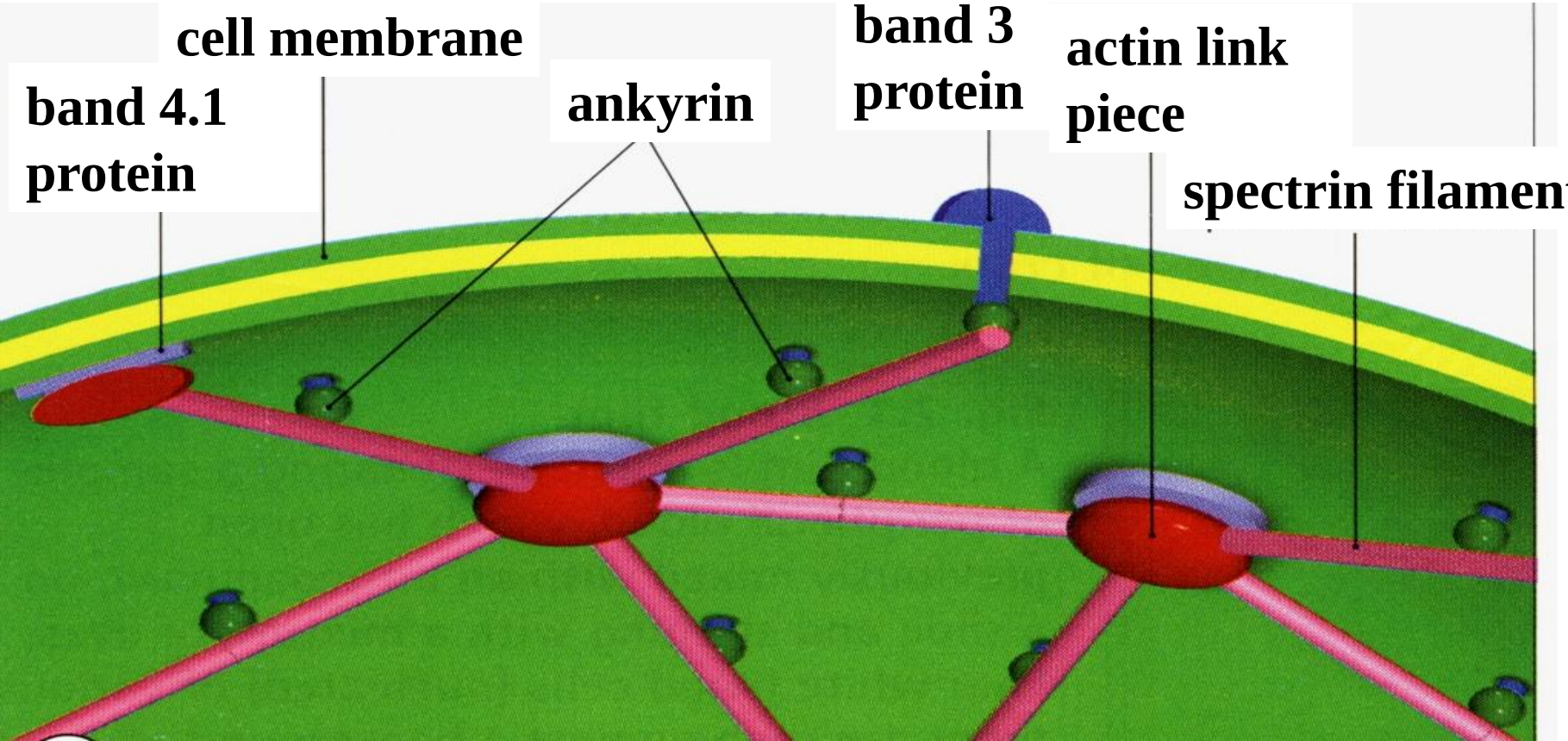
Functionally red cells are highly deformable and are able to squeeze through small blood vessels down to 3-4 micrometers in diameter. The cells membrane is braced by an actin-spectrin containing cytoskeletal meshwork which is largely responsible for the maintaining the distinctive bioconcave shape.

Erythrocytes, SEM



Mature red blood cells

Cytoskeleton, which maintains its distinct shape. A filamentous skeleton of the protein spectrin is anchored to the cell membrane by three main proteins (band 3 protein, ankyrin and band 4 protein) with short actin pieces, about 15 actin monomeres long, linking spectrin to the band 4.1 protein. Other proteins are also involved, but have been omitted for clarity.



CLINICAL CORRELATIONS

On the basis of the amino acid sequences, there are four normal human polypeptide chains of Hb, designated alpha, beta, gamma and delta. The principal hemoglobin of the fetus, the fetal Hb (HbF), composed of the two alpha and two gamma chains is replaced shortly after birth by adult HbA. There are two types of normal adult Hb (HbA1: alpfa2, beta2; and the much rarer form HbA2: alpha2gamma2). In the adult around 96% of the Hb is HbA1, 2% HbA2 and 2% HbF.

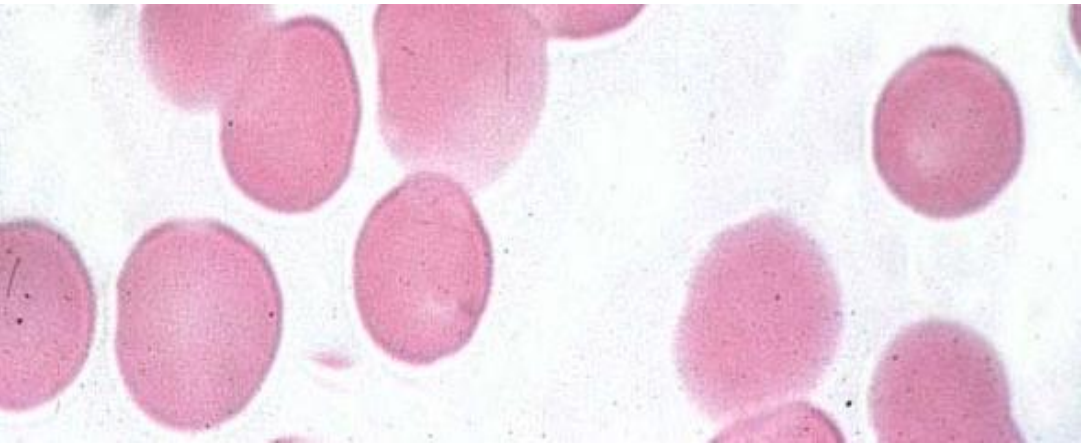
Any significant lowering of the blood concentration of hemoglobin due either to reduction in the total number of circulating erythrocytes or to decrease in their individual hemoglobin content, is described as an anemia (greek: without blood).

Several hereditary diseases result from defects in the genes encoding the hemoglobin polypeptide chains. Diseases referred to as thalassemia are marked by decreased synthesis of one or more hemoglobin chains. In beta-thalassemia synthesis of the beta-chains is impaired. In the homozygous form of the disease (persons from Mediterranean descent) HbA is missing and high levels of HbF persist after bitrth. As one of the globin chains of Hb is usually absent or inadequately produced and the erythrocytes are typically pale and small with reduced life expectancy.

CLINICAL CORRELATIONS

In the case of genetically transmitted condition called sickle cell anemia, erythrocytes become deformed into elongated crescents (sickles) in the process of yielding their oxygen. A single amino acid substitution in hemoglobin cauases this protein to polymerize and crystallize as it gives up its oxygen. In ß-chain valine is incorporated into the sequence instead of glutamate, forming the abnormal hemoglobin HbS. When the oxygen tension is reduced (e.g., during strenuous exercise), HbS changes shape, producing abnormal-shaped (crescent-shaped) erythrocytes that are less pliant, more fragile, and more prone to hemolysis than normal cells with premature cell destruction. Sickle cell anemia is prevalent in the black population, especially in those whose ancestors lived in regions of Africa where malaria is endemic. In the United States, about 1 of 600 newborn African-American babies is stricken with this condition.

Normally the internal surface of the RBC cell membrane is braced by cytoskeletal proteins via interconnections between ankyrin and spectrin. Defects in the cytoskeletal components of erythrocytes result in various conditions marked by abnormal shaped cells. Hereditary spherocytosis, for instance, is caused by synthesis of an abnormal spectrin that exhibits defective binding to band 4.1 protein while normal ankirin binding to spectrin is absent. In hereditary spherocytosis RBC do not form their normal biconcave disk shape, but appear round and convex. They are abnormally fragile and do not resist changes in osmotic pressure. This abnormally rapid breakdown of RBC is called hemolysis. Red blood cells of patients with this condition are more easily deformed and transport less oxygen compared with normal erythrocytes. Moreover, these spherocytes are preferentially destroyed in the spleen, leading to anemia.



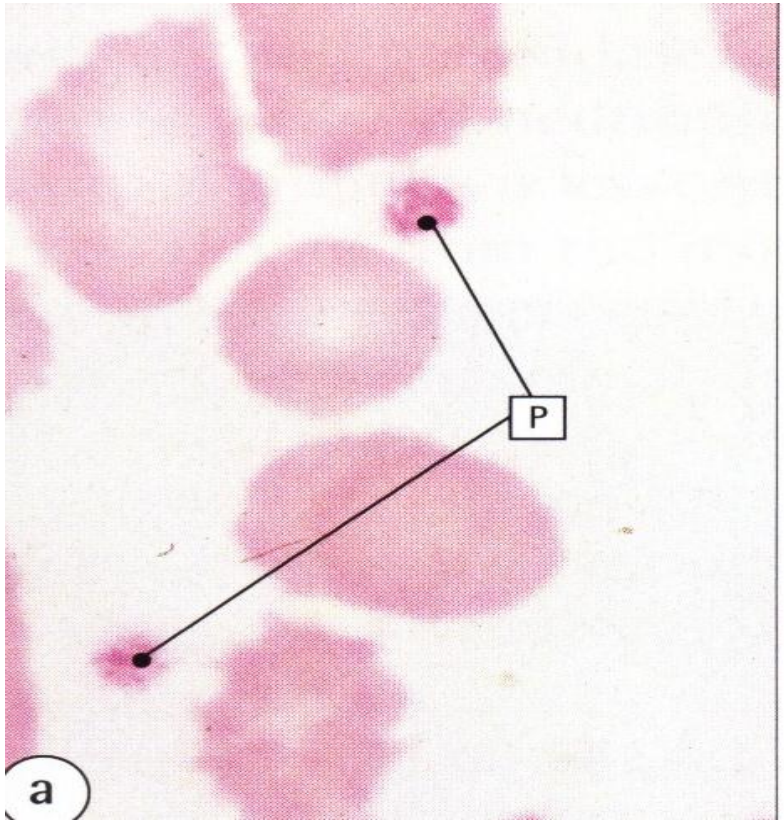
Micrograph showing the abnormal round convex-shaped blood cell of hereditary spherocytosis.

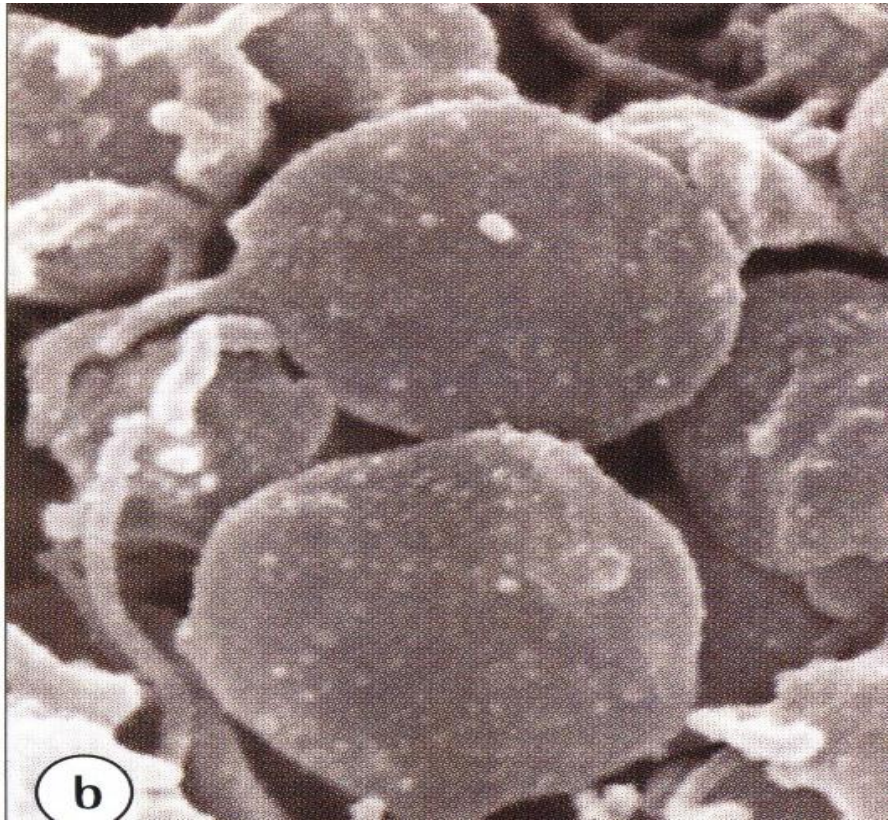
HEREDITARY SPHEROCYTOSIS

RED BLOOD CELLS:

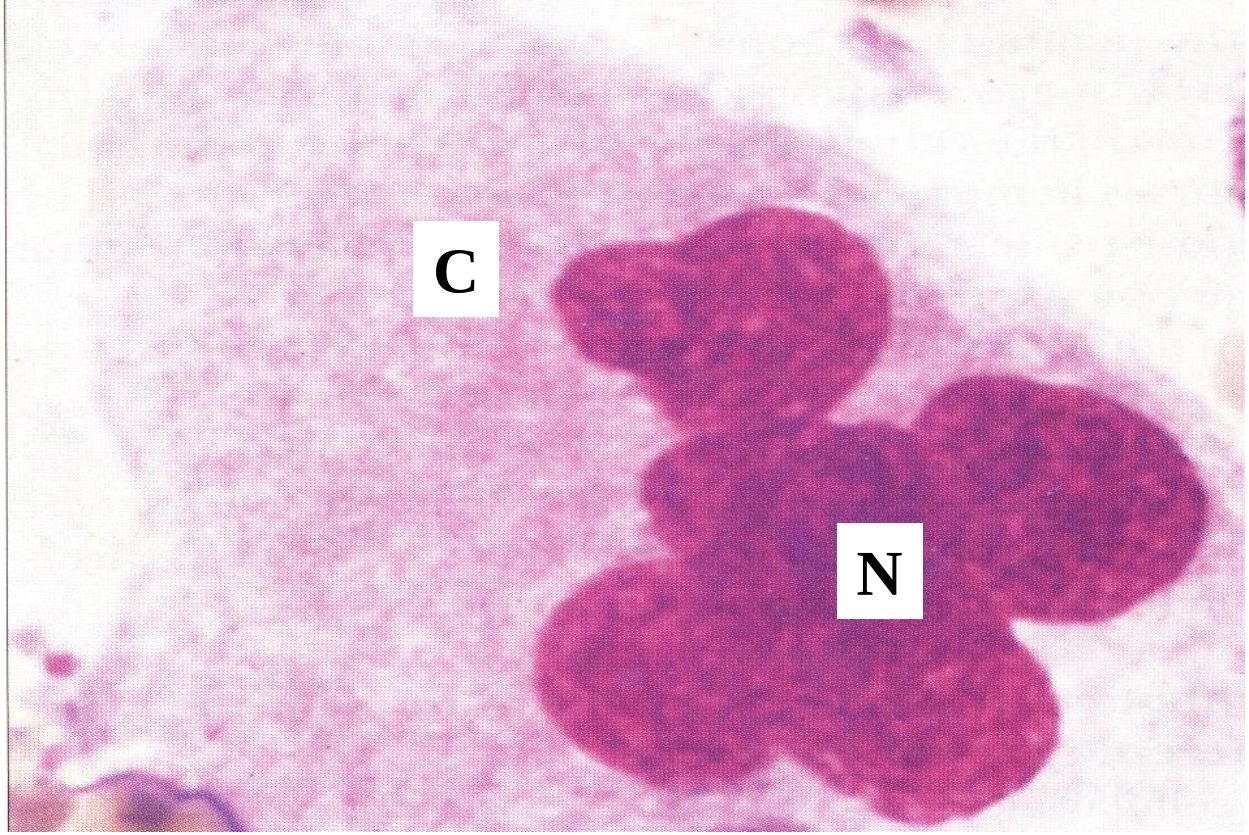
- biconcave shape for high surface area/volume ratio
- main function is oxygen carriage
- contain hemoglobin
- have no cell organelles
- cell membrane is braced by an actin-containing cytoskeleton which maintains shape.

PLATELETS





a)Platelets are 1.5-3.5 mcm in diameter in peripheral blood.
b)Scanning electronmicrograph showing the smooth disk shape of an inactive platelet. The surface canalicular pores (see below) cannot be seen at this magnification. The platelet plasmalemma has numerous receptor molecules and a thick glycocalyx (15-20 nm) that includes cell adhesion molecules for platelet adhesion.



Megakaryocyte, H & E

Platelets are small cell fragments derived from megakaryocytes and are important in hemostasis.

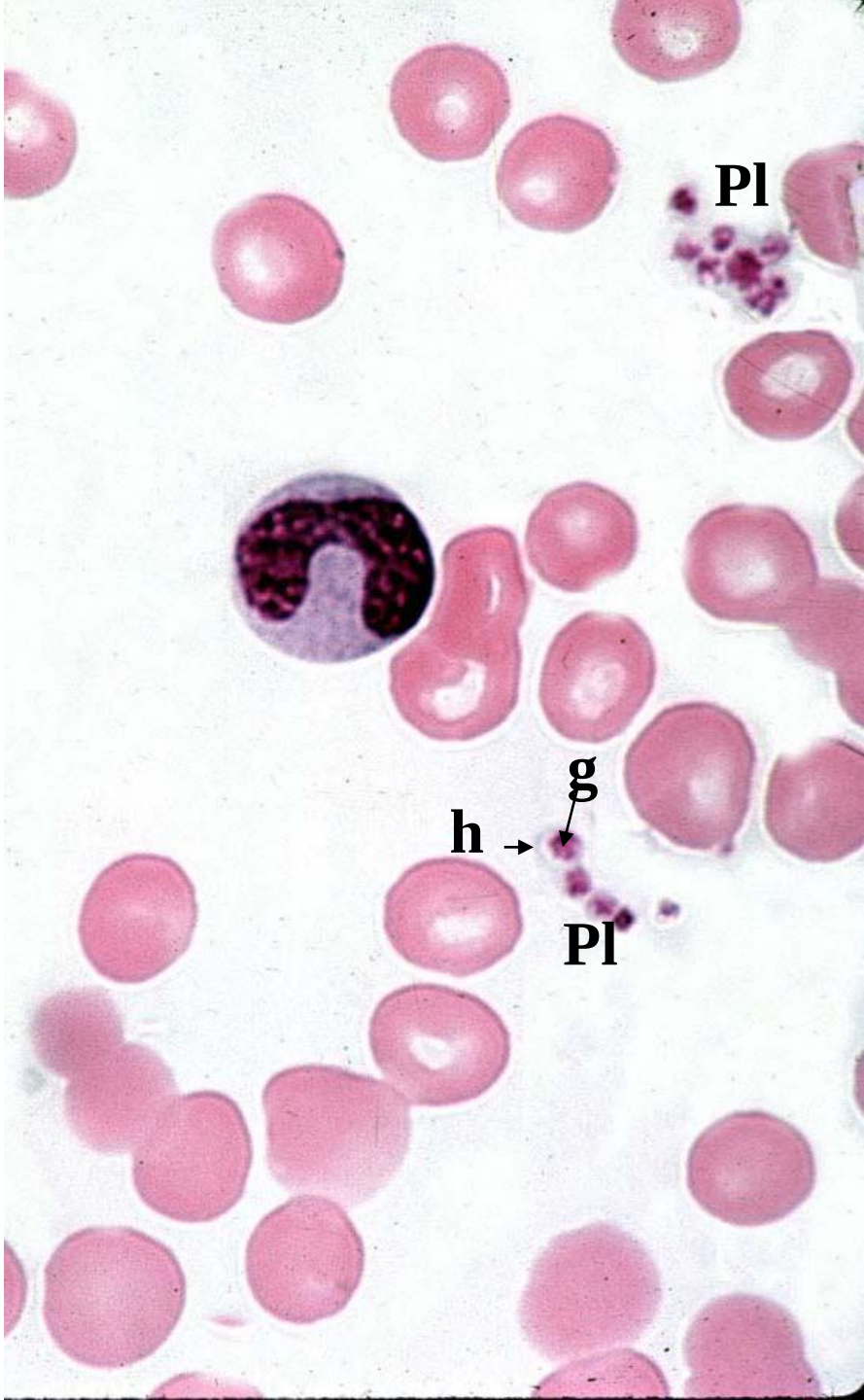
Megakaryocytes are huge polyploid cells 30-100 mcm in diameter, with a large irregular multilobular nucleus (N) which contains dispersed chromatin, and is devoid of nucleoli. Their extensive cytoplasm (C) is filled with fine basophilic granules reflecting their profusion of cytoplasmic organelles.

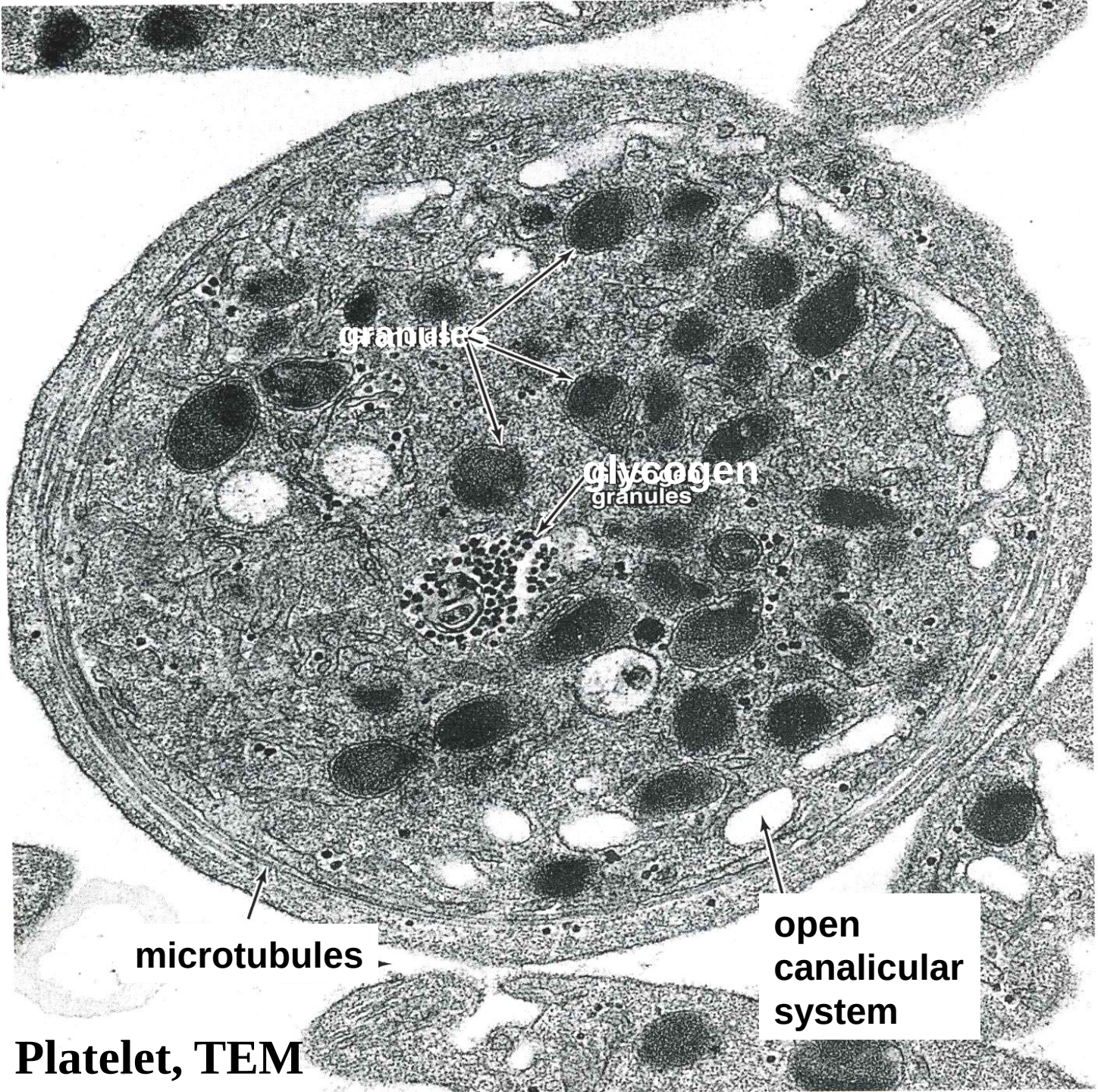
With light microscopy the cell margin is often difficult to define clearly due to the presence of numerous disaggregating platelets, cytoplasmic processes, ruffles and blebs.

PLATELETS

Platelets (P) (thrombocytes) are small, disk-shaped, anuclear cells, and are formed by the cytoplasmic fragmentation of huge precursor cells (megakaryocytes) in the bone marrow. Platelets contain mitochondria, microtubules, glycogen granules, occasional Golgi elements and ribosomes, as well as enzyme systems for aerobic and anaerobic respiration.

The platelets (Pl) possess a central dense region, the granulomere (g), and a peripheral light region, the hyalomere (h). The white blood cell in the center of the field is a monocyte (M). Their most conspicuous organelles, are their granules (4 types) located in the granulomere. Platelets also contain two tubular systems (dense and surface opening).



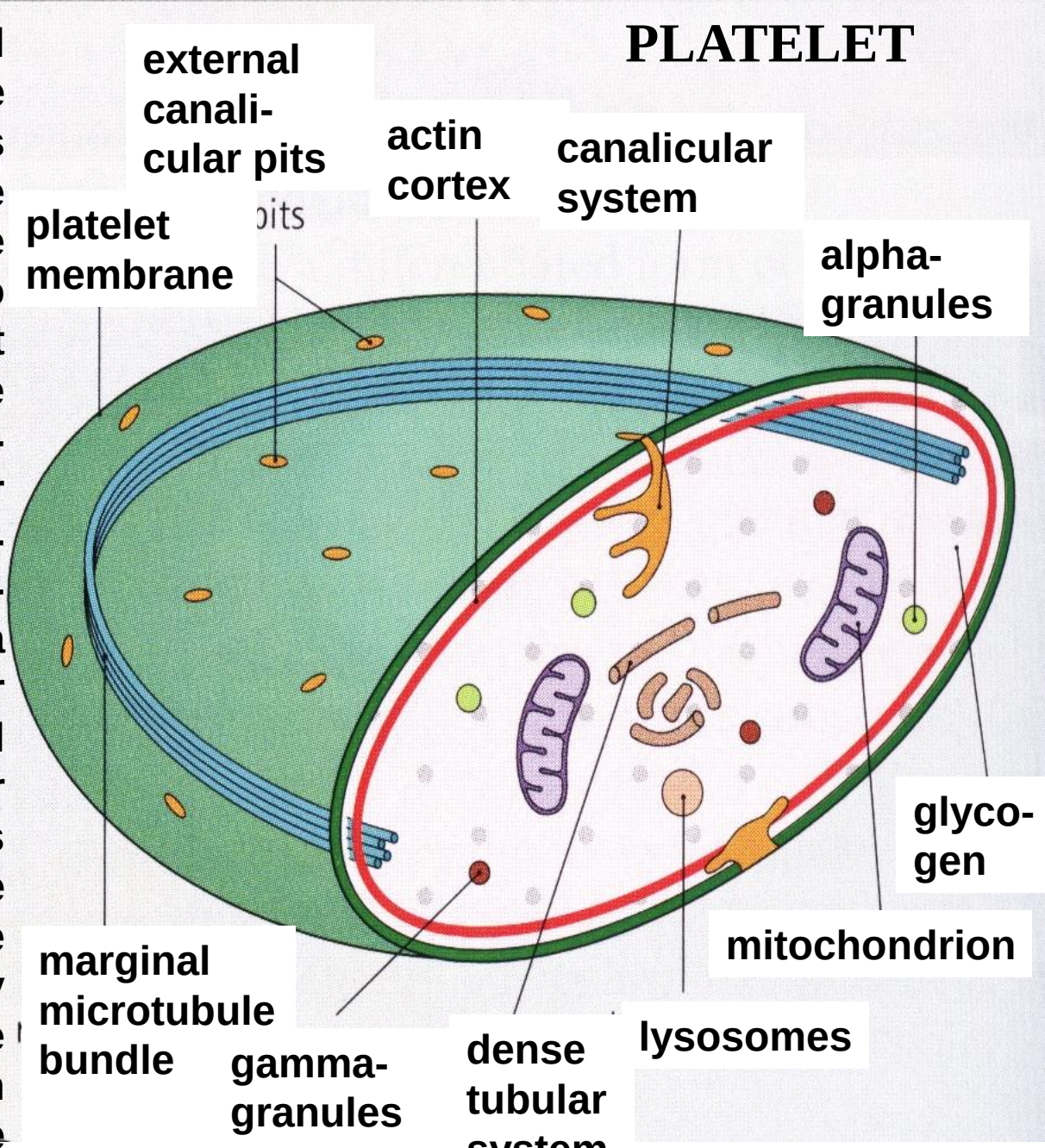


Platelet, TEM

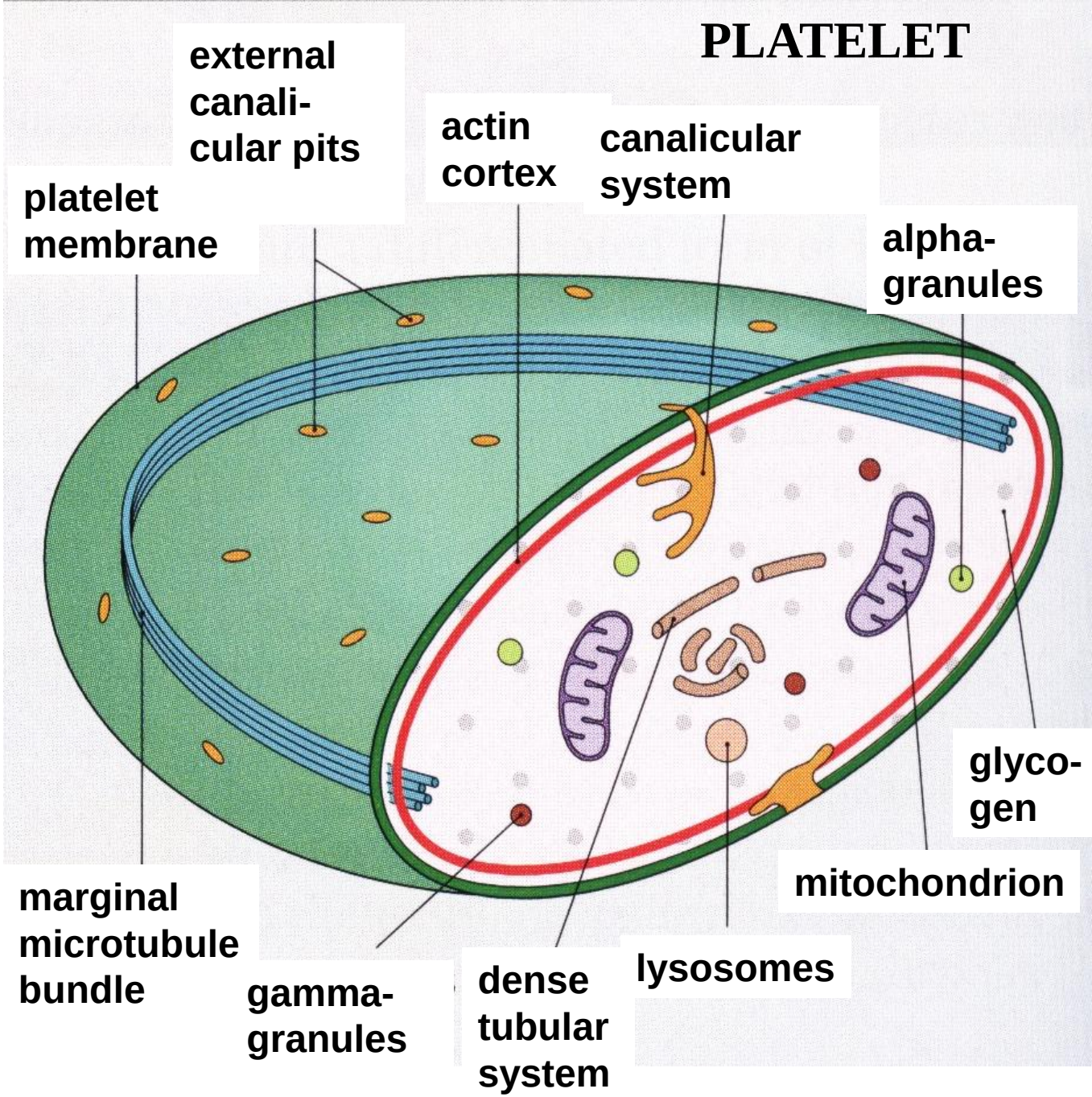
Electron micrograph displays 10-15 microtubules arranged parallel to each other and forming a ring within the hyalomere. The microtubules assist platelets in maintaining their discoid morphology.

Associated wiht the marginal bundle of microtubules are actin & myosin monomertes which can rapidly assemble forming a contractile apparatus. In addition two tubular systems are present in the hyalomere, the surface-opening (connecting) and dense tubular system. Platelet cell membrane, contains many external pits which connect a surface-opening canalicular system with the external environment.This canalicular system secretes the contents of the alpha granule, while contractile proteins in the actin cortex (previously called thrombosthenin), are involved in clot retraction and extrusion of granule contents.

PLATELET



Microtubules of the marginal microtubule bundle polymerize into component filaments at the onset of platelet aggregation.
Deep to the marginal band of microtubules and also scattered throughout the cytoplasm is the dense tubular system (DTS) consisting of narrow membranous tubules containing a homogenous electron-opaque substance.



GRANULES OF THE PLATELETS

- Alpha granules are variable in size and shape and contain important groups of proteins (platelet exclusive proteins, coagulation factors and other proteins);
- Dense granules (gamma granules) are electron-dense and appear to contain serotonin, which is not synthesized by platelets but absorbed from the plasma;
- Lambda-granules (lysosomes) are membrane-ound vesicles containing lysosomal enzymes (e.g., acid hydrolases)
- Peroxisomes are few in number and have peroxidase (probably catalase) activity.

PLATELET FUNCTION

If the endothelial lining of the blood vessel is disrupted and platelets come in contact with the subendothelial collagen, they become activated, release the contents of their granules, adhere to the damaged region of the vessel wall (platelet adhesion), and adhere to each other (platelet aggregation). Interactions of the tissue factors, plasma-borne factos, and platelet-drived factors form a blood clot.

FUNCTIONS OF THE PLATELETS

Platelets aggregate together and degranulate in hemostasis. Platelets are essential to normal hemostasis, undergoing aggregation in the process. Hemostasis is achieved by the following steps:

After loss of the lining endothelium of blood vessels, platelets adhere to exposed collagen by interacting with glycoprotein receptors for Von Willebrand factor attached to it.

Platelet actin, myosin and microtubules cause reversible platelet moulding and adhesion along a broad surface. They then irreversibly release the contents of their granules through the canalicular system, in a secretion reaction, and synthesize thromboxane.

Thromboxane, ADP and Ca++ ions mediate adhesion of other platelets. Platelet phospholipids (with Ca++ ions) activate the blood clotting cascade, leading to the formation of fibrin.

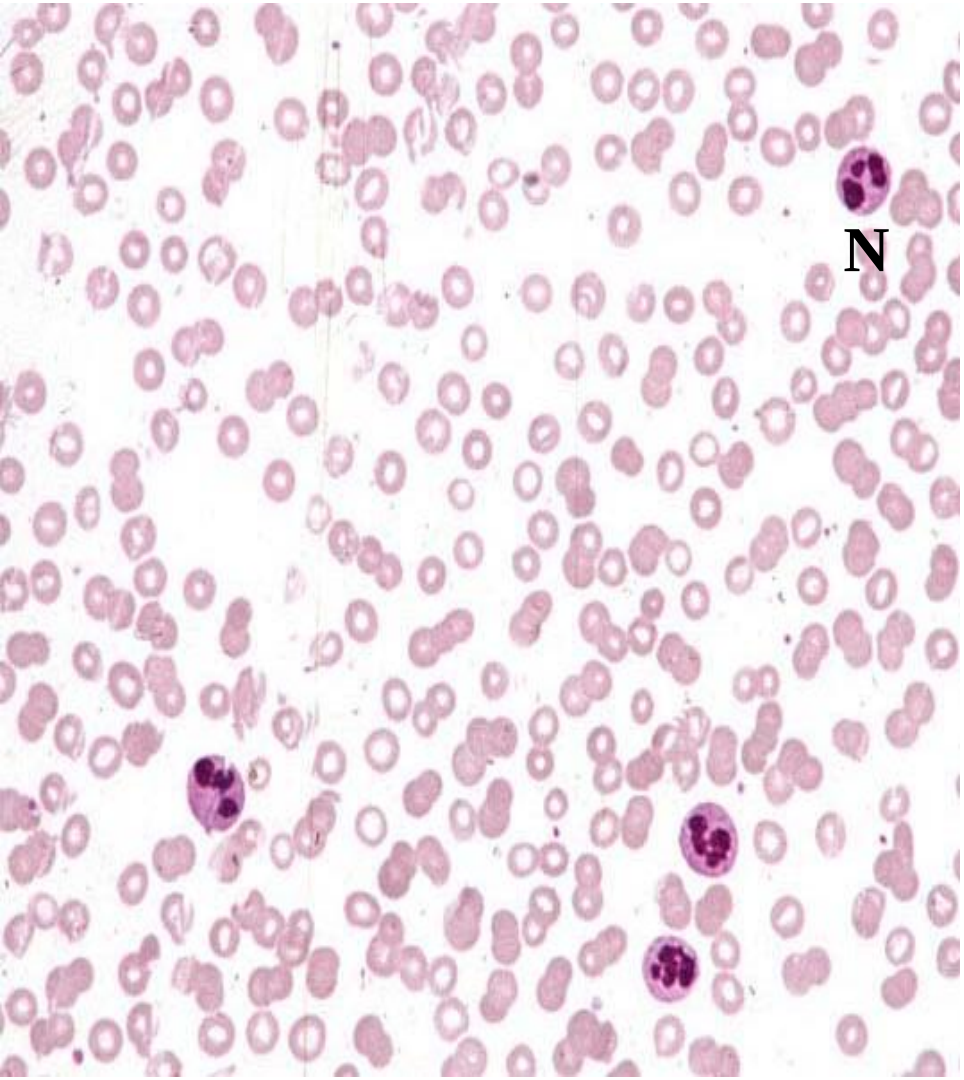
Platelet Tubules and Granules

Structure (size)	Location	Contents	Function
Surface-opening tubule system	Hyalomere		Expedites rapid uptake and release of molecules from activated platelets
Dense tubular system	Hyalomere		Probably sequesters calcium ions to prevent platelet “stickiness”
α-Granules (300-500 nm)	Granulo-mere	Fibrinogen, platelet-derived growth factor, platelet thromboplastin, thrombospondin, coagulation factor	Contained factor facilitate vessel repair, platelet aggregation, and coagulation of blood
δ-Granules (dense bodies) (250-300 nm)	Granulo-mere	Calcium, ADP, ATP, serotonin, histamine, pyrophosphatase	Contained factors facilitate platelet aggregation and adhesion, as well as vasoconstriction
λ-Granules (lysosomes) (200-250 nm)	Granulo-mere	Hydrolytic enzymes	Contained enzymes aid clot resorption

CLINICAL CORRELATIONS

In patients with thrombocytopenia, the blood level of platelets is decreased. The condition becomes serious when the platelet level is below 50,000/mm3. Bleeding is common in these patients because of the failure of platelets to seal over microscopic breaches in vessel walls resulting from minor trauma. The bleeding is generalized and occurs from small vessels, resulting in purplish splotches on the skin. Other manifestations in the skin include larger bruise-like patches (ecchymoses).

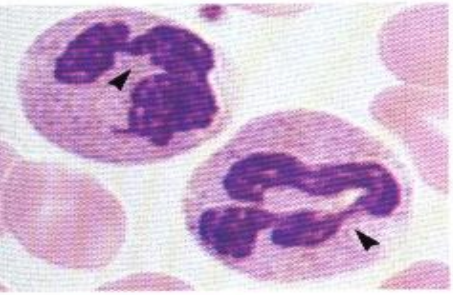
This condition is believed to be an autoimmune disease, in which antibodies are formed to one's own platelets and these antibodies destroy the platelets.



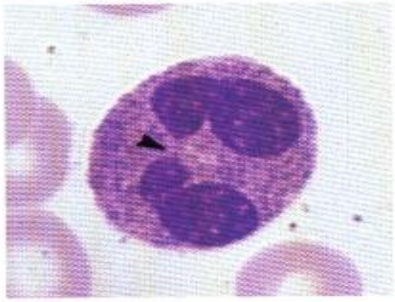
Blood smear. Human. Wright stain.

This normal blood smear presents erythrocytes (RBC), neutrophils (N) and platelets. RBC far outnumber the platelets and they, in turn, are much more numerous than white blood cells (WBC). Since neutrophils constitute the highest percentage of WBC, they are the ones most frequently encountered of the WBC population.

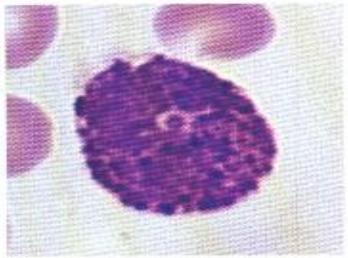
WBC



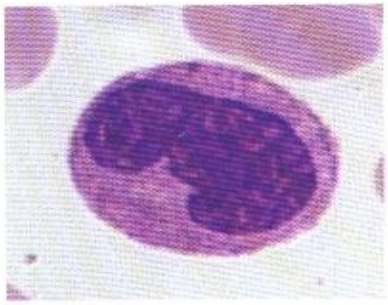
Neutrophils display a somewhat granular cytoplasm and lobulated (arrowhead) nuclei.



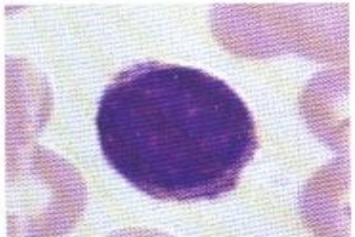
Eosinophils are recognized by their large pink granules and their sausage-shaped nucleus. Observe the slender connecting link(arrowhead) between the two lobes of the nucleus.



Basophils are characterized by their dense dark large granules

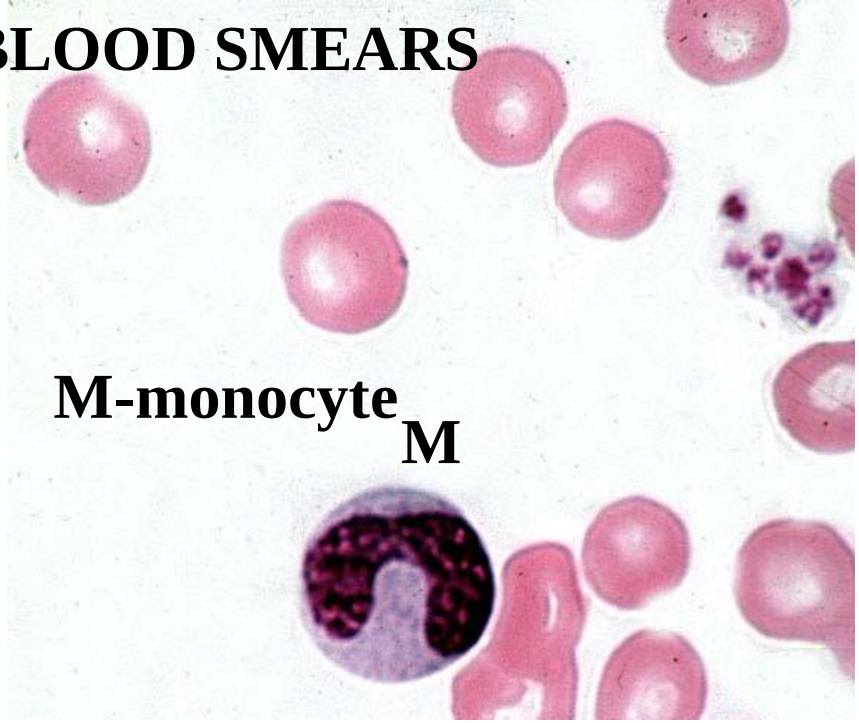


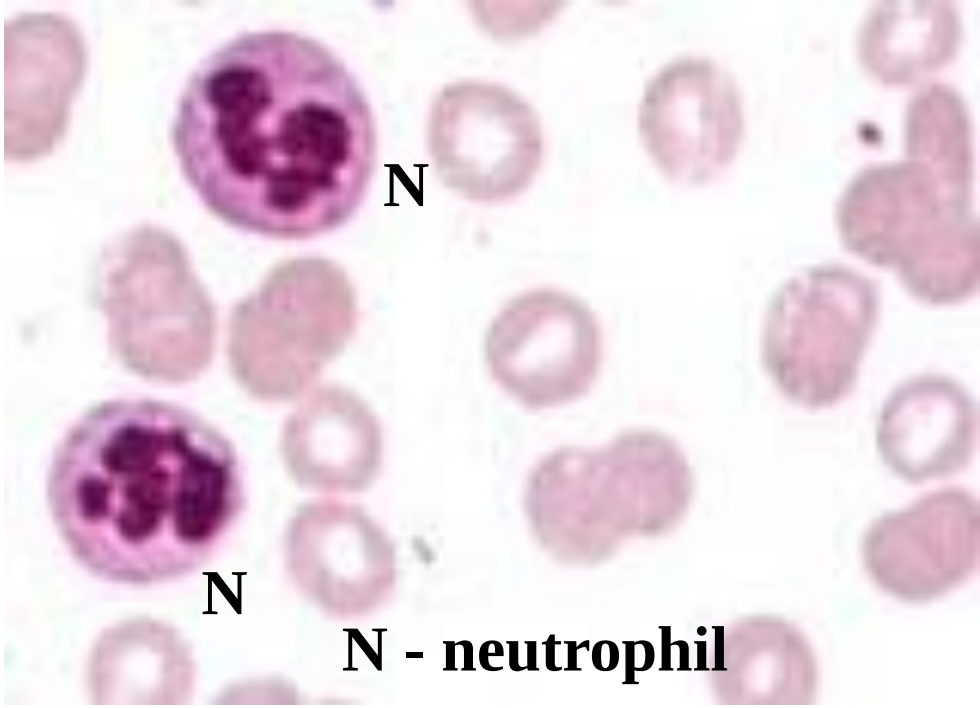
Monocytes are characterized by their large size, accentric kidney-shaped nucleus, and lack of specific granules.



Lymphocytes are small cells that possess a single, large, acentrically located nucleus and a narrow rim of light blue cytoplasm.

BLOOD SMEARS

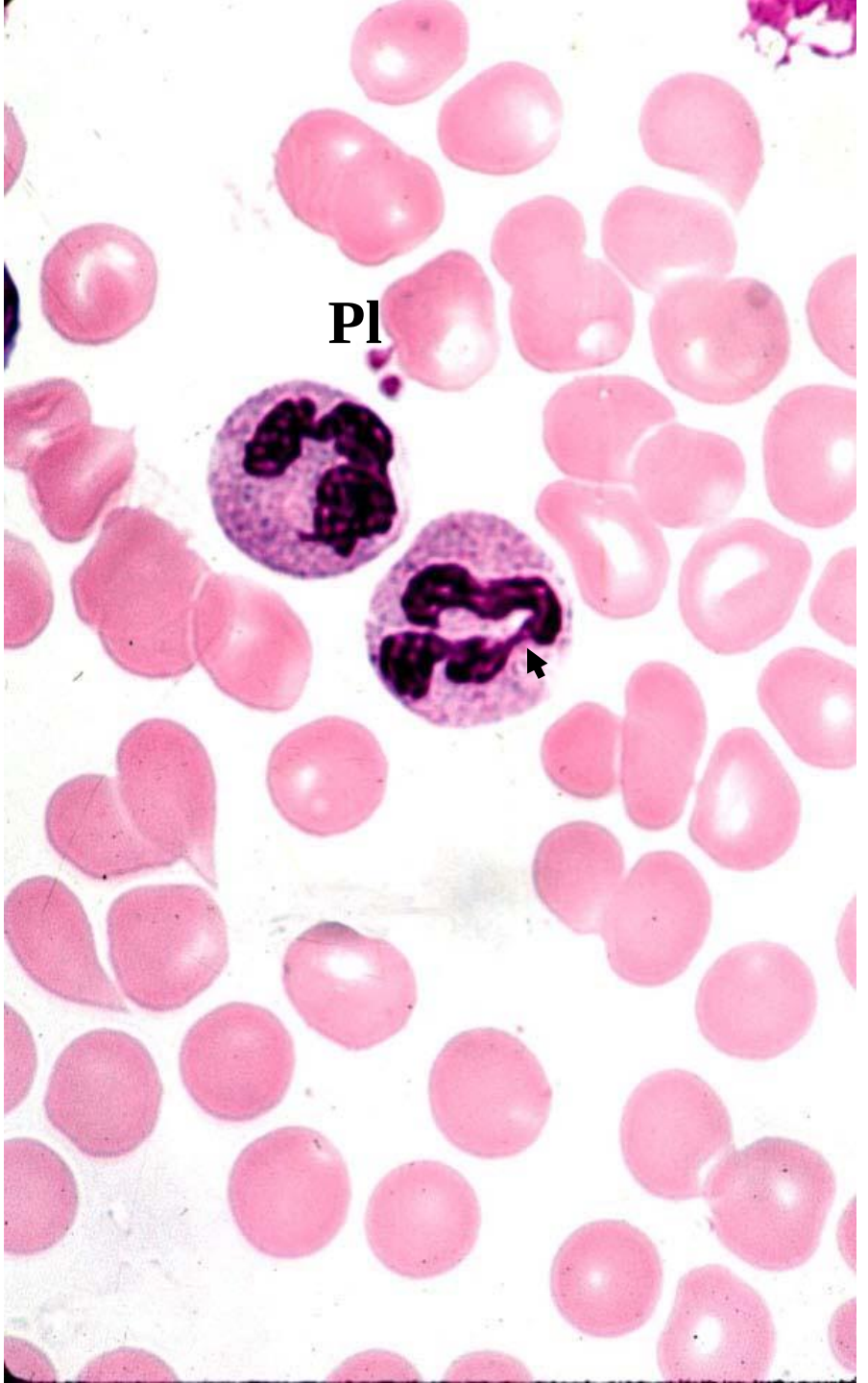




According to the type of granules in their cytoplasm and the shape of their nuclei, leukocytes are divided into two groups: granulocytes (polymorphonuclear cells because of the multilobed nuclei) and agranulocytes (mononuclear cell). Both granulocytes and agranulocytes are spherical while suspended in the blood plasma, but some become ameboid after leaving the blood vessels and invading the tissues. Basophils, eosinophils and neutrophils are known as granulocytes because their cytoplasm contains prominent granules, and may also be referred to as myeloid cells because of their origin from the bone marrow, while agranulocytes include lymphocytes (lymphoid cells) and monocytes (myeloid cells). Agranulocytes are found mainly in tissues such as lymph node or spleen. In the tissues monocytes transform into macrophages and basophils become mast cells.

Neutrophils. Human. x 1325.

Neutrophils, the most populous of the leukocytes, are 9-12 micrometers in diameter, displaying a light pink cytoplasm housing many azurophilic and smaller specific granules. The specific granules do not stain well, hence the name of these cells. The nucleus is dark blue, coarse, and multilobed with most being two- to three-lobed with thin connecting strands (arrowheads). Note the numerous RBC and the two platelets (Pl).



MATURATION OF NEUTROPHILS

Immature neutrophils that have recently entered the blood circulation have a non-segmented nucleus in the shape of a horse-shoe (band or stab forms). An increased amount of band neutrophils in the blood indicates a higher production of neutrophils, probably in response to bacterial infection. Neutrophils with more than 5 lobes are called hypersegmented and are typically old cells. In some pathologic conditions young cells appear with 5 or more lobes.

4

Neutrophilic metamyelocyte

0-0.5%

5

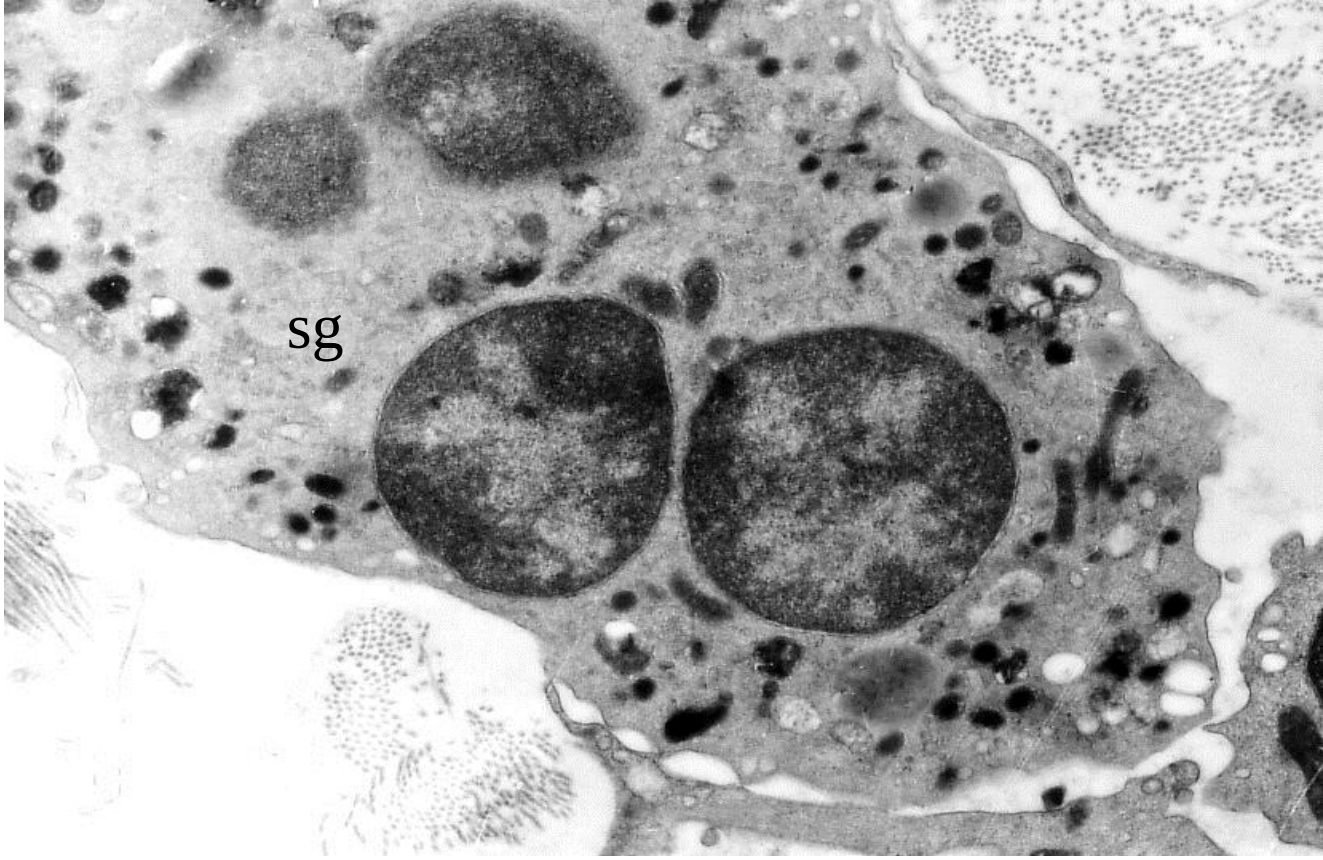
Neutrophilic stab cell

3-5%

Neutrophil

40-70%

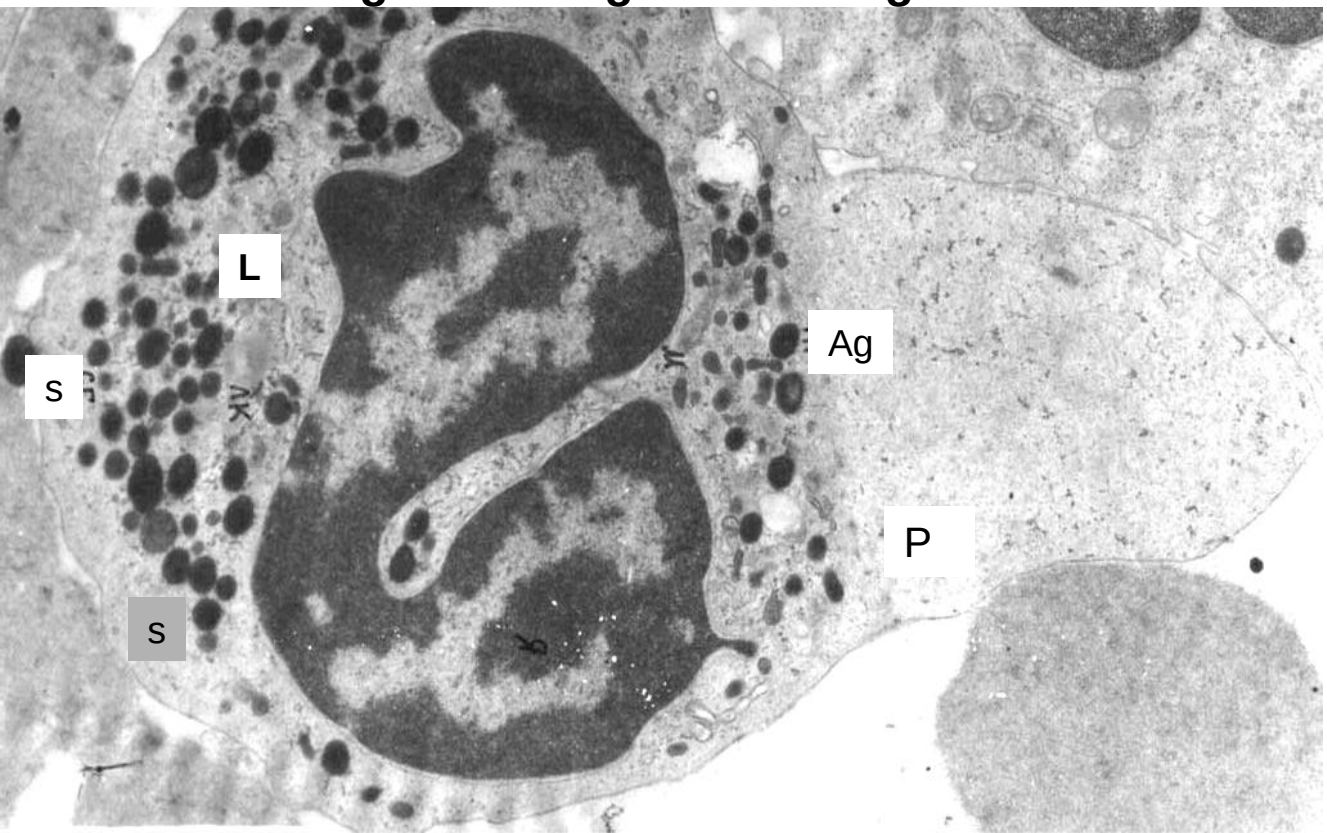
NEUTROPHIL, TEM



Neutrophils are the most abundant of the circulating white cells. They circulate in a resting state but, with appropriate activation, leave the blood and enter tissues where they become highly motile, phagocytic cells. Their primary function is to ingest and destroy invading microorganisms in tissues. They play a central role in the early stages of the acute inflammatory response to tissue injury and are the major constituent of pus.

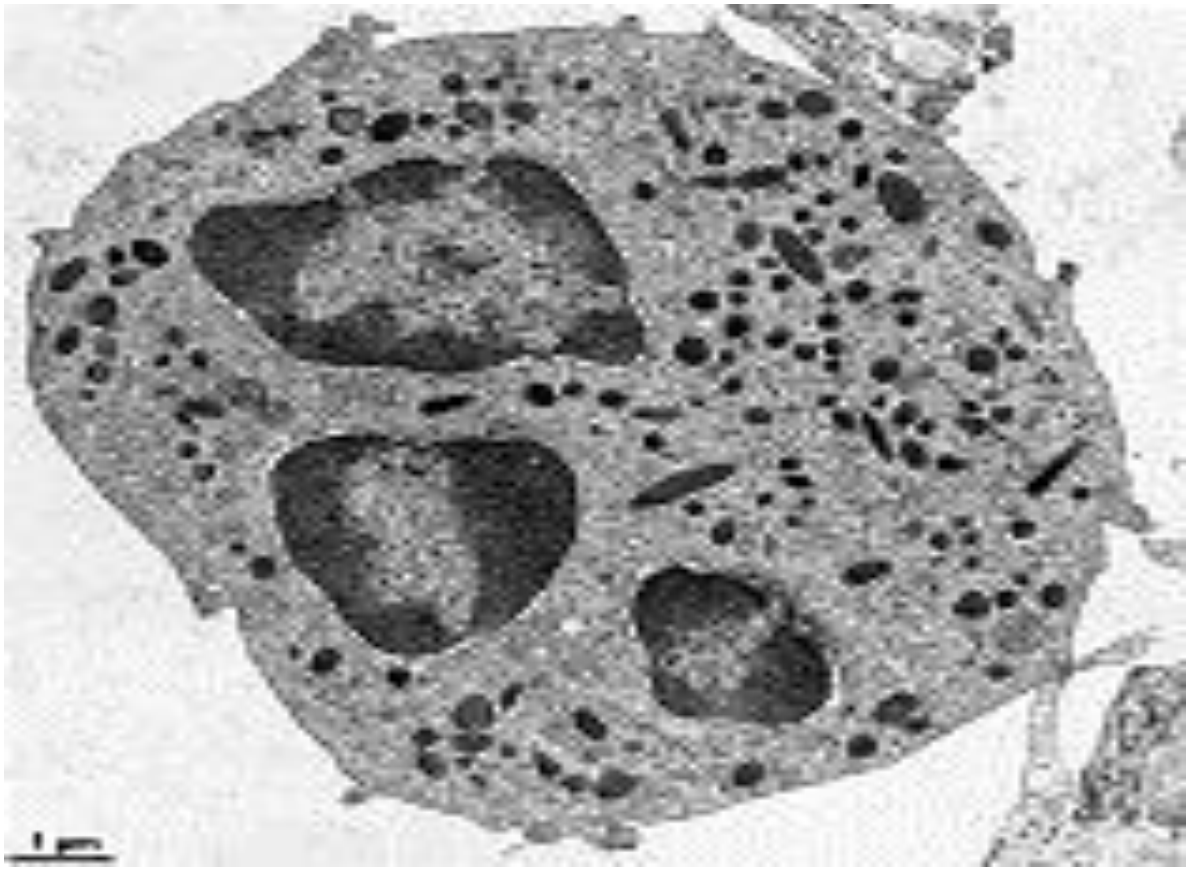
Neutrophils contain three types of granules.

Primary granules are similar to lysosomes in other cells. They are first granules to appear during neutrophil formation, but as the cell matures, their number falls with respect to secondary granules making them difficult to see with light microscopy. With EM, they are large and electron-dense. As with lysosomes, primary granules contain acid hydrolases, but in addition they also contain antibacterial and digestive substances, most notably myeloperoxidease, which can be detected by peroxidease stain, and elastase. Myeloperoxidase is therefore a useful light microscopic marker not only for these granules, but also in establishing cell lineage in the diagnosis of leukemias.



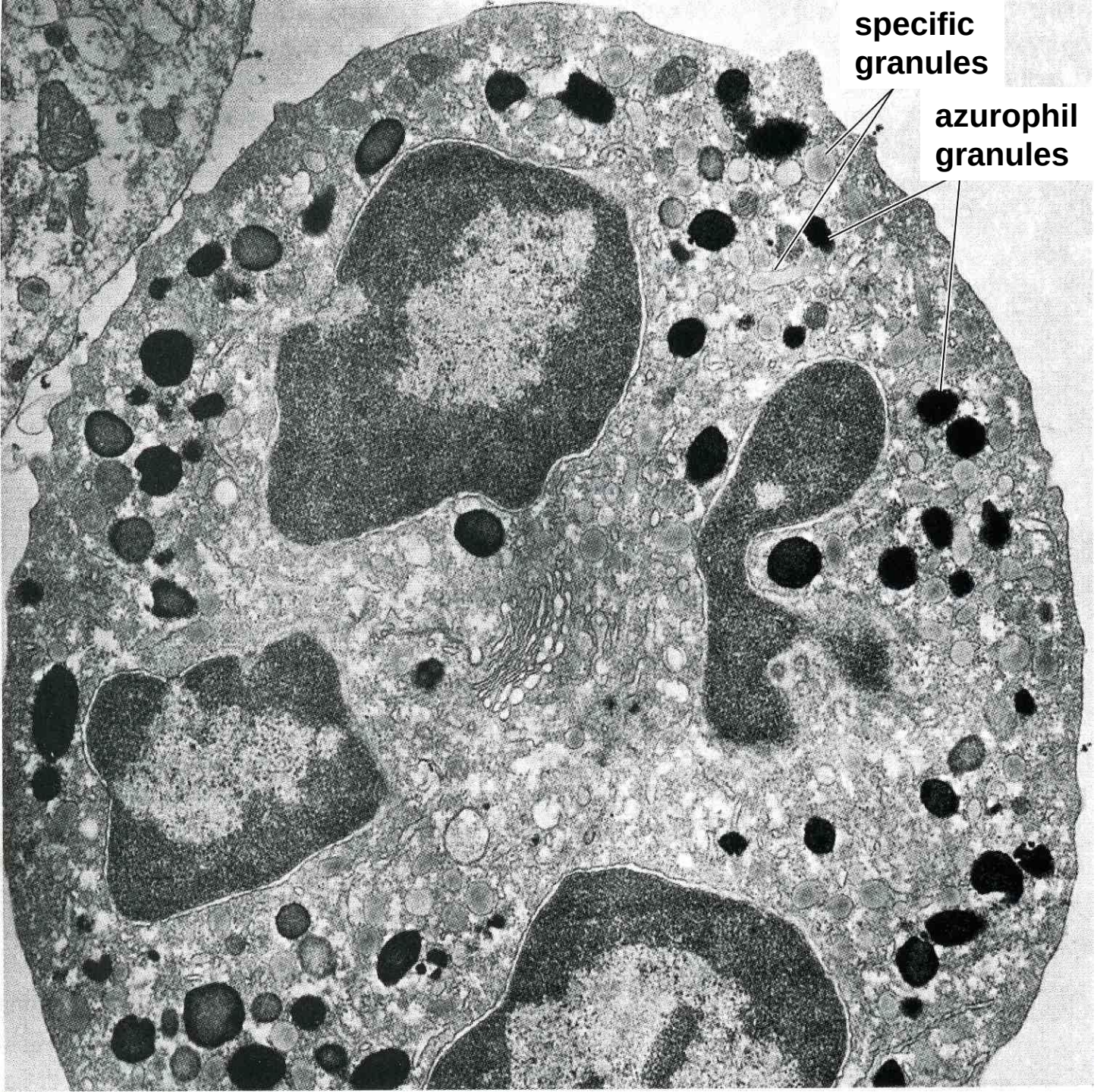
Secondary granules are specific to neutrophils and twice as numerous as primary granules. With a diameter of 0.2-0.8 mcm (i.e. smaller than primary granules), they are barely visible with light microscope. They contain collagenase (type IV), lysozyme, phagocytin, etc.

Neutrophil, TEM, x10,000



Ultrastructural studies have shown secondary granules to be of variable size, shape and density and to contain substances involved in the mobilization of inflammatory mediators and complement activation. These substances are secreted into the extracellular environment.

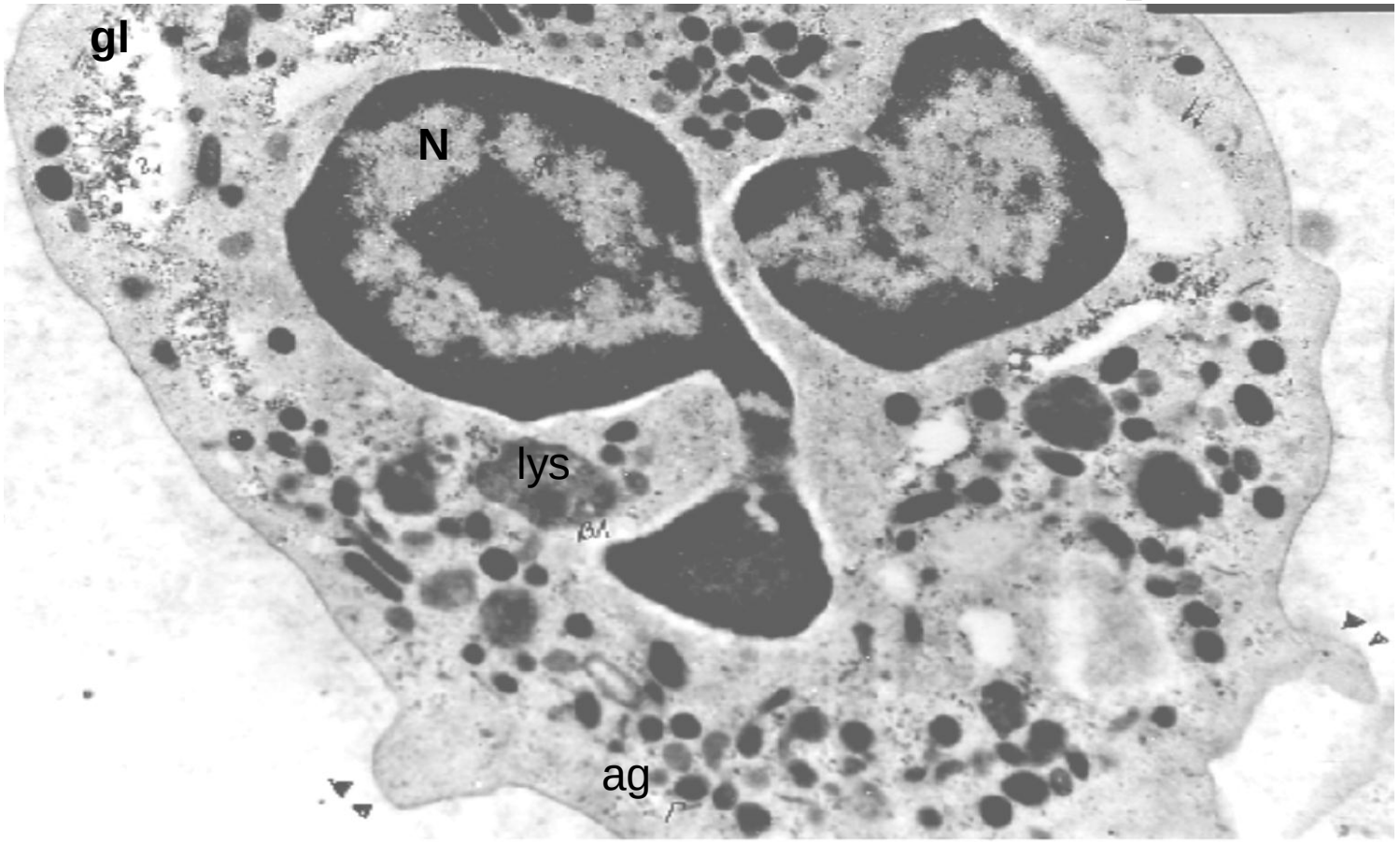
Tertiary granules have only recently been described and contain enzymes (e.g.gelatinase) secreted into extracellular environment. They also insert some glycoproteins into cell membranes, and this may promote cellular adhesion and hence may be involved in the phagocytic process.



NEUTROPHIL,TEM

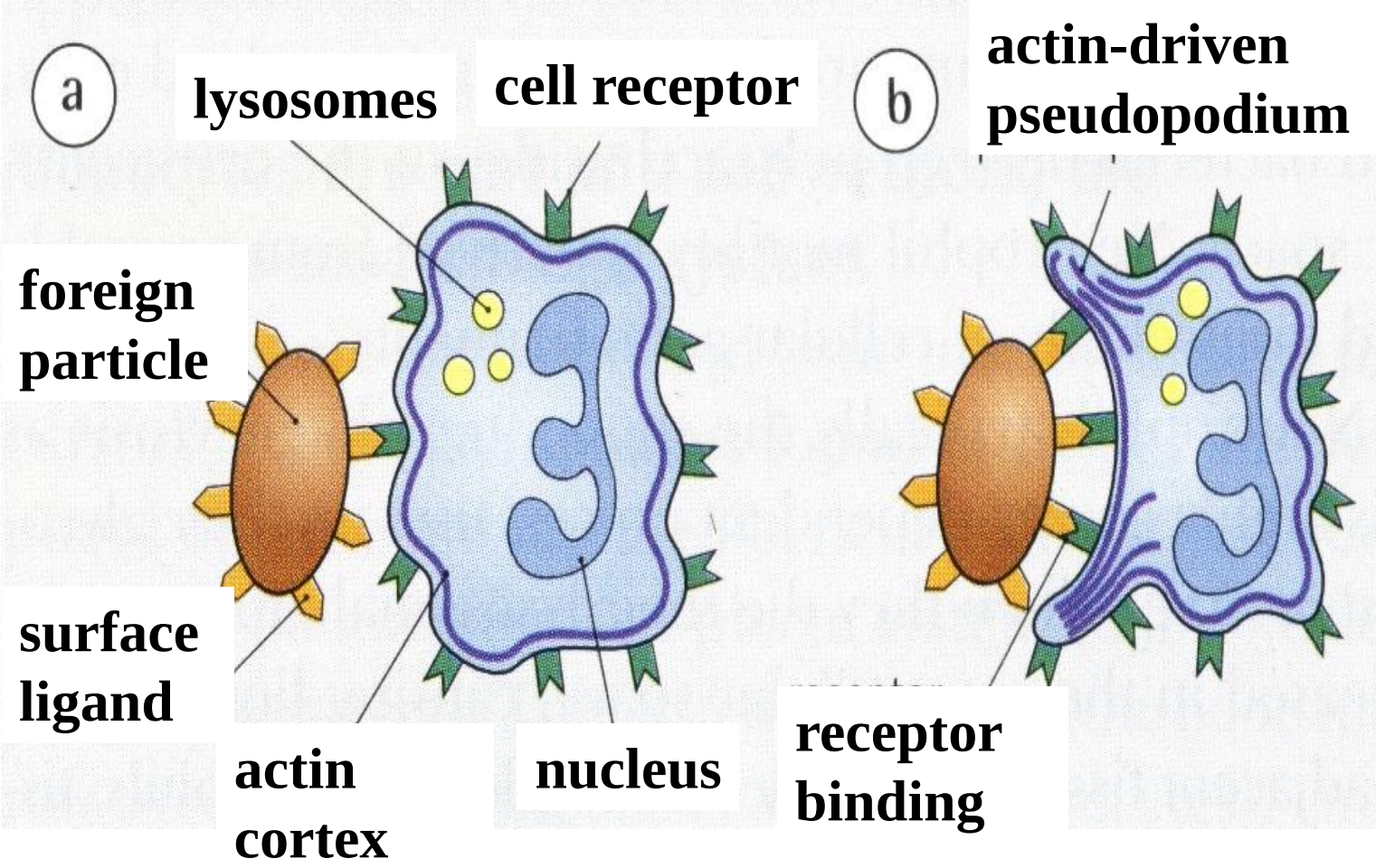
The neutrophil cytoplasm contains few organelles apart from granules. There are only a few scattered profiles of rough endoplasmatic reticulum and free ribosomes, with the remnants of the Golgi complex involved in granule packaging earlier in development. While mitochondria (m) are also few, they provide about 50% of energy needs.

Once activated, neutrophils need to be able to operate in devascularized tissue where oxygen and glucose may be in short supply. They therefore contain abundant glycogen for anaerobic metabolism, which occurs mainly via the glycolytic pathway. Energy production may also take place via the hexose monophosphate shunt, but this is used to generate microbicidal oxidants rather than for general cellular upkeep.



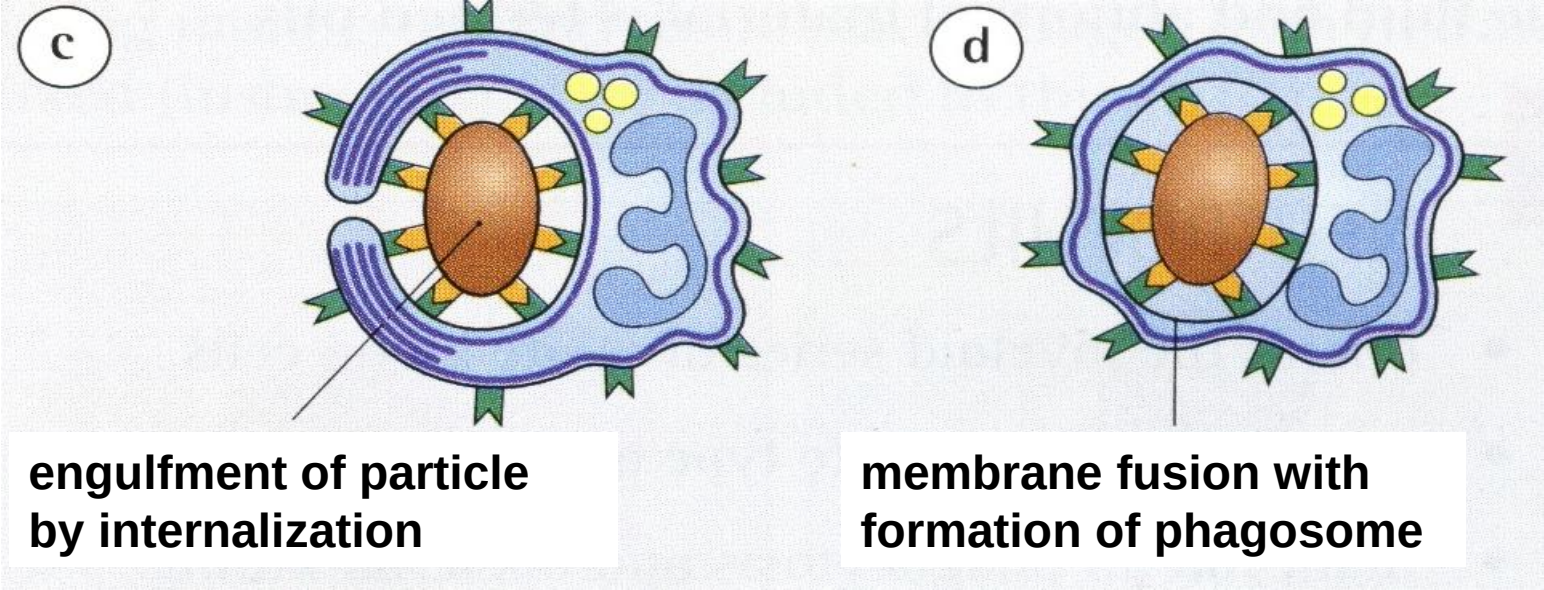
NEUTROPHILS ARE ADAPTED FOR ANAEROBIC METABOLISM

Neutrophil Phagocytosis



a) Neutrophils have membrane receptors, mainly for the Fc portion of antibodies, complement factors bound to foreign particles, and bacterial polysaccharides. Neutrophils do not phagocytose material to which they do not bind.

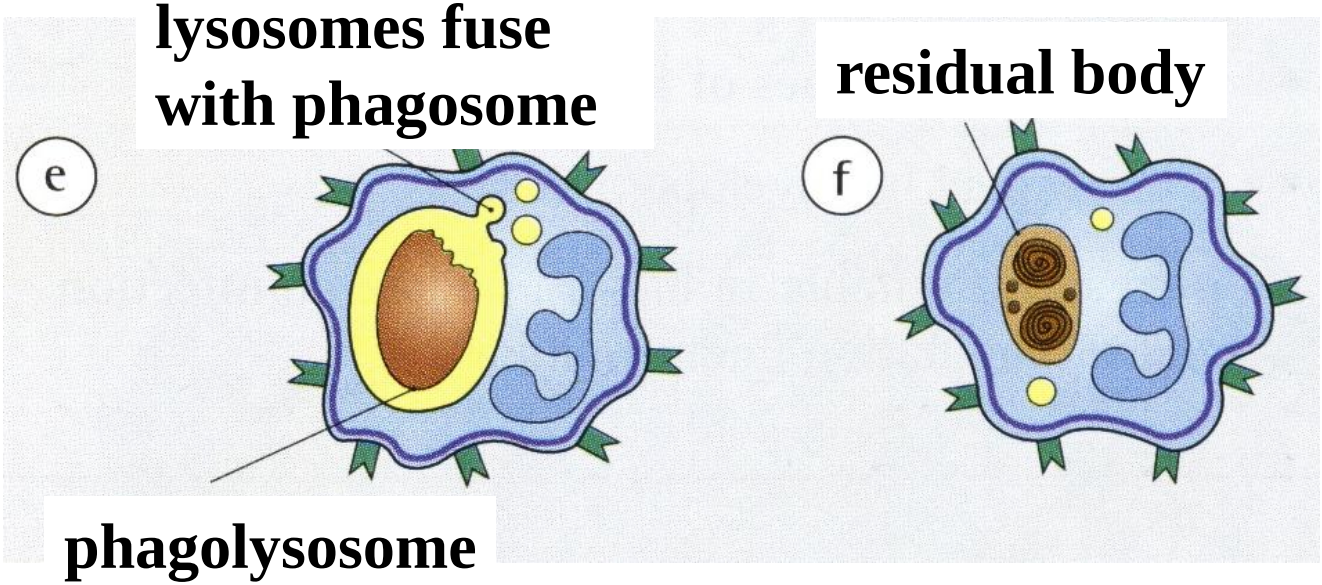
b) As the first step in phagocytosis the neutrophil binds to the abnormal particle by its specific receptors. The cell pushes out pseudopodia to surround the particle driven by assembly and disassembly of actin filaments.



NEUTROPHIL PHAGOCYTOSIS

c)The pseudopodia fuse to enclose completely the abnormal particle and from an endocytic vesicle. Special proteins probably allow final sealing of the membrane.

d)The internalized particle in the endocytotic vesicle is called phagosome.



NEUTROPHIL PHAGOCYTOSIS

e) The phagosome fuses with neutrophil granules, particularly primary granules, which discharge their contents, exposing the particle to a potent mixture of lysosomal enzymes. If the particle is a bacterium, killing is enhanced by hydrogen peroxide and superoxide generated by the enzymatic reduction of oxygen

f) Foreign particle destruction is associted with formation of a residual body containing degraded material.

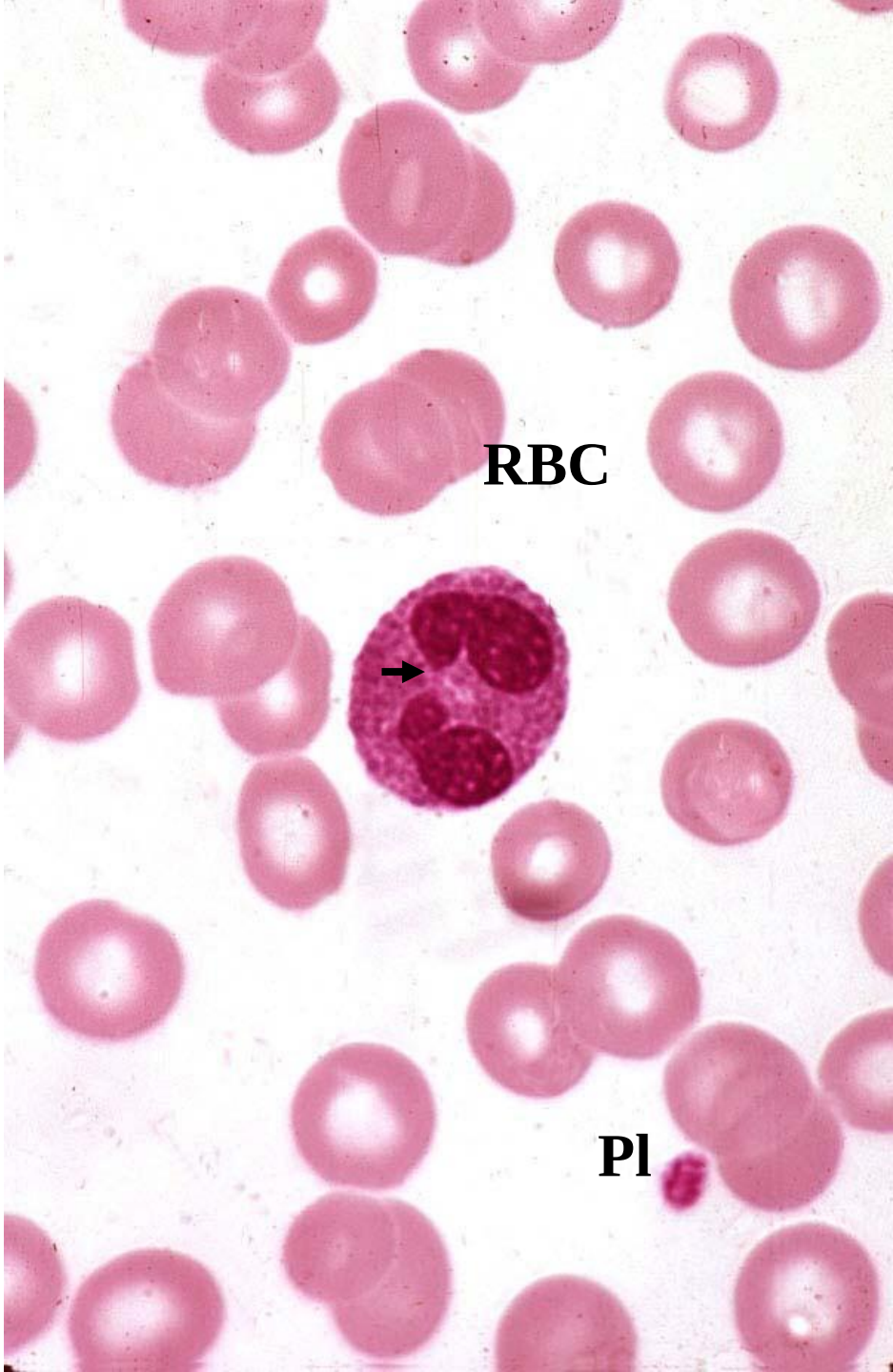
CLINICAL CORRELATIONS

During phagocytosis a burst of oxygen consumption leads to the formation of superoxide anion which is a short-lived free radical that kills the microorganisms ingested by the neutrophils.

Children with hereditary deficiency of NADPH oxidase are subject to persistent bacterial infections because their neutrophils cannot form a respiratory burst response to the bacterial challenge. Their neutrophils cannot generate superoxide, hydrogen peroxide, or hypochlorous acid during phagocytosis of bacteria.

Eosinophils. Human. x 1325.

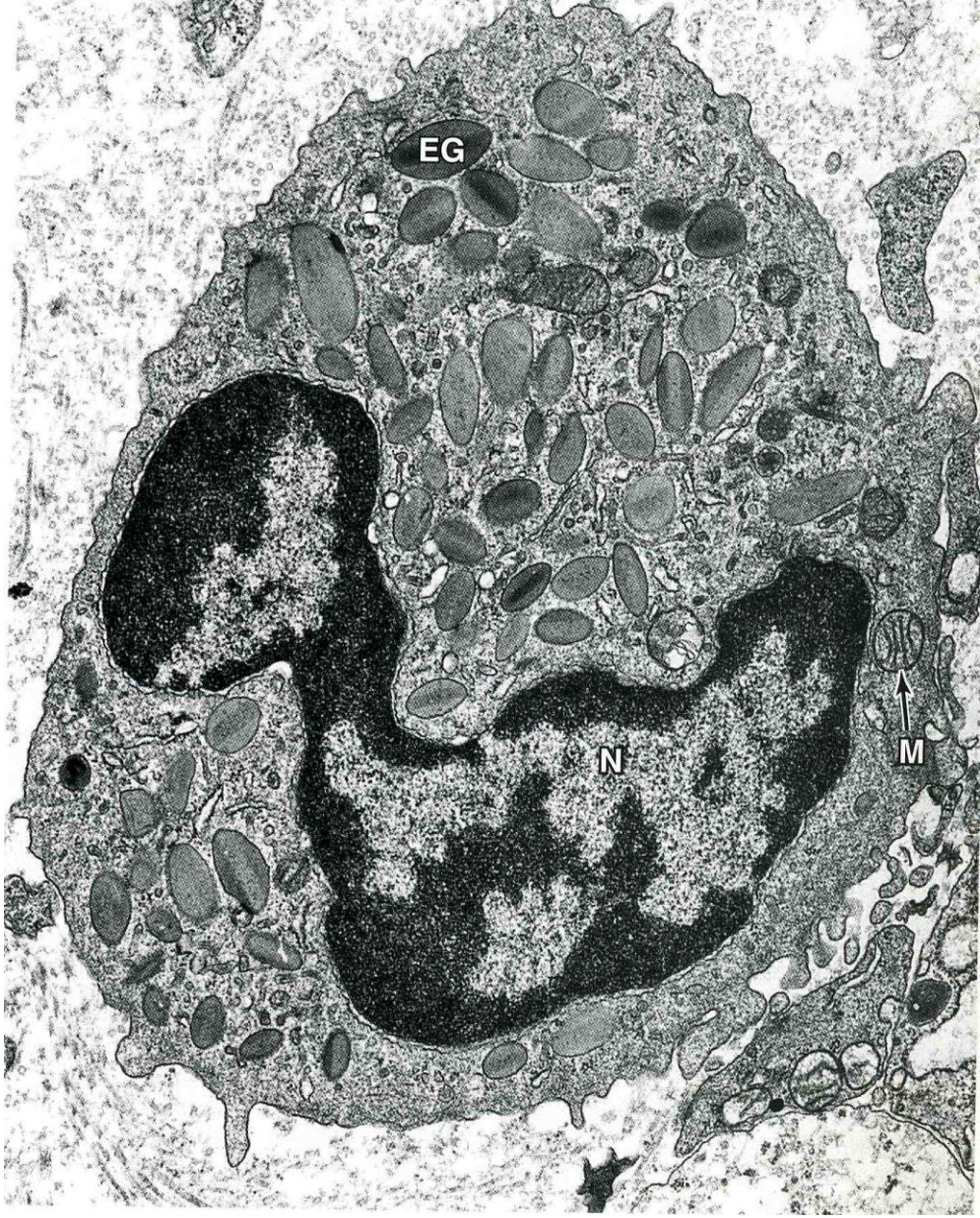
Eosinophils are 10-14 micrometers in diameter and possess numerous refractile, spherical, large, reddish-orange specific granules. Azurophilic granules are also present. The nucleus, which is brownish-black, is bilobed, resembling sausage links united by a thin connecting strand (arrowhead). Note the numerous RBC) and the small cluster of platelets (Pl).



Eosinophils contain specific granules (EG) (200 per cell) which are stained by eosin.

The specific granules contain a crystalline core (internum) that lie parallel to the long axis of the granule. It contains major basic protein with a large number of arginine residues. This protein constitutes 50% of the total granule protein and accounts for the eosinophilia of the granules. This protein functions in killing of parasituc worms such as shistosomes. The less dense material surrounding the internum is known as externum or matrix.

Azurophil granules (lysosomes like in neutrophils) contain acid hydrolases. They function both in the destruction of parasite worms and in the hydrolysis of the immune complexes internalized by eosinophils.



EOSINOPHIL, TEM

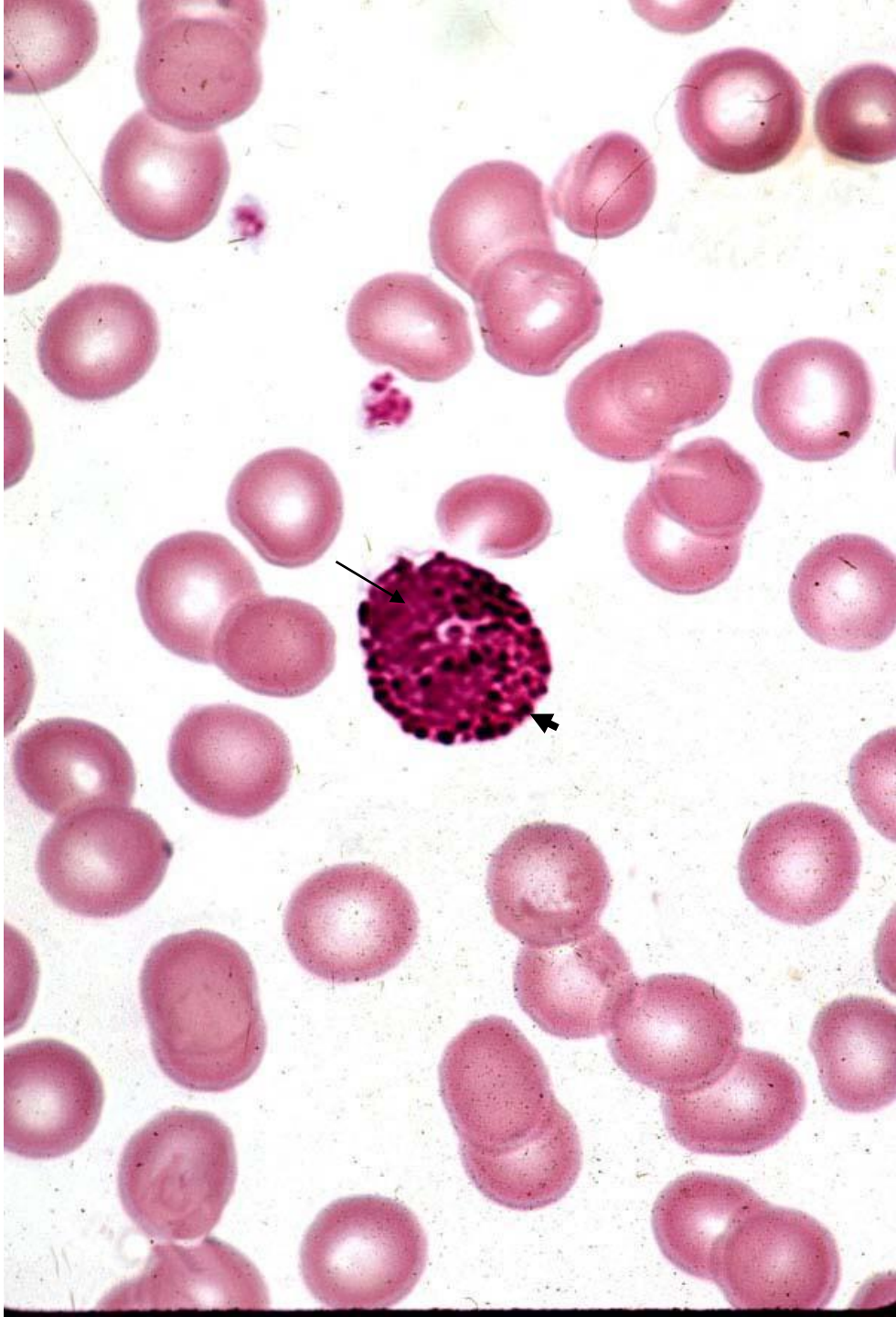
CLINICAL CORRELATIONS

Eosinophils contain IgE on its surface. Eosinophils produce substances which modulate inflammation by inactivating the leukotriens and histamine produced by the other cells. They also phagocytose antigen-antibody complexes. The inactivate and accumulate histamine.

An increase in the number of eosinophils in blood is associated with allergic reactions and helminthic (parasitic) infections. They participate in the anti-allergic reactions.

In patients getting treatment by corticosteroids the number of eosinophils is decreased.

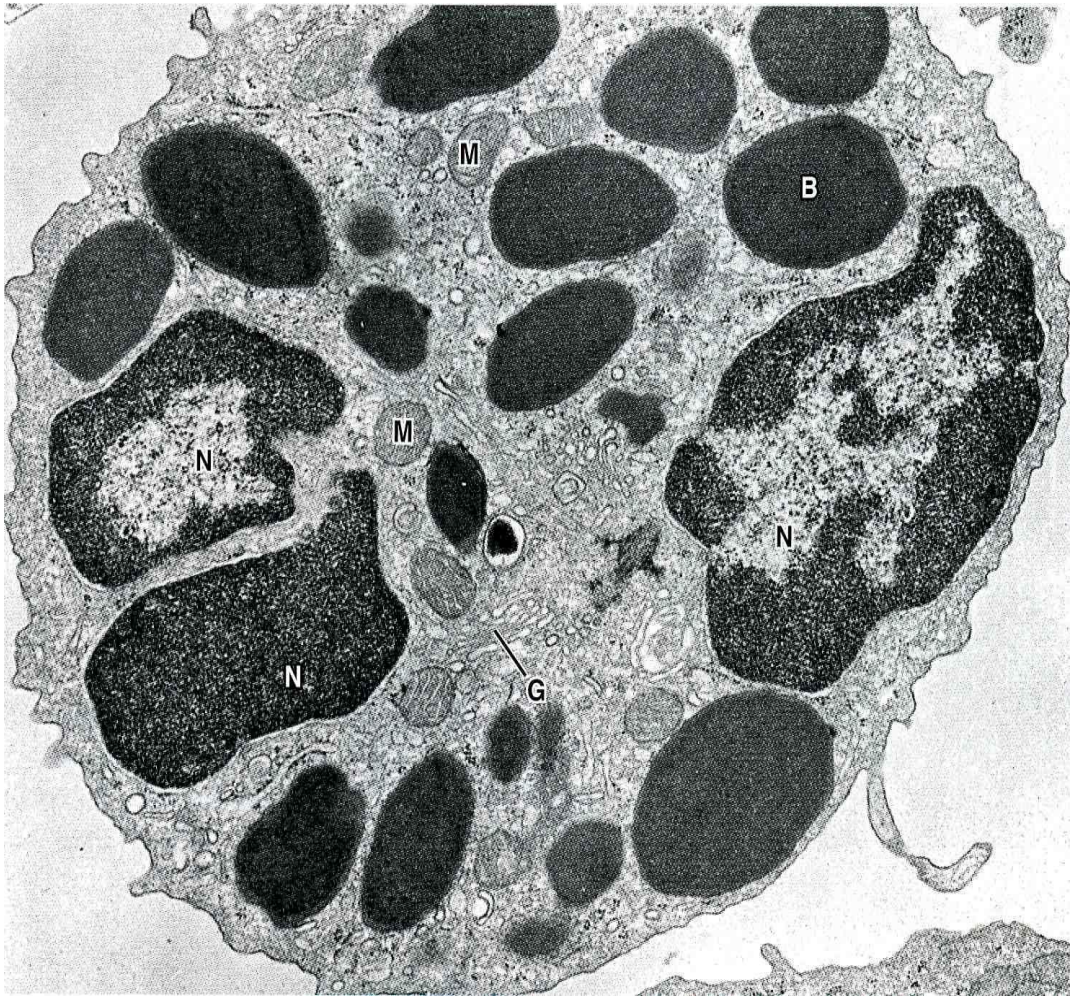
Basophils. Human. x 1325. Basophils, the least numerous of all leukocytes, are 8-10 micrometers in diameter. Frequently, their cytoplasm is so filled with dark, large, basophilic specific granules (arrowheads) that they appear to press against the cell membrane, giving it an angular appearance. The specific granules usually mask the azurophilic granules as well as the light blue nucleus (arrow).



Basophils have several surface receptors on their plasmalemma, including IgE receptors.

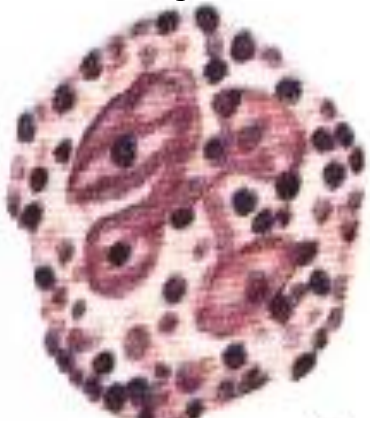
Basophils may supplement the functions of the mast cells in immediate hypersensitivity reactions by migrating (under special circumstances) into connective tissue. Basophils can liberate their granule content in responce to certain antigens. In spite of many similarities mast cells and basophils are different cells and they originate from different stem cells (on the contrary to the mast cells basophils contain peroxidase). Basophils are antagonistic to eosinophils.

Basophil, TEM



The specific granules of the basophils (0.5 mcm in diameter) stain metachromatically (change the color of the stain used) with the basic dye of the usual blood stains. This is due to presence of heparin. Specific granules of the basophils are fewer and more irregular in shape and size than in the other granulocytes. Degranulating in the inflammatory and allergic reactions they control blood clotting and vascular permeability. Basophils contain heparin, histamine, leukotriens and serotonin. Leukotriens are slowly acting substances of anaphylaxy forming during allergic reaction in the presence of IgE secreted by mast cells and other leukocytes.

GRANULOCYTES



basophil

neutrophil

eosinophil

CLINICAL CORRELATIONS

In responce to the presence of some antigens plasma cell manufacture and secrete IgE. The Fc portions of the IgE molecules become attached to the receptors of basophils without any effect, but next time when the same antigen enters the body, it is bound to the IgE molecules on the surface of the basophils.

1.Binding of antigen to the IgE molecules on the surface of a basophil (and mast cell) causes the cell to release the contents of iyts specific granules into the extracellular space.
2.In addition, phospholipases act on certain phospholipids of the basophil plasmalemma to form arachidonic acids. Arachidonic acids are metabolized to produce leukotriens C4,D4 and E4.
3.The release of histamine causes vasodilataion, smooth muscle contraction in the bronchial tree and leakiness of blood vessels.
4.Leukotriens have similar effects, but these actions are slower and more persistent than those associated with histamine. In addition leukotriens activate leukocytes, causing them to migrate to the site of antigenic challenge.

GRANULOCYTES			
Features	**Neutrophils**	**Eosinophils**	**Basophils**
Number/mm^3 **% of WBCs**	**3500-7000** **45-70 %**	**150-400** **1-5 %**	**50-100** **< 1 %**
Diameter (*μ*m) **(section)** **(smear)**	**8-9** **9-12**	**9-11** **10-14**	**7-8** **8-10**
Nucleus	**Three to four lobes**	**Two lobes (sausage shaped)**	**S-shaped**
Specific granules	**0.1 *μ*m. light pink***	**1-1.5 *μ*m. dark pink***	**0.5 *μ*m. blue/ black***
Contents of specific granules	**Type IV collagenase, phospholipase A_2, lacto-ferrin, lyso-zyme, phagocytin, alkaline phosphatase**	**Aryl sulfatase, histaminase, *β*-glucoronidase, acid phosphatase, phospholipase, major basic protein, eosinophil cationic protein, neurotoxin, ribonuclease, cathepsin. peroxidase**	**Histamine, heparin, eosinophil chemotactic factor, neutrophil chemotactic factor, peroxidase**

GRANULOCYTES			
Features	**Neutrophils**	**Eosinophils**	**Basophils**
Surface markers	**Fc receptors, platelet-activating factor receptor, leukotriene B_4 receptor, leukocyte cell adhesion molecule-1**	**IgE receptors, eosinophil chemotactic factor receptor**	**IgE receptors**
Life span	**< 1 week**	**< 2 week**	**1-2 years (in murines)**
Function	**Phagocytosis and destruction of bacteria**	**Phagocytosis of antigen-antibody complex; destruction of parasites**	**Similar to mast cells to mediate inflammatory responses**

CLINICAL CORRELATIONS

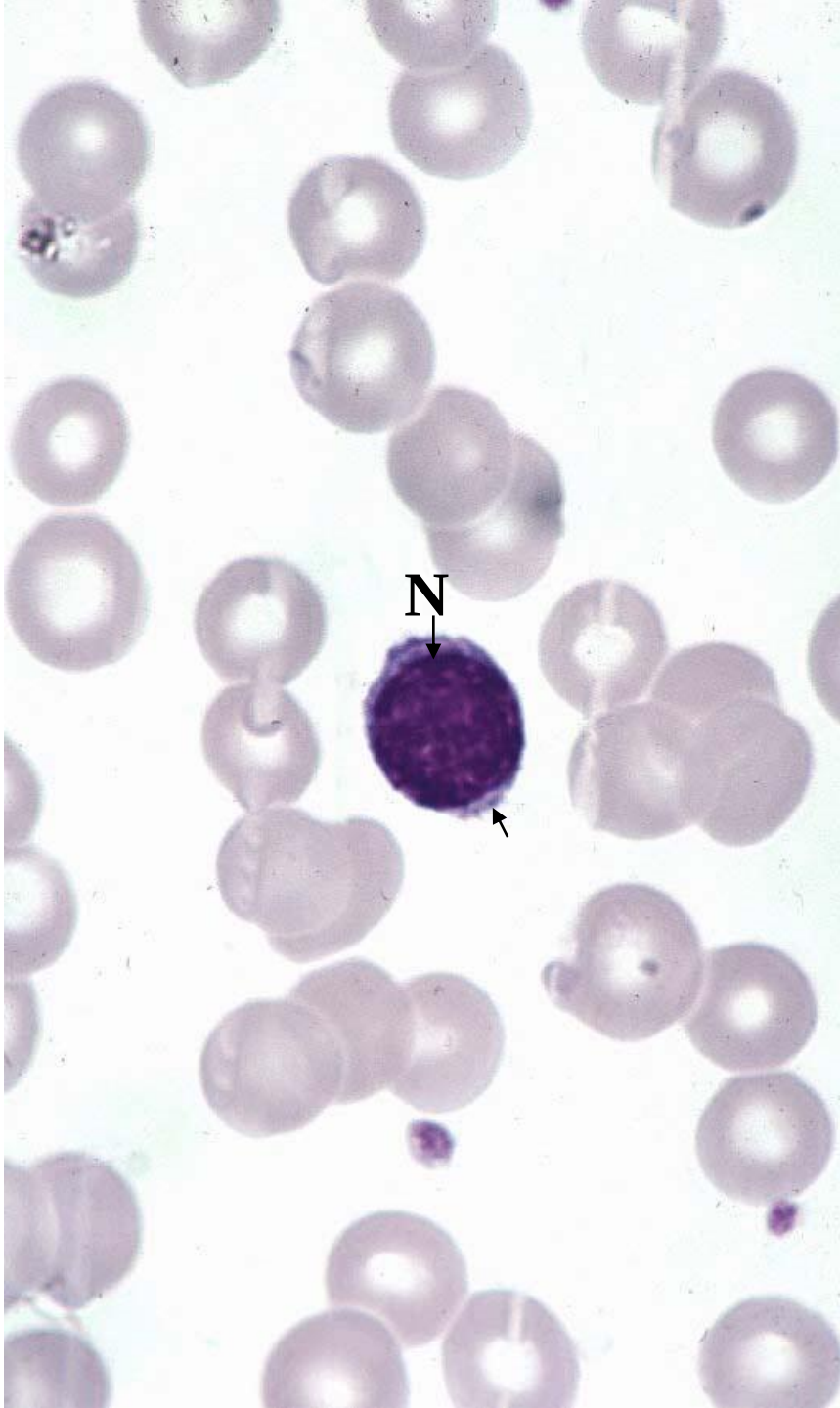
Connective tissue cells in the vicinity of antigen-antibody complexes release the pharmacological agents histamine and IL-5, causing increased formation and release of eosinophils from the bone marrow. On the contrary, elevation of blood corticosteroid levels depresses the number of eosinophils in circulation.

In certain allergic people, a second exposure to the same allergen may result in an intense generalized response. A large number of basophils (and mast cells) degranulate, resulting in widespread vasodilation and sweeping reduction in blood volume (because of vessel leakiness). Thus, the person goes into circulatory shock. The smooth muscles of the bronchial tree constrict, causing respiratory insufficiency. The combined effect is a life-threatening condition known as anaphylactic shock.

Lymphocytes. Human. x 1325.

Lymphocytes are small cells (8-10 micrometers in diameter) that possess a single large, acentrically located round nucleus (N) and a narrow rim of light blue cytoplasm (arrowhead). The nucleus is dense with a great deal of heterochromatin. Azurophilic granules (lysosomes) may be evident in the cytoplasm. On the basis of size lymphocytes may be described as small, medium (12-15 mcm in diameter) and large (15-18 mcm).

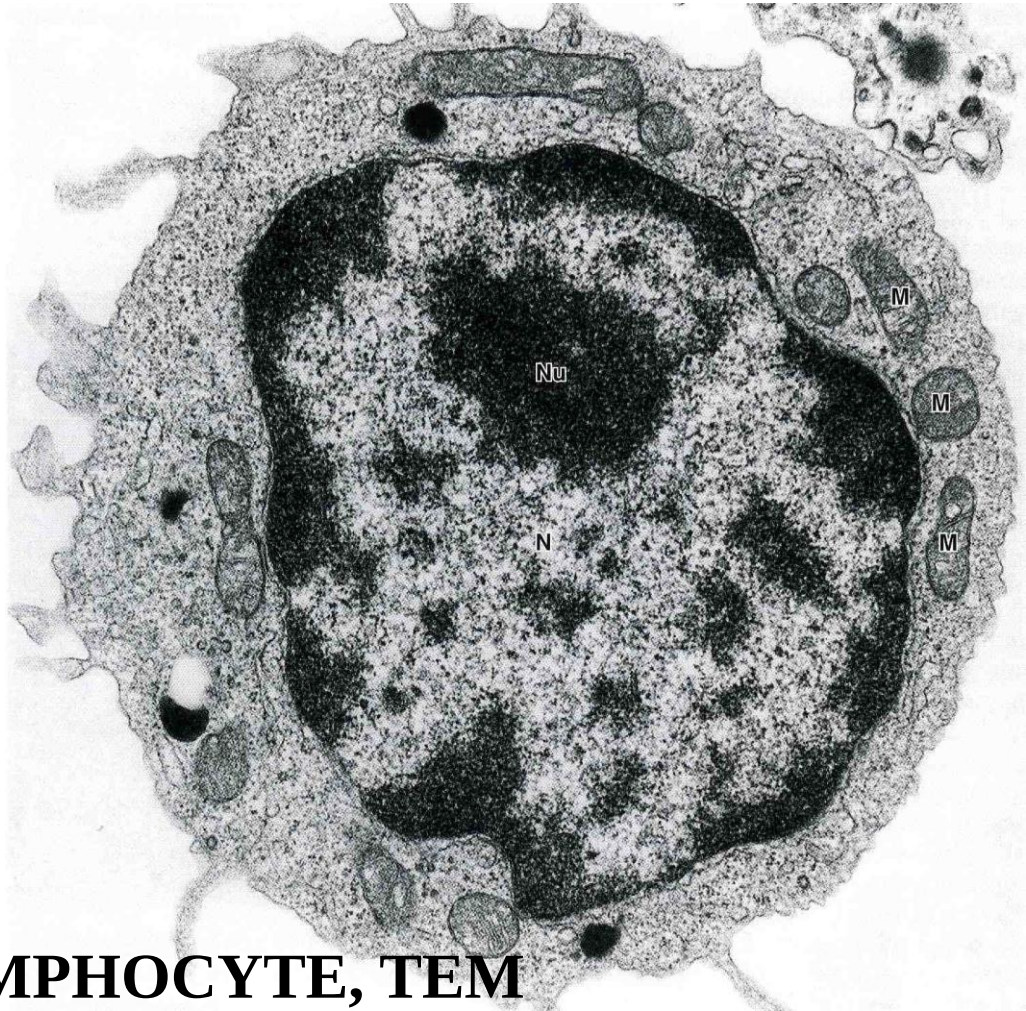
Lymphocytes are present in small numbers in most CT, except at sites of chronic inflammation where they are abundant.



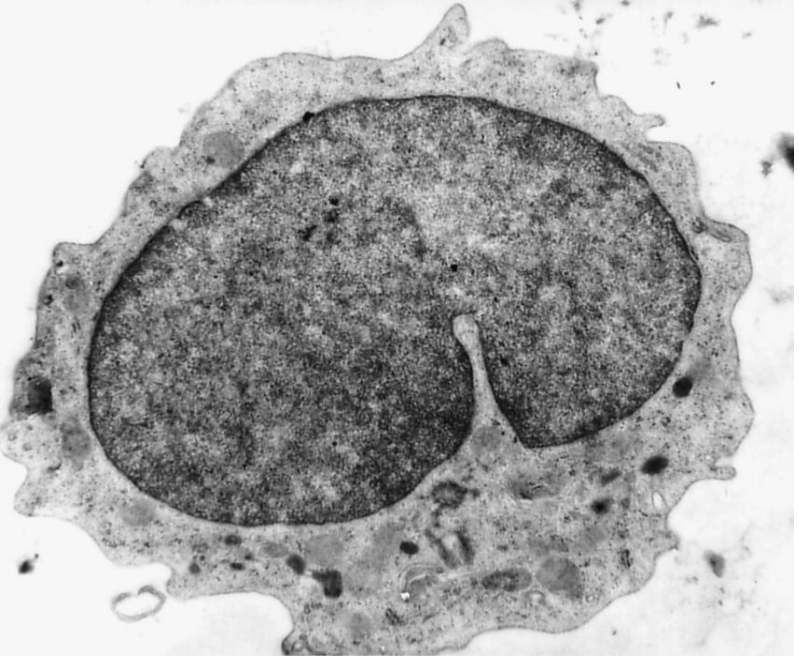
Electron micrograph displays a a scant amount of peripheral cytoplasm housing a few mitochondria, a small Golgi apparatus, and a few profiles of RER. A small number of lysosomes representing azurophil granules 0.5 mcm in diameter, and an abundant supply of ribosomes are also evident.

There are three types of lymphocytes: T lymphocytes, B lymphocytes and null cells. Morphologically they are not identifiable, but immunohisto-chemical staining may discriminate the types differentiating the surface markers (CD). Approximately 80% of the circulationg lymphocytes are T-cell, 15% are B-cells and the remainder are null cells. Their life spans also differ widely: some T-cells may live for years while B-cells may die in a few months.

Null cells are composed of two distinct populations: circulating stem cells which give rise to all of the formed elements of blood and natural killer cells (NK cells) which can kill some foreign and virally altered cells without the infuence of thymus or T cells.



LYMPHOCYTE, TEM



Medium lymphocyte, TEM

In general B-lymphocytes are responsible for the humoral immunity while T lymphocytes are responsible for the cellular immunity. Lymphocytes have no function in the bloodstream, but in the connective tissue they are responsible for proper functioning of the immune system.

To be immunologically competent they migrate to specific body compartment to mature and to express specific surface markers and receptors. B cells enter as yet unidentified regions of the bone marrow whereas T-cells migrate to the cortex of the thymus. Once they have become immunologically competent, lymphocytes leave their respective sites and of maturation, enter lymphoid system and undergo mitosis forming a clone of identical cells. All members of a particular clone can recognize and respond to the same antigen.

After stimulation by a specific antigen, both T- and B-cells proliferate and differe-ntiate into 2 subpopu-lations: memory cells and effector cells.

TYPES OF LYMPHOCYTES

1.Memory cells do not participate in the immune responce but remain as part of the clone with an immunological memory, ready to mount a response against a subsequent exposure to a particular antigen or foreign substance.

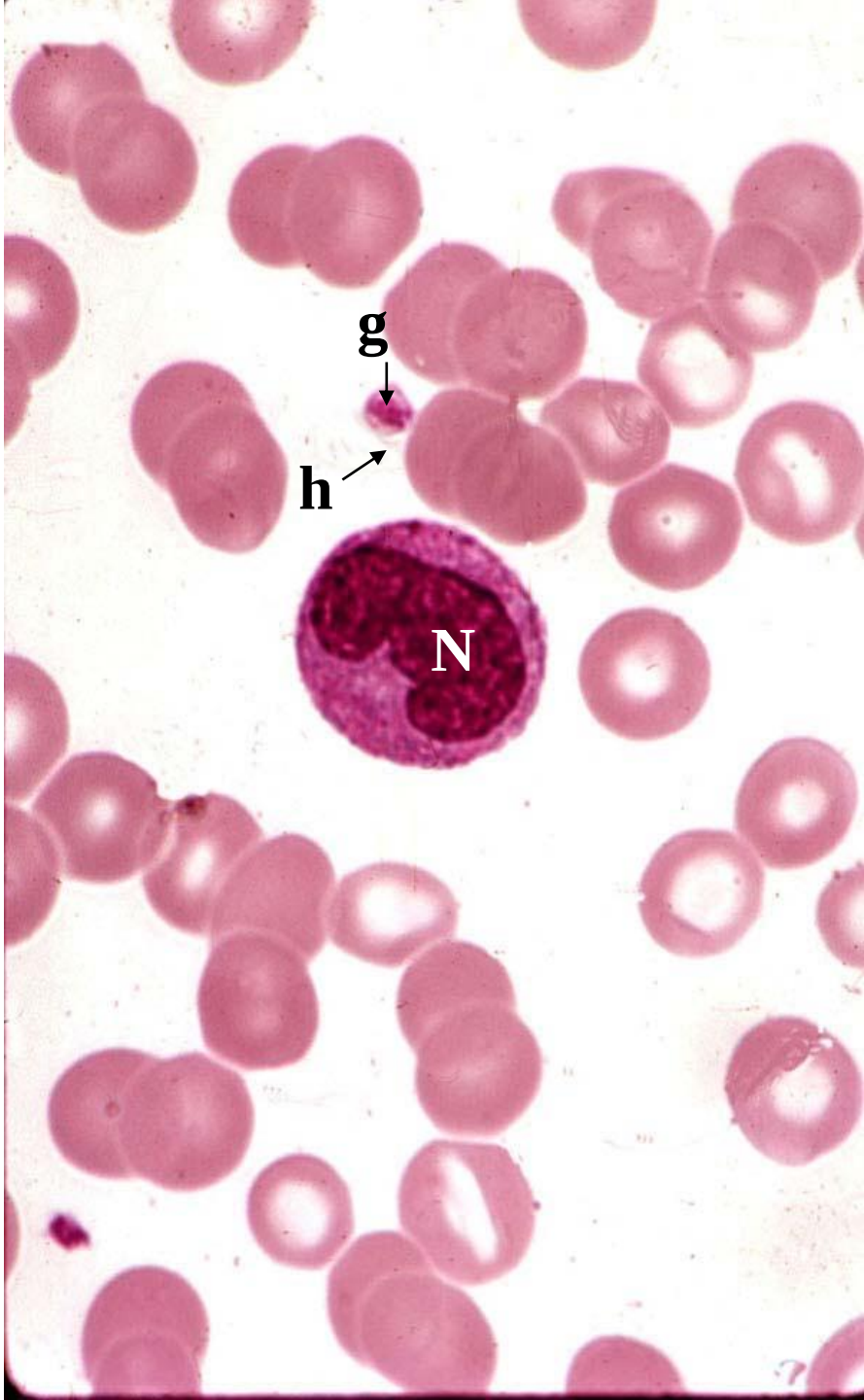
2.Effector cells can be classified as B cells and T cells (and their subtypes). They are imminocompetent cells that can perform their immune functions, that is, eliminating antigens.

B cells are responsible for the humorally mediated immune system, that is, they differentiate into plasma cells which produce antibodies against antigens. T cells are responsible for the for the cellularly mediated immune system. Some T cells differentiate into cytotoxic Tcells (T kilers cells), which make physical contact with and kill foreign or virally altered cells. In addition certain T cells are responsible for the initiation and development (T-helpers) or for the suppression (T-suppressors) of most humorally and cellularly mediated immune responses. They accomplish this by releasing signaling molecules known as cytokines (lymphokines) that elicit specific responses from other cells of the immune system.

Monocytes. Human. x 1325.

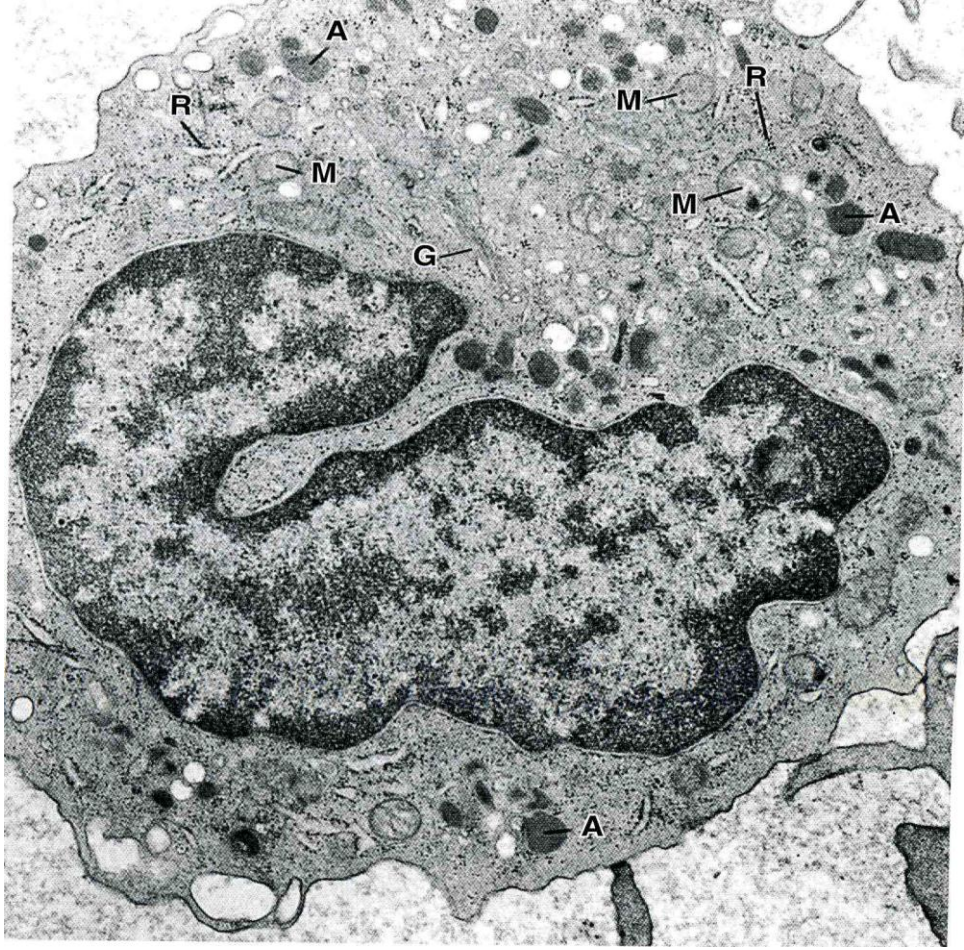
Monocytes are the largest of all circulating blood cells (12-15 micrometers in diameter). They have a considerable amount of grayish-blue cytoplasm containing numerous azurophilic granules but no specific granules. These cells are also characterized by their large size, accentric, kidney-shaped nucleus (N) that possesses a coarse chromatin network with clear spaces. Lobes of the nucleus are superimposed on themselves, and their outlines frequently appear to be distinctly demarcated. Observe the platelet with its clearly evident granulomere(g) and hyalomere (h).

Macrophages enter the connective tissue spaces where they are known as macrophages.



Monocytes have a large acentric kidney-shaped nucleus that frequently has a “moth-eaten” soap-bubbly appearance and whose lobe-like extensions seem to overlap one another. The chromatin network is coarse but not overly dense, and typically two nucleoli are present, although they ae not always evident in smears. Both hetero- and euchromatin are displayed in the nucleus. The cytoplasm contains deposits of glycogen granules, a few profiles of RER, some mitochondria (M), free ribosomes (R), and numerous lysosomes. The periphery of the cell displays microtubules, microfilaments, pinocytotic vesicles and filopodia.

Monocytes stay in the circulation for only a few days, then they migrate into the connective tissue to differentiate into macrophages.



MONOCYTE, TEM

Macrophages phagocytose unwanted particular matter, produce cytokines that are required for the inflammatory and immune response, and present epitopes to T-lymphocytes.

FUNCTIONS OF THE MACROPHAGES

1.Macrophages are avid phagocytes, and as members of mononuclear phagocyte system they phagocytose and destroy dead and defunct cells (such as senescent erythrocytes) as well as antigens and foreign particulate matter (suh as bacteria). The destruction occurs within the phagosomes through both enzymatic digestion and the formation of superoxide, hydrogen peroxide, and hypochlorous acid.

2.Macrophages produce cytokines that activate the inflammatory response as well as the proliferation and maturation of other cells.

3.Certain macrophages known as antigen-presenting cells, phagocytose antigens and present their most antigenic portions, epitopes, in conjunction with the integral proteins, class II human leukocyte antigen (class II HLA, also known as major histocompatibility complex antigen, MHC II), to immunocompetent cells.

4.In response to large foreign particulate matter, macrophages fuse with one another, forming foreign body giant cells that ae large enough to phagocytose the foreign particle.

AGRANULOCYTES

Features	Lymphocytes	Monocytes
Number/mm^3 % of WBCs	1500-2500 20-35 %	200-800 3-9 %
Diameter (μm) (section) (smear)	7-8 8-10	10-12 12-15
Nucleus	Round	Kidney-shaped
Specific granules	None	None
Contents of specific granules	None	None
Surface markers	*T-cells:* T-cell receptors, CD molecules, IL receptors *B-cells:* surface immunoglobulins	Class II HLA, Fc receptors
Life span	Few months to several years	Few days in blood, several months in connective tissue
Function	*T-cells:* cell-mediated immune response *B-cells:* humorally mediated immune response	Differentiate into macrophage: phagocytosis, presentation of antigens