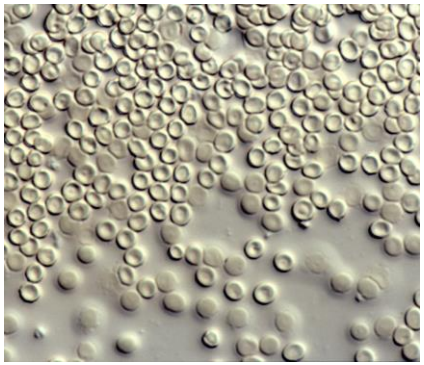
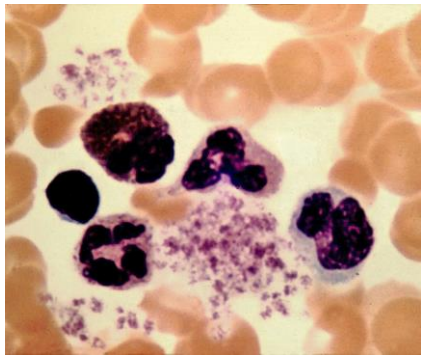
Manchester Microscopical & Natural History Society

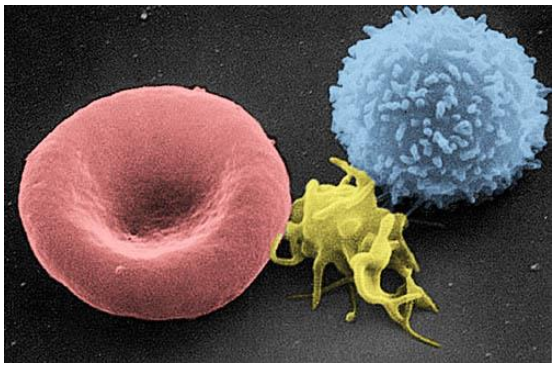
Established 1880

www.manchestermicroscopical.org.uk

Microscopy of Blood





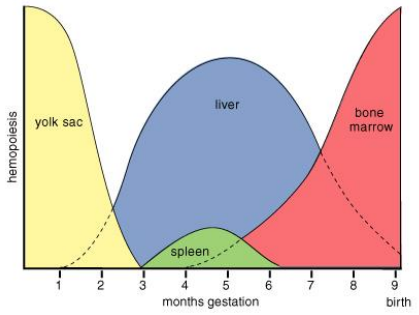


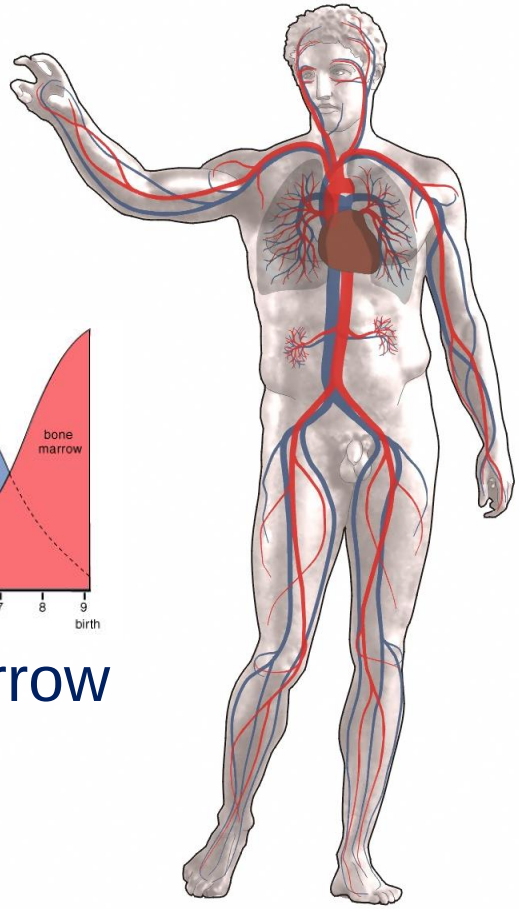
Hancock's Half Hour - 36. The Blood Donor - BBC iPlayer

***Mike Mahon**, October 26th, 2024*

Blood - Overview

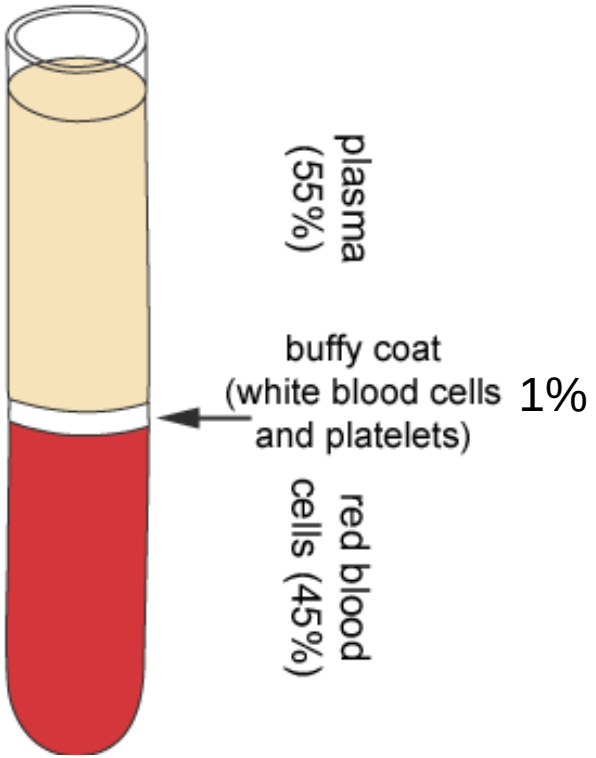
- Human
  - 5 litres or 10 pints
  - 10% body weight
  - 80% body cells
  - Circulates every 60 seconds
  - Oxygenation
  - $C0_2$ removal
  - Nutrients
  - Hormone transport
  - Defence & Repair
  - Produced by Yolk Sac / Liver / Bone Marrow
  - Filtered by Liver, Kidney, Spleen
  - Blood Groups A, B, AB, O … Rh+, Rh-





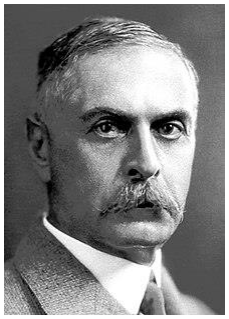
Blood - Analysis

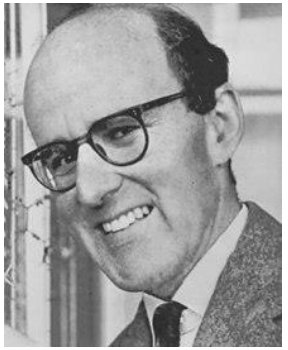
- **Haematocrit**
  - 45% males, 40% females
  - 5 million cells/µl (mm^3)
- **Biochemistry** ~500 tests !
  - Hb ~14 g/dl
- **Microscopy** (Pathology)
  - Differential White Cell Count
  - LM
  - EM



Discovery of Blood Cells

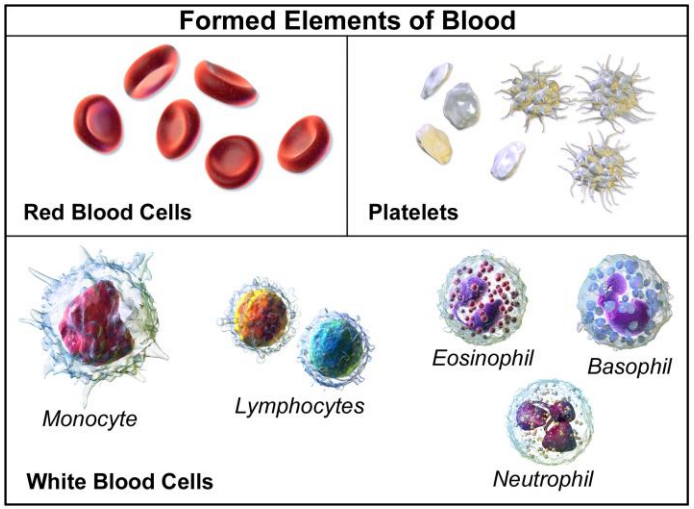
- Swammerdam 1658
- Leuwenhoek 1674





- Iron (Menghini, 1740)
- Blood Groups A,B,O (Landsteiner, 1901)
- Haemoglobin X-ray Crystallography (Max Perutz, 1959)

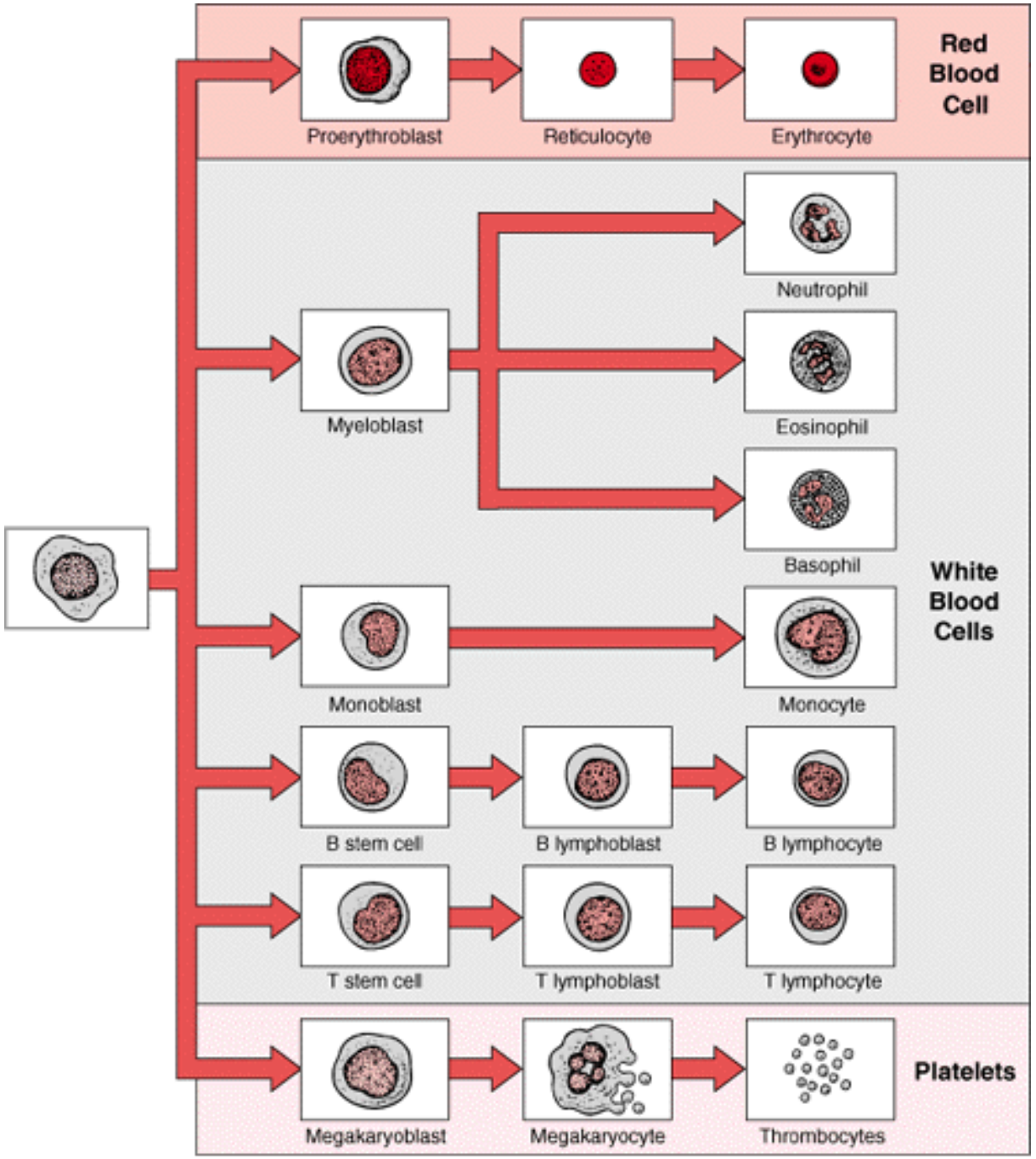
Blood - composition



- **Plasma**
- **Cells** ... n=7
  - **Red Blood Cells** (Erythrocytes)
  - **White Blood Cells** (Leucocytes) … n=5
    - **Granular** (Neutrophils, Eosinophils, Basophils)
    - **Non-Granular** (Monocytes, Lymphocytes)
  - **Platelets** (Thrombocytes)

Morphology - Functions – Number - Size

Bone Marrow

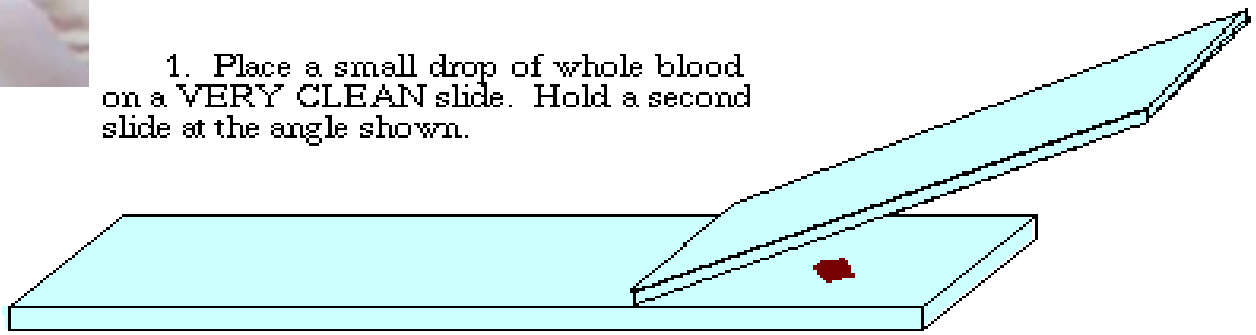


~ 7 days

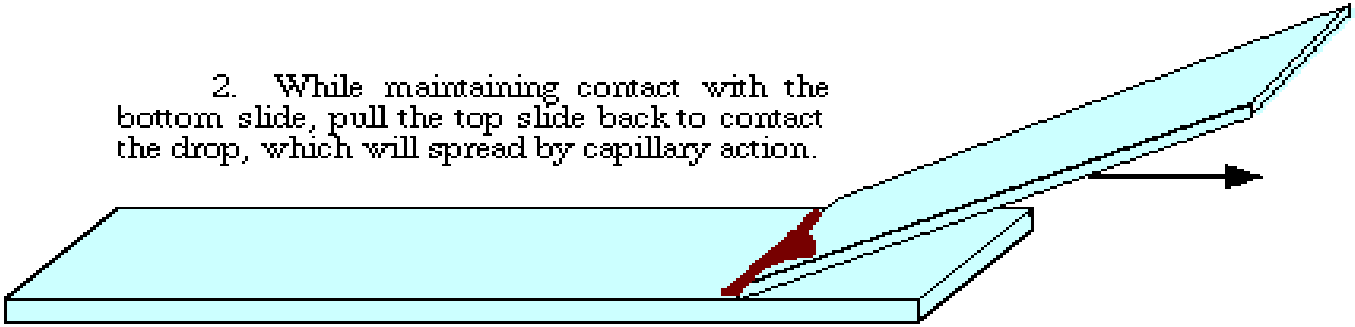
2.4million/s

Blood Smear

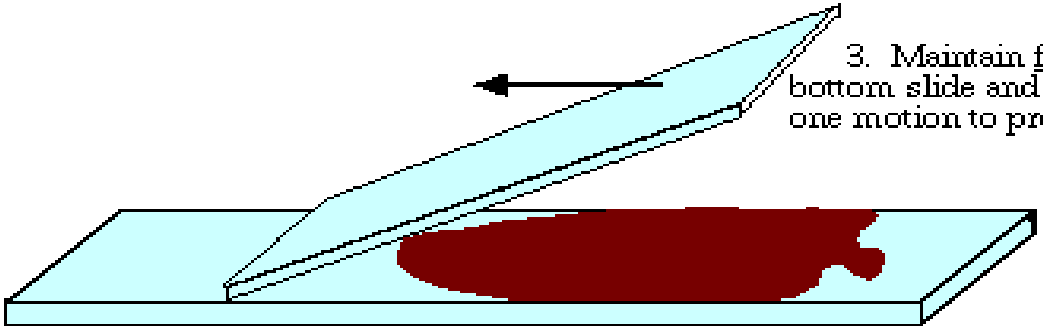
1. Place a small drop of whole blood on a VERY CLEAN slide. Hold a second slide at the angle shown.



2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



3. Maintain <u>firm</u> contact with the bottom slide and push the top slide in one motion to produce the smear.



- Air **Dried** and **Fixed** in 100% Methanol - **Dry**

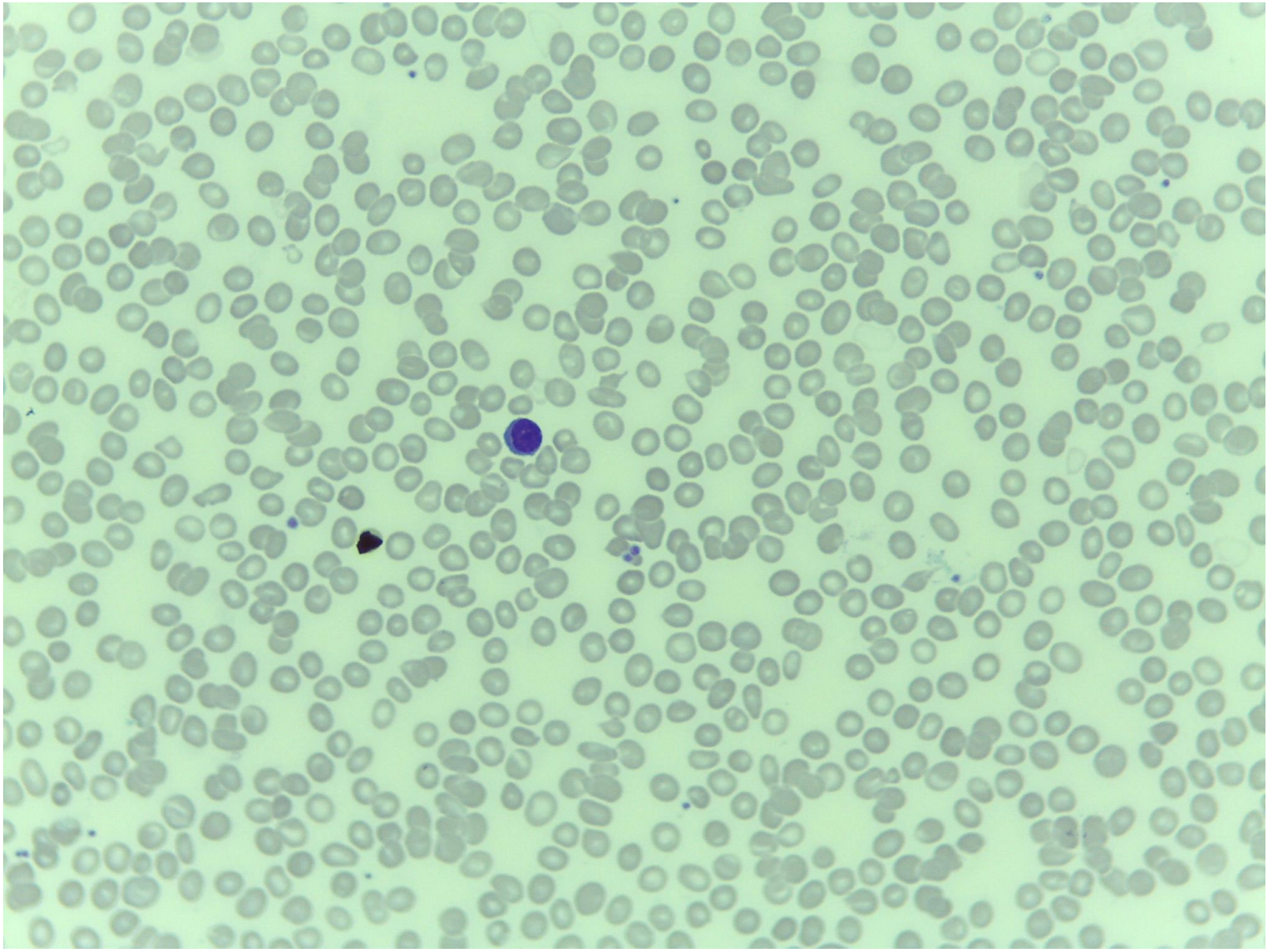
- **Stains**
  - Romanowsky
  - Wright – Giemsa
  - May – Grunwald
  - Leishman

Sticks to ..

BLUE **Methylene Blue – Basic** **Acids**

RED **Eosin - Acidic** **Bases**

Red Blood Cells - Erythrocytes

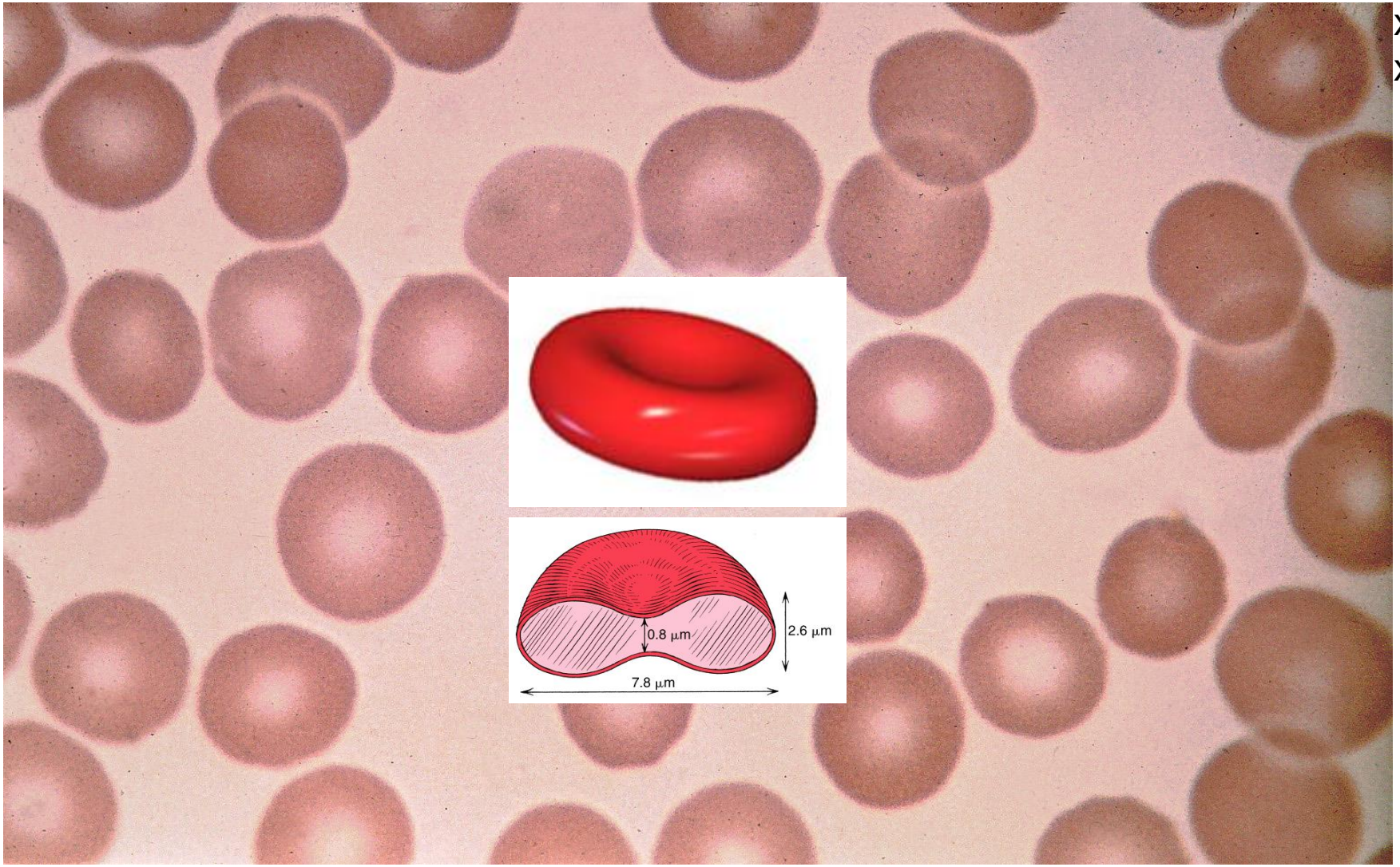


X10

50µm

Red Blood Cells - Erythrocytes

Use x40 or x100

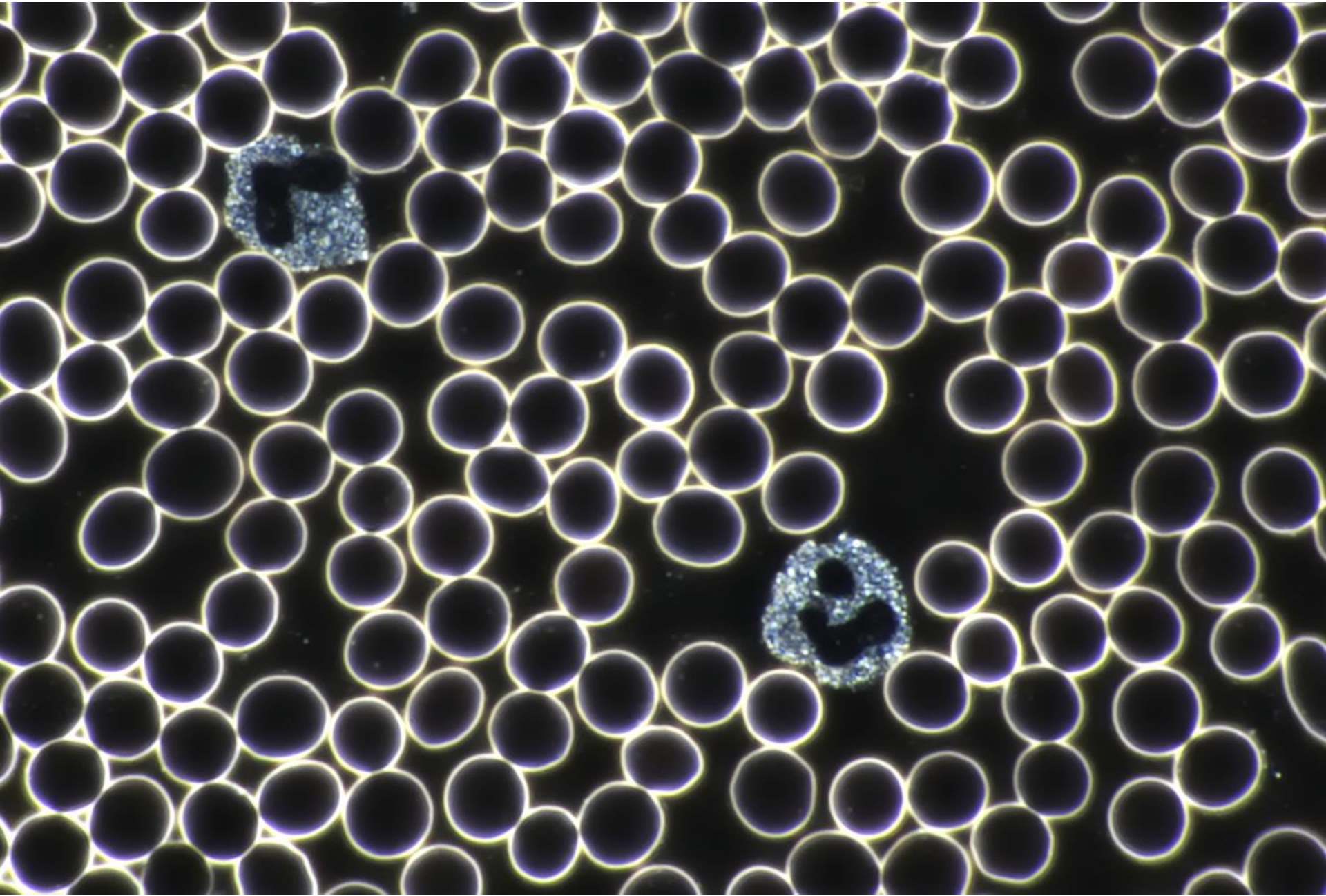


Biconcave Disc **7.7** x 2.6/0.8µm, SA 140µm^2, Vol 90fl

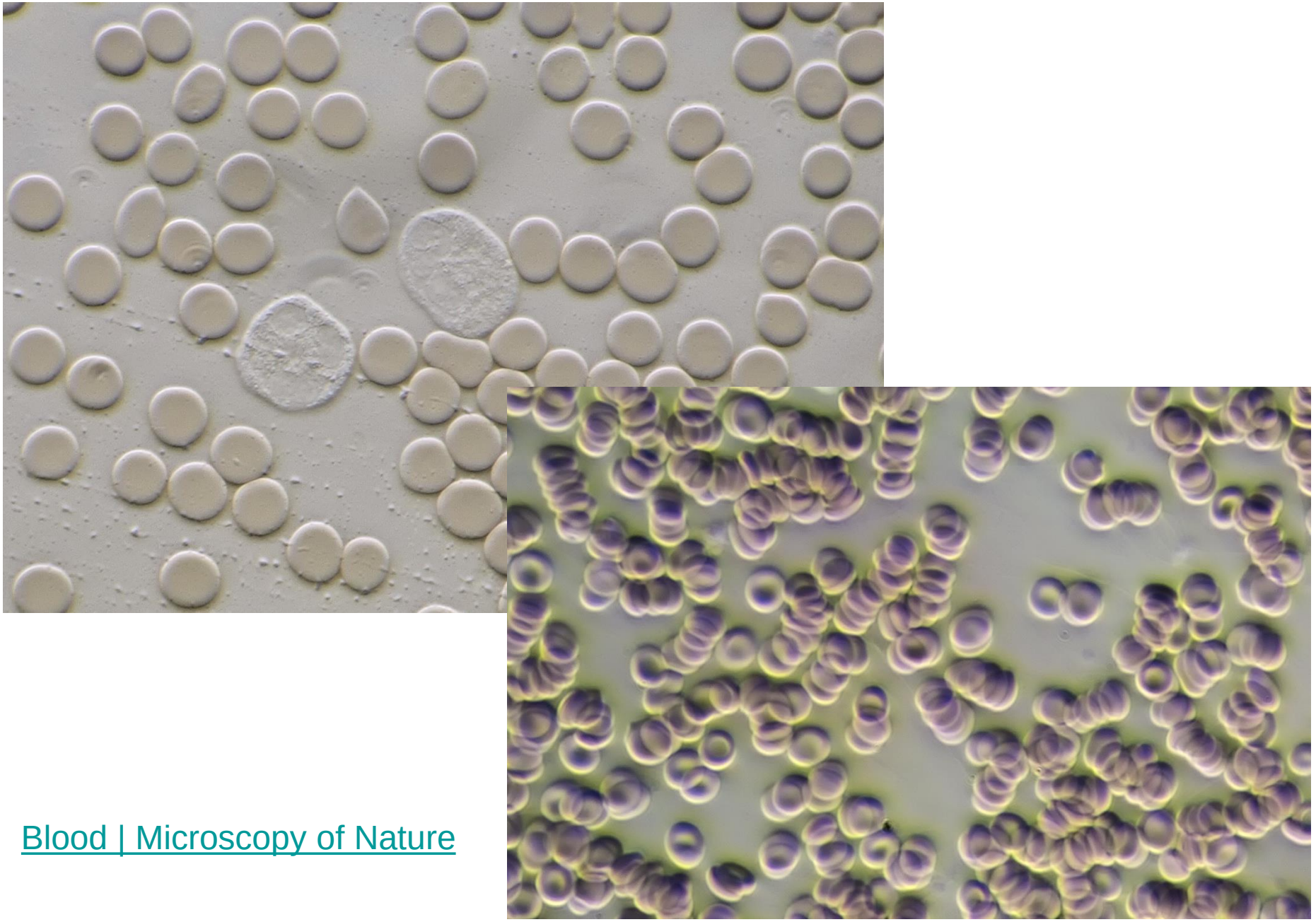
Numbers …….. 25 trillion or 5 trillion /l or 5 million /µl ^{3}

7 days maturation
120 days life cycle

Darkfield Microscopy

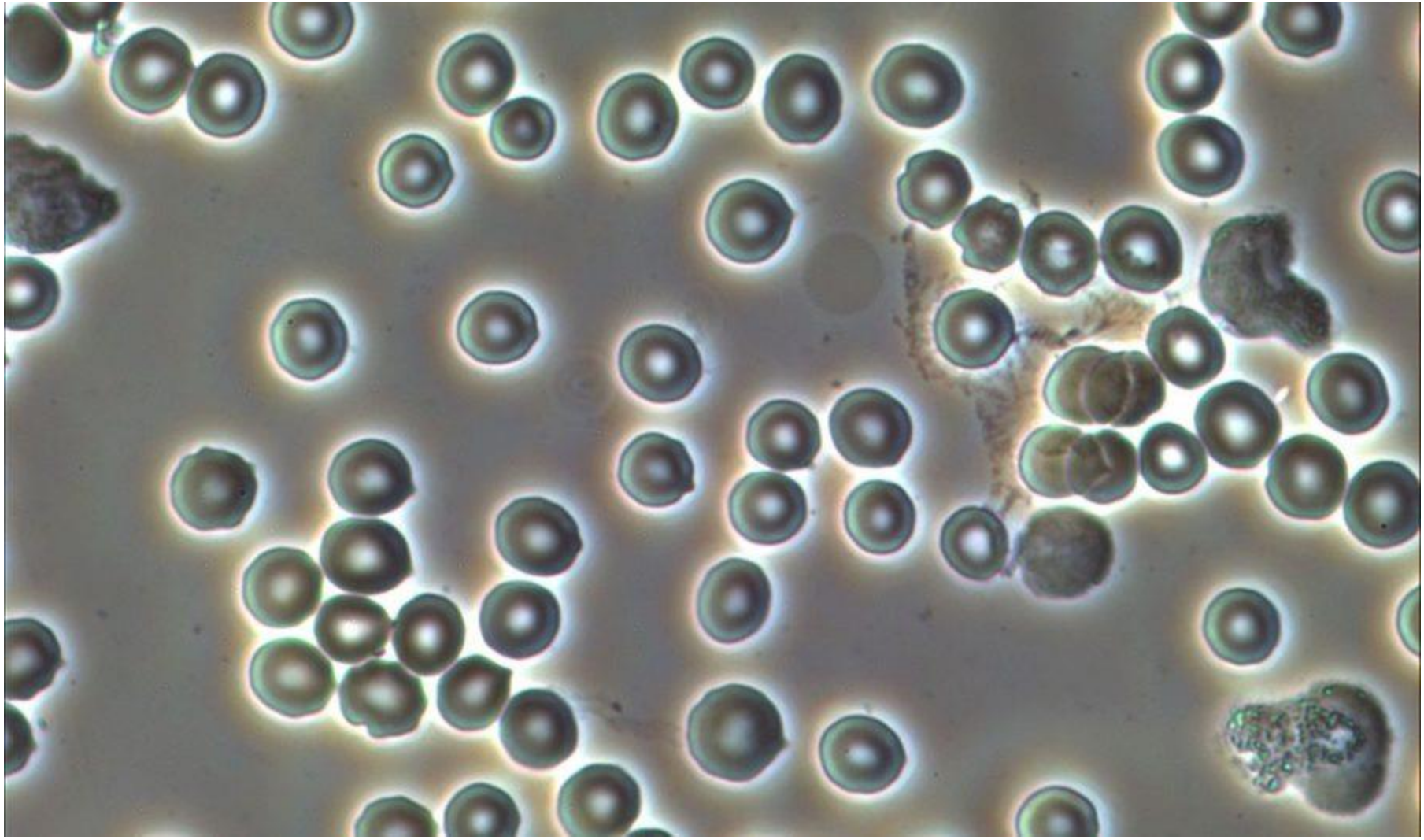


Oblique Illumination



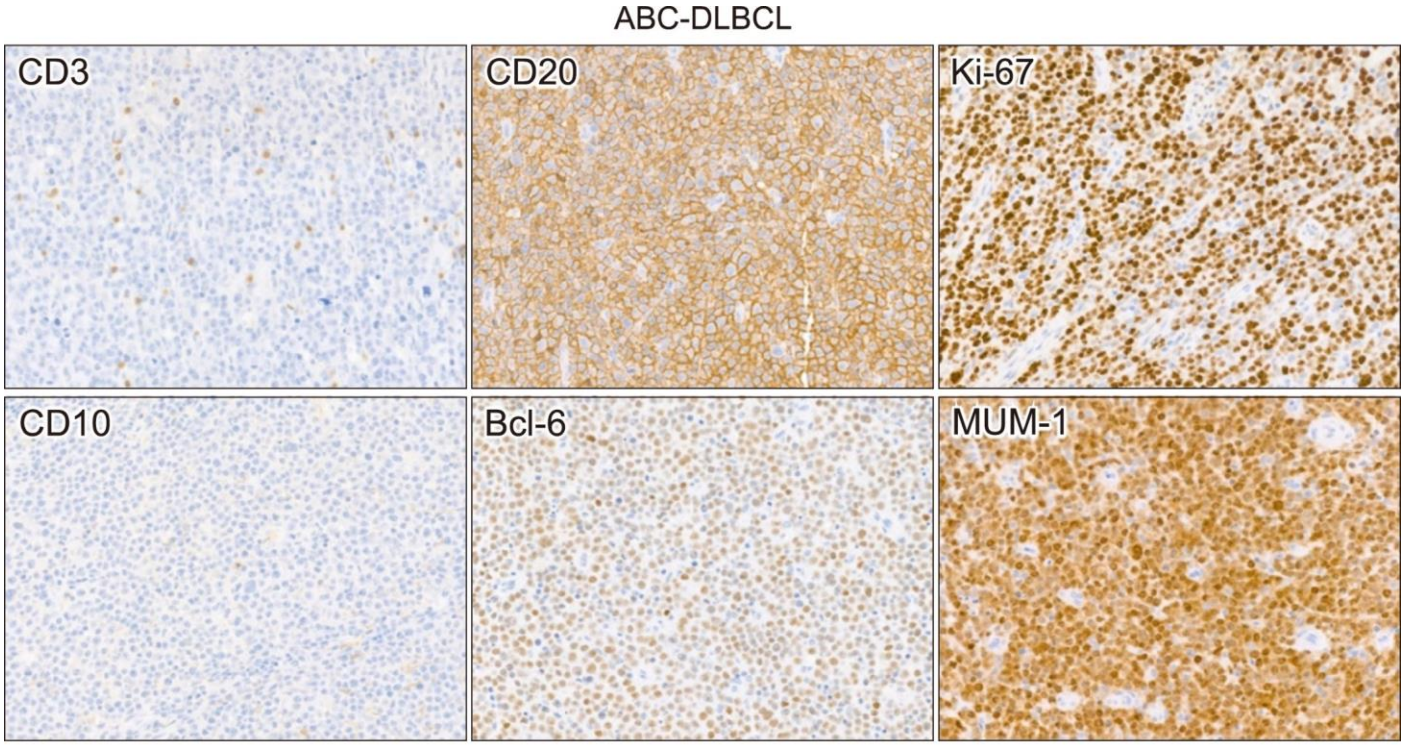
Blood | Microscopy of Nature

Phase Contrast Microscopy



DIC Microscopy

LM - Immunocytochemistry



Super-resolution Microscopy of Red Blood Cells

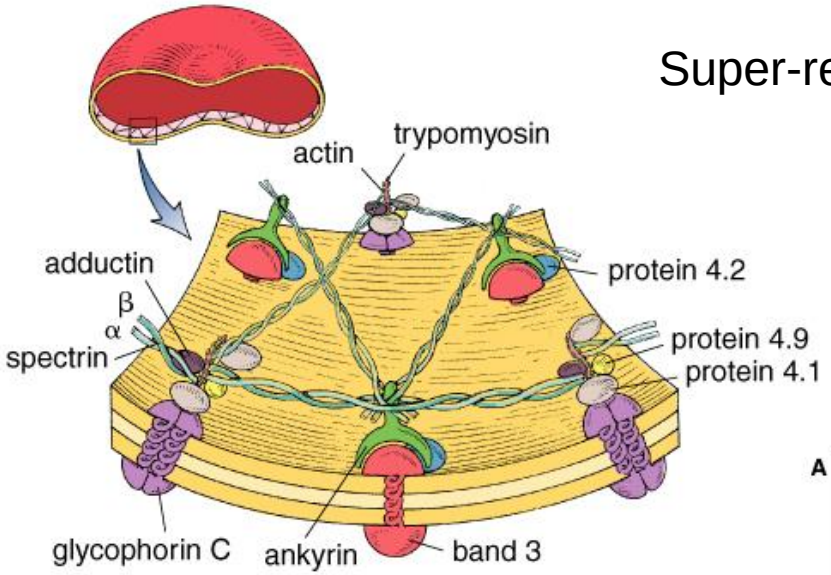
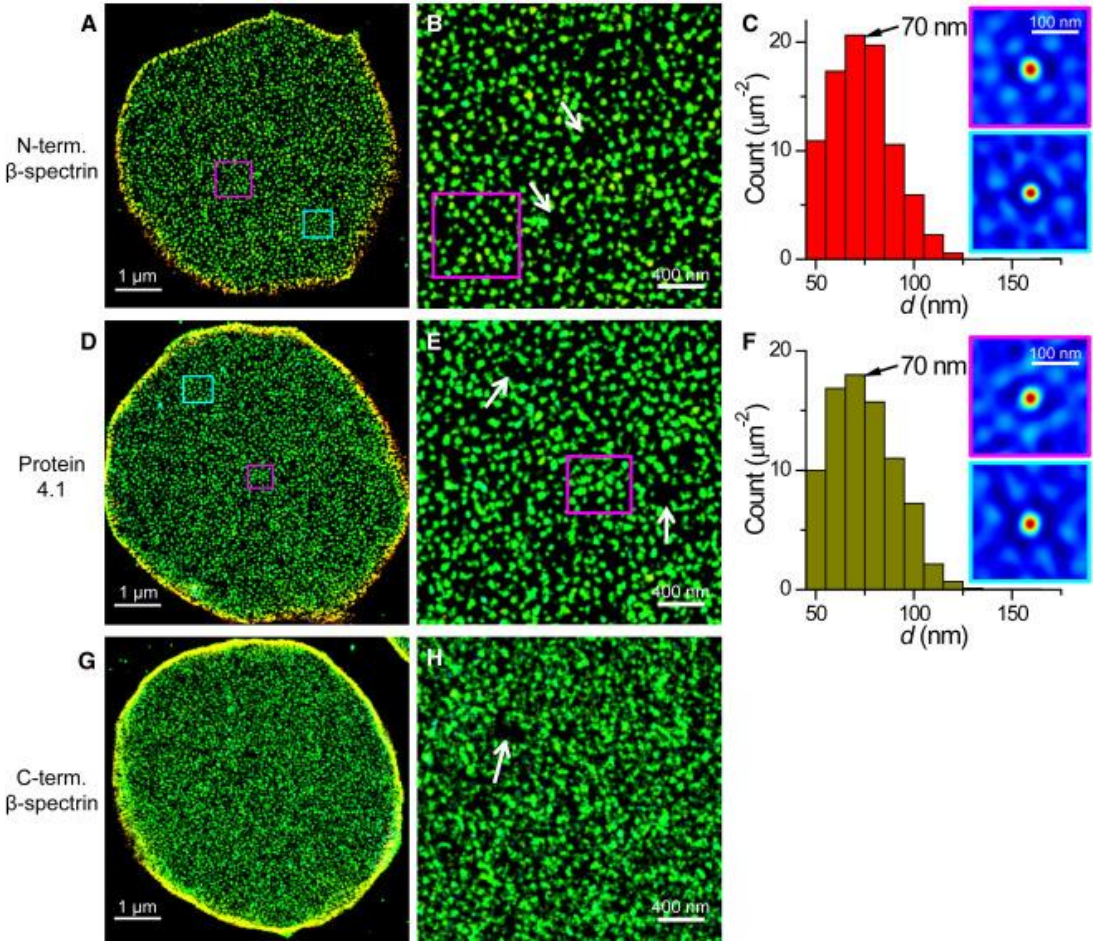
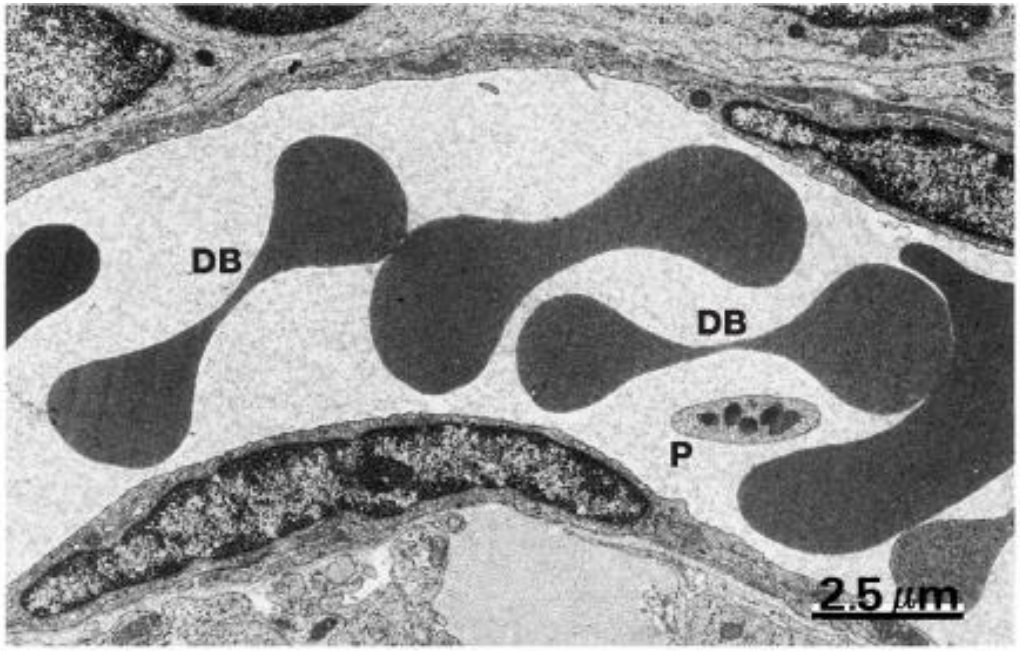


Figure 9.4. **Erythrocyte membrane organization.**

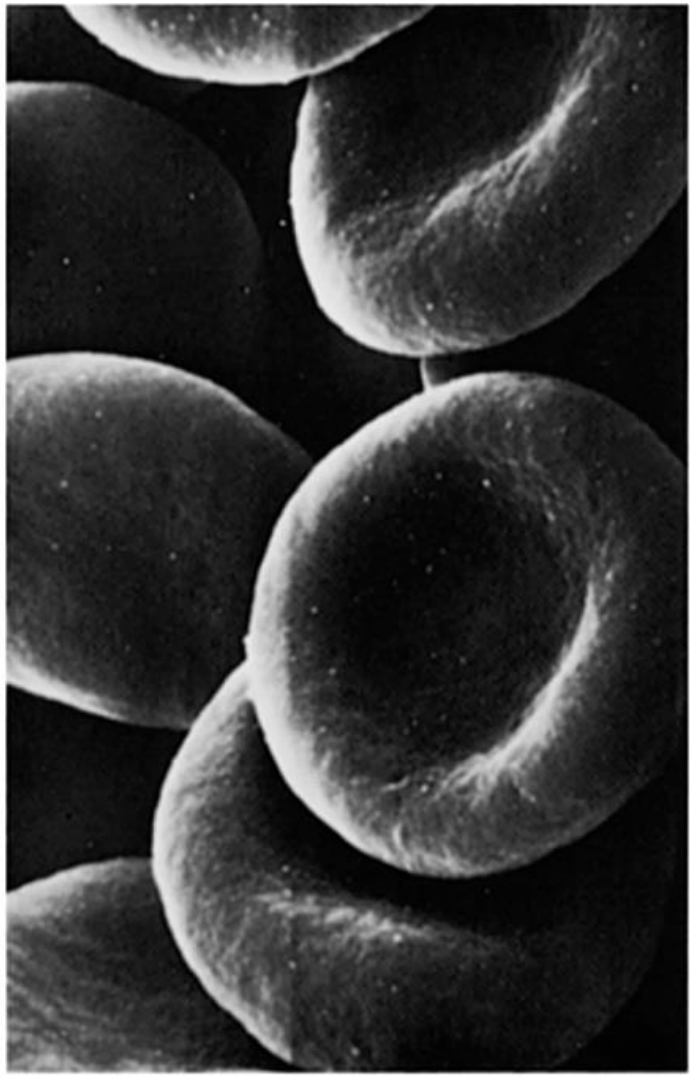


Electron Microscopy TEM

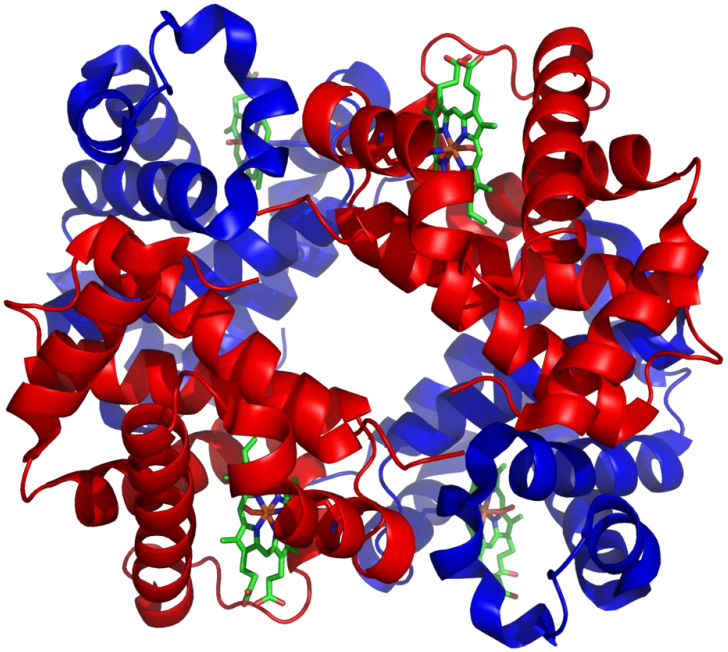
Highly distensible

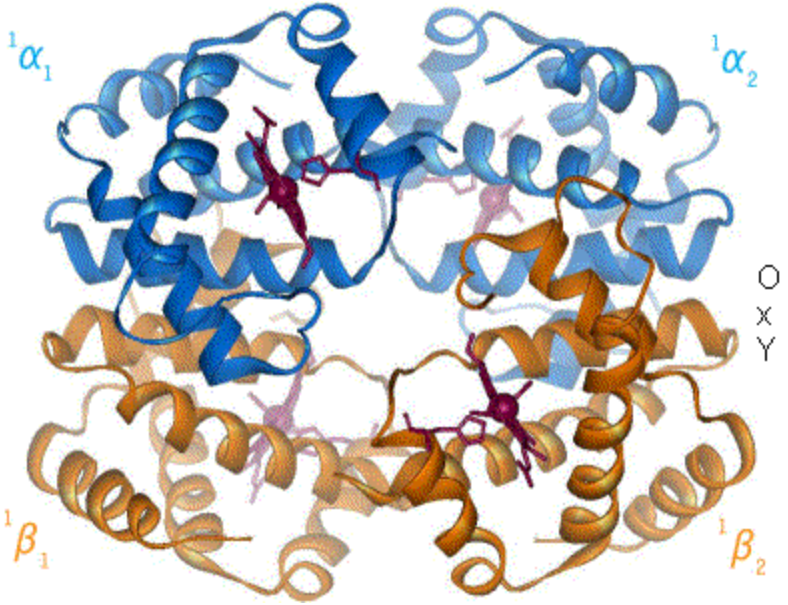


Electron Microscopy SEM

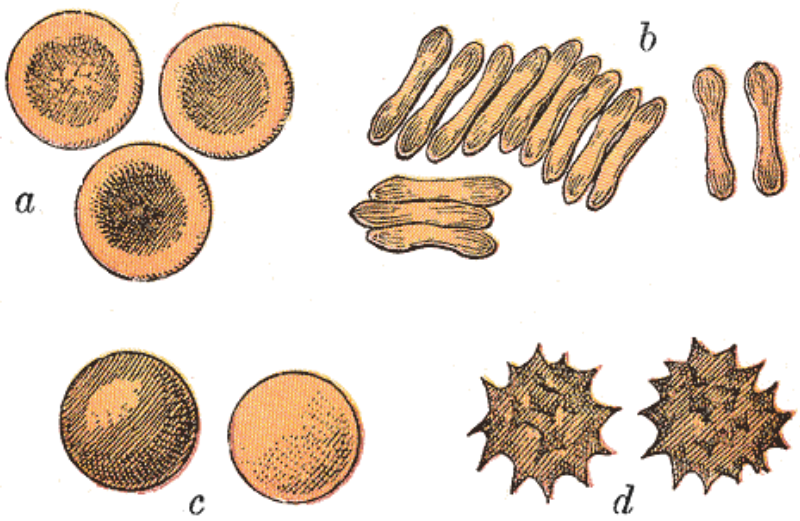


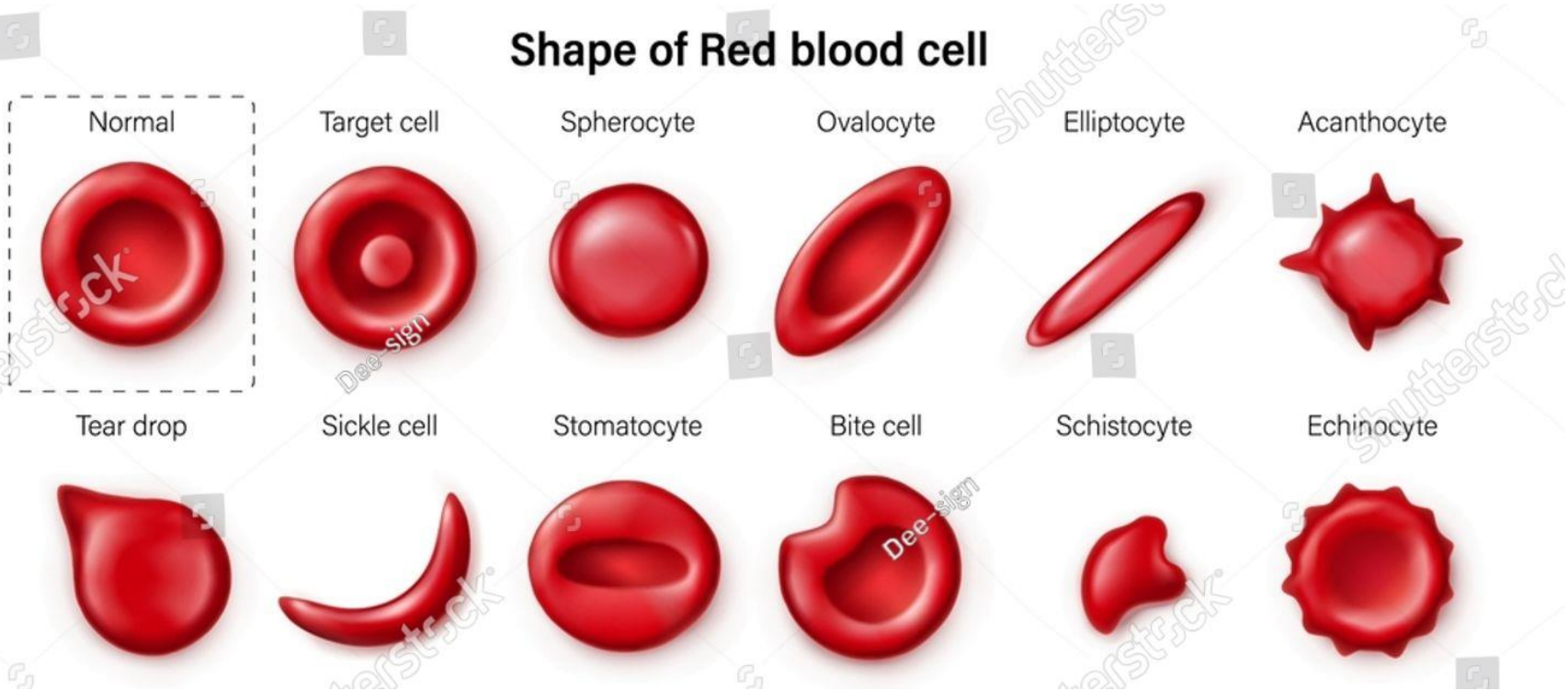
270 million haemoglobin molecules per erythrocyte !

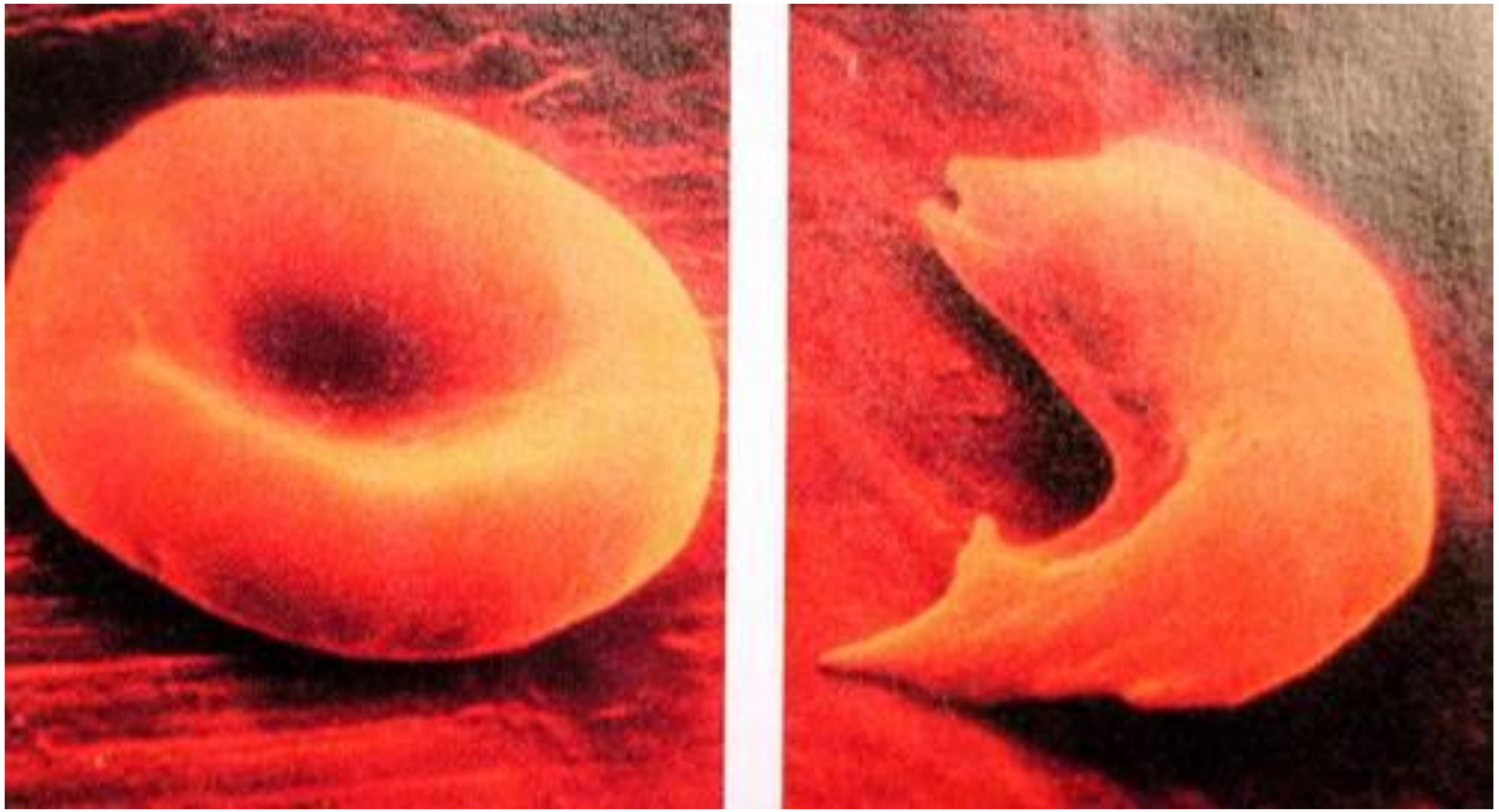


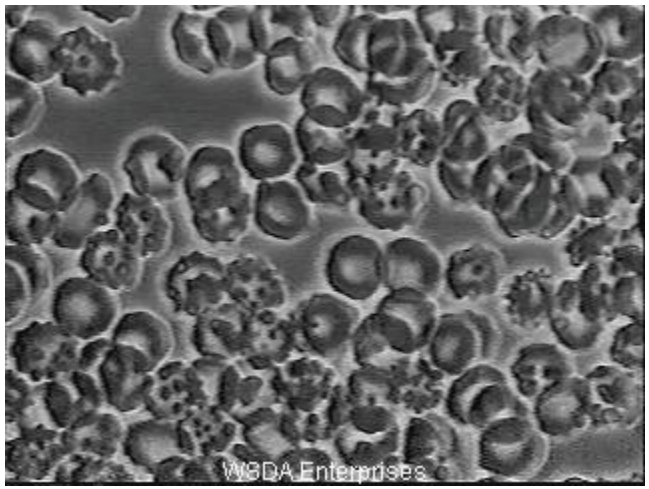


O_2 and CO_2 transport

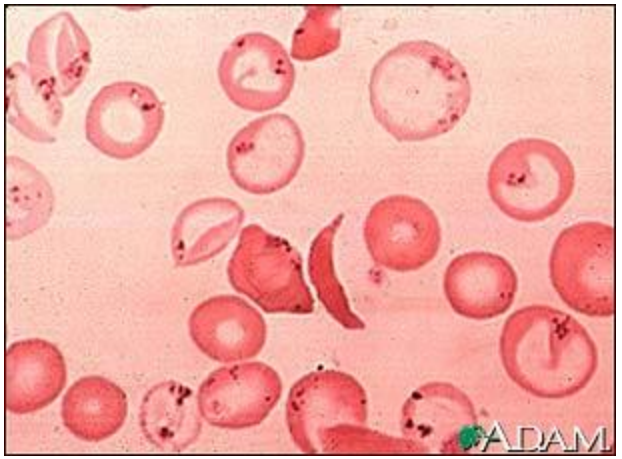

a
b
c
d


Shape of Red blood cell
Normal
Target cell
Spherocyte
Ovalocyte
Elliptocyte
Acanthocyte
Tear drop
Sickle cell
Stomatocyte
Bite cell
Schistocyte
Echinocyte



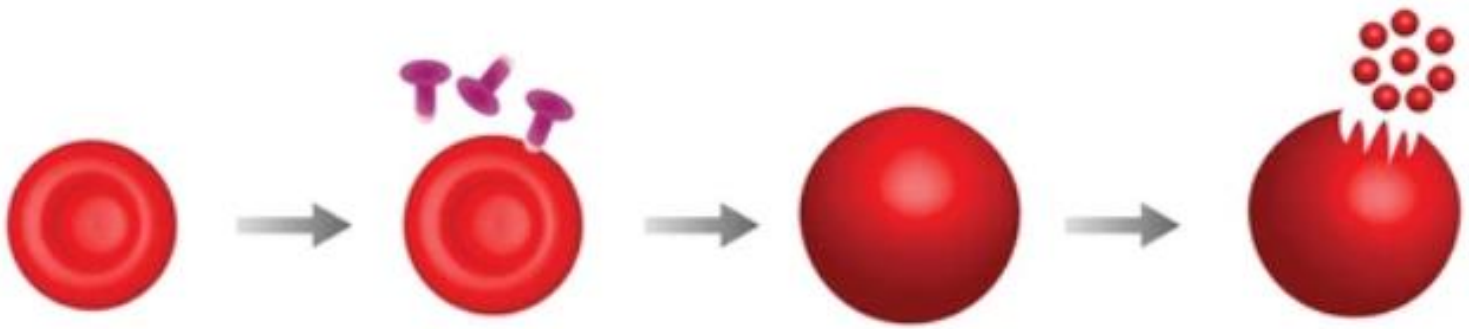


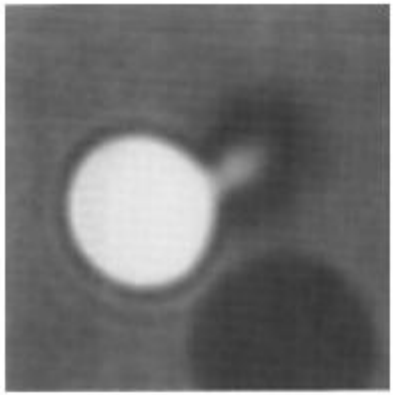
Abnormal Shapes



Sickle cell anaemia

Haemolysis





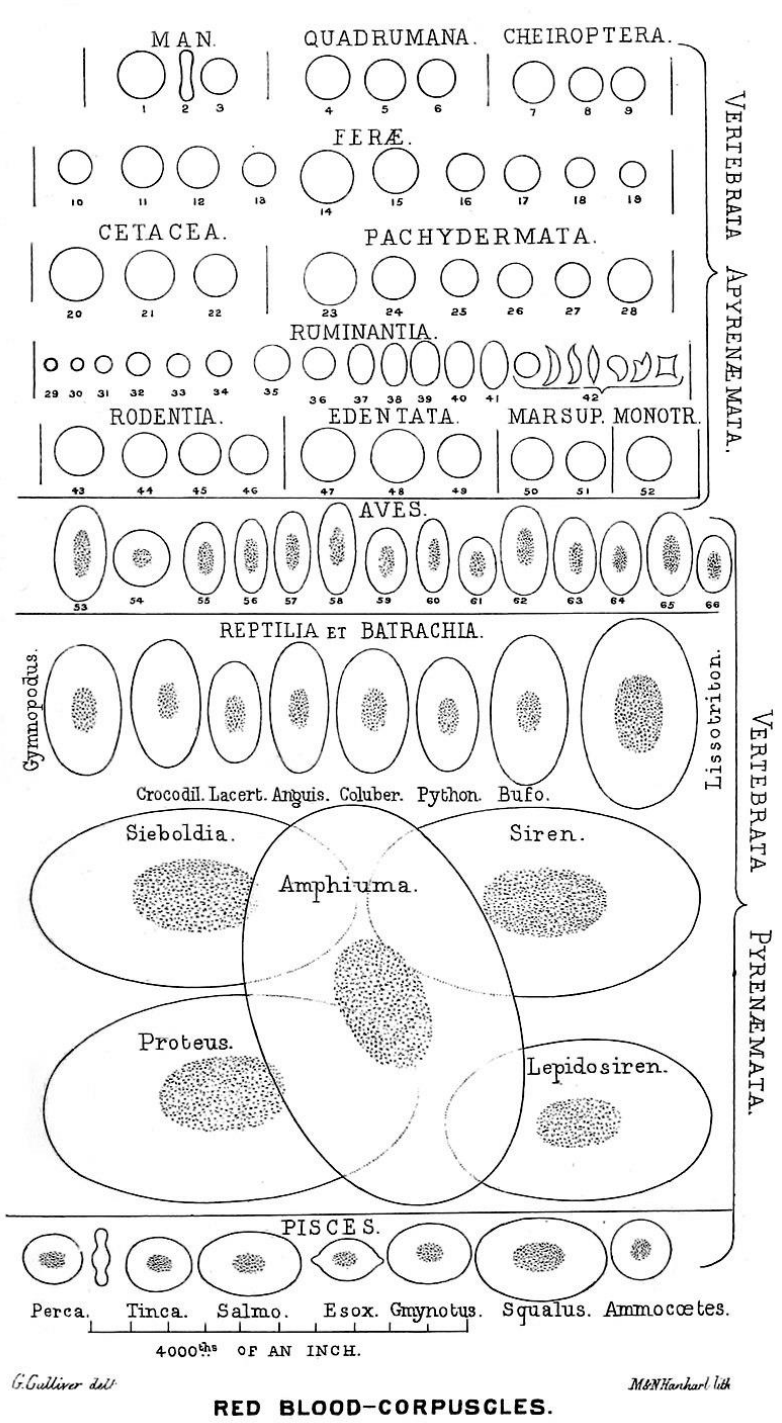
Non-Human

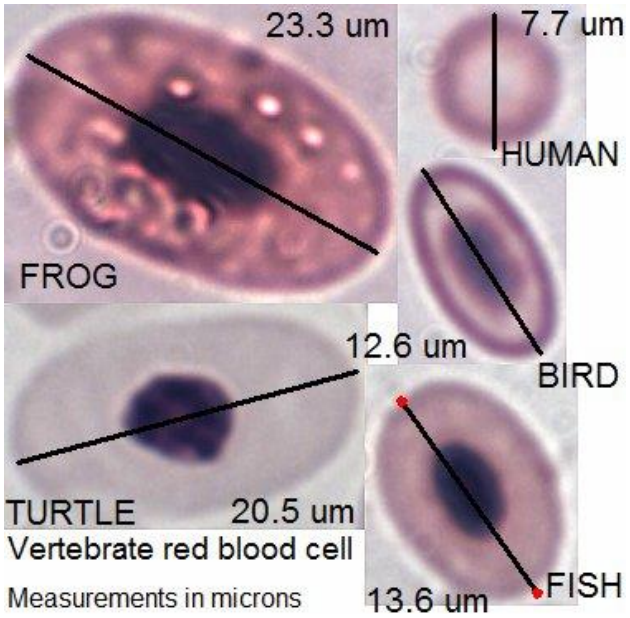
Vary in …

Size

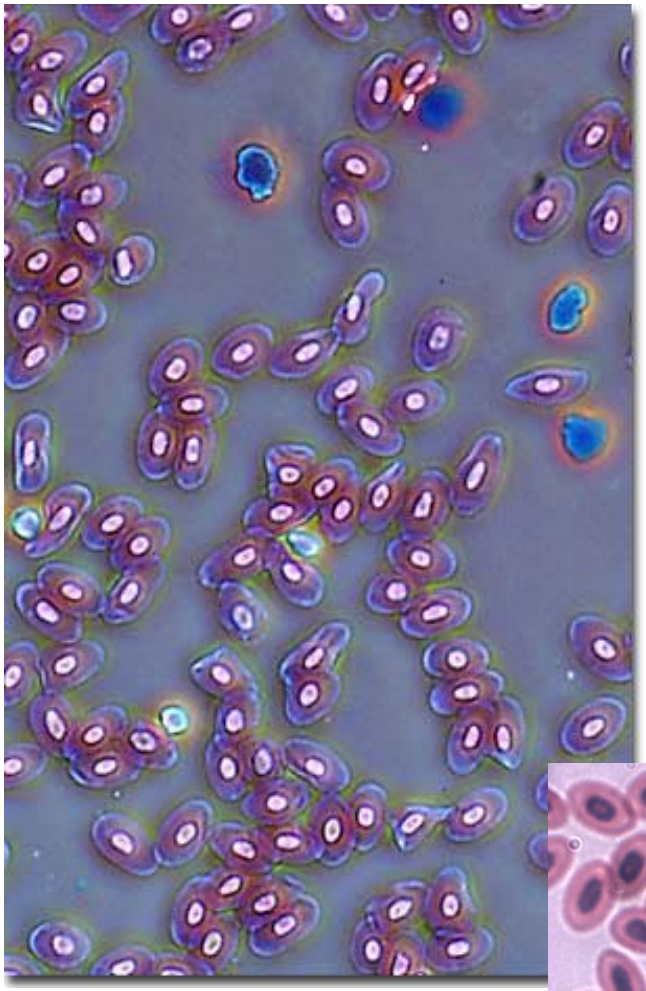
Shape

Nucleation

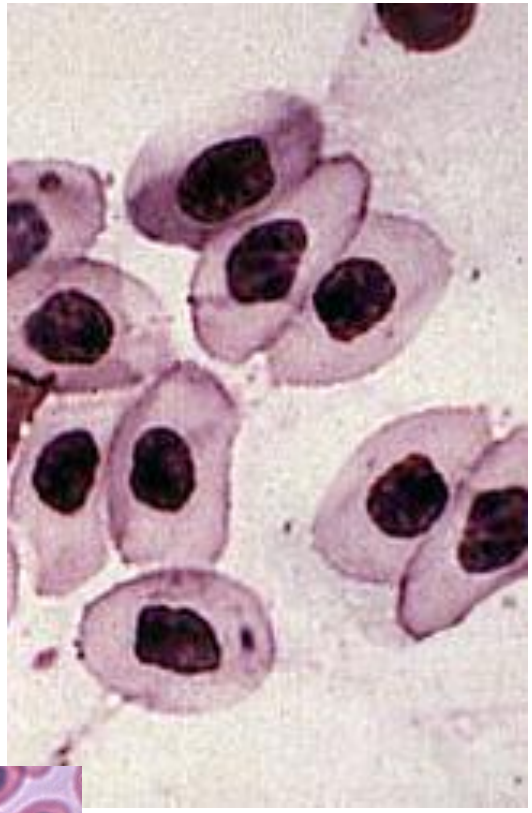


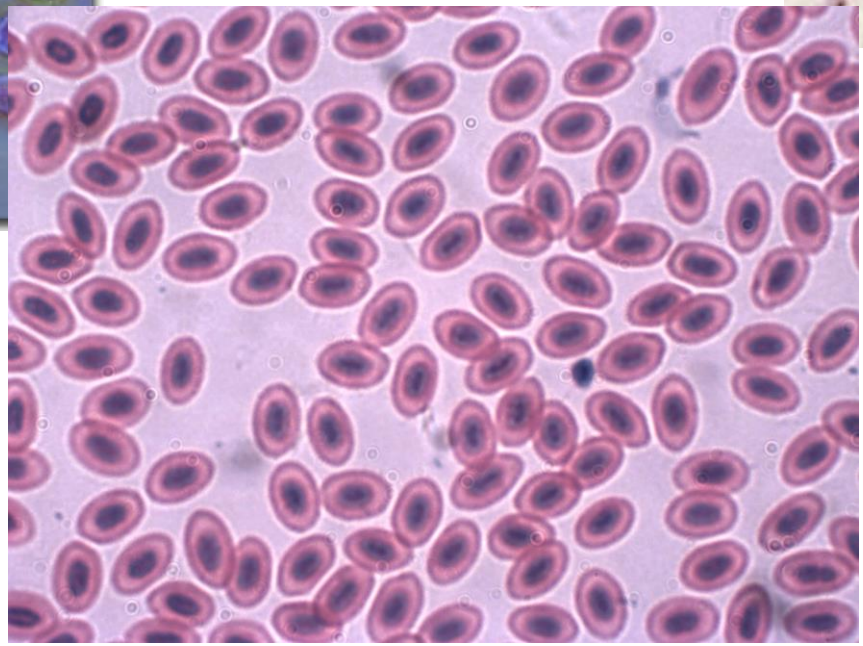


Nuclei !!



Frog Blood RBCs



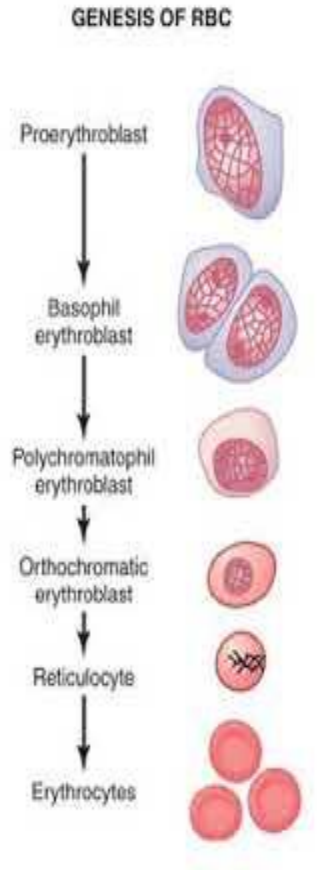


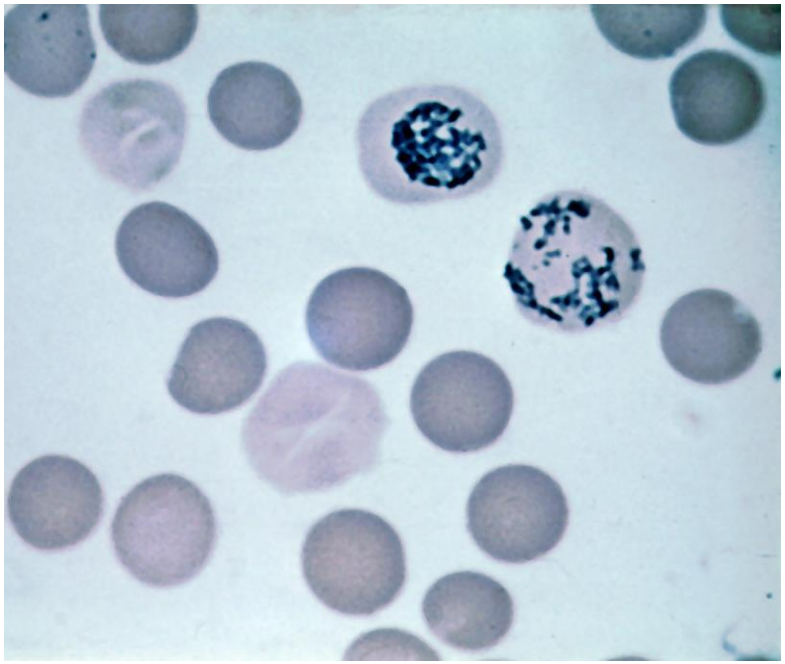
Chicken RBCs

Reticulocytes

<1%

Immature
Red Blood Cells in
circulating blood





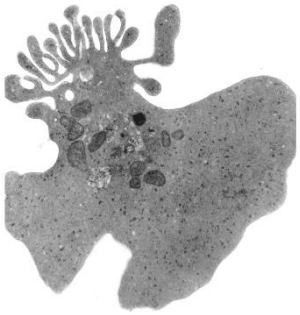
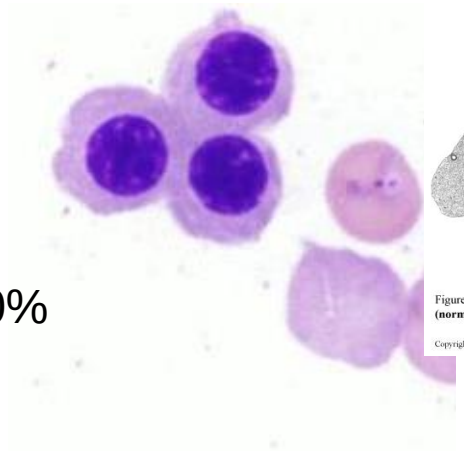


Figure 9.20. **EM of a polychromatophilic erythrocyte (reticulocyte).** X16,500.

⇧ in anaemia

Normoblasts 0%



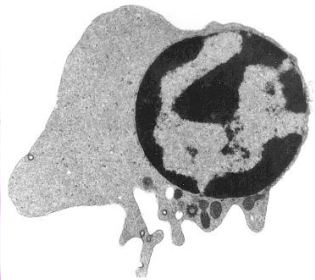
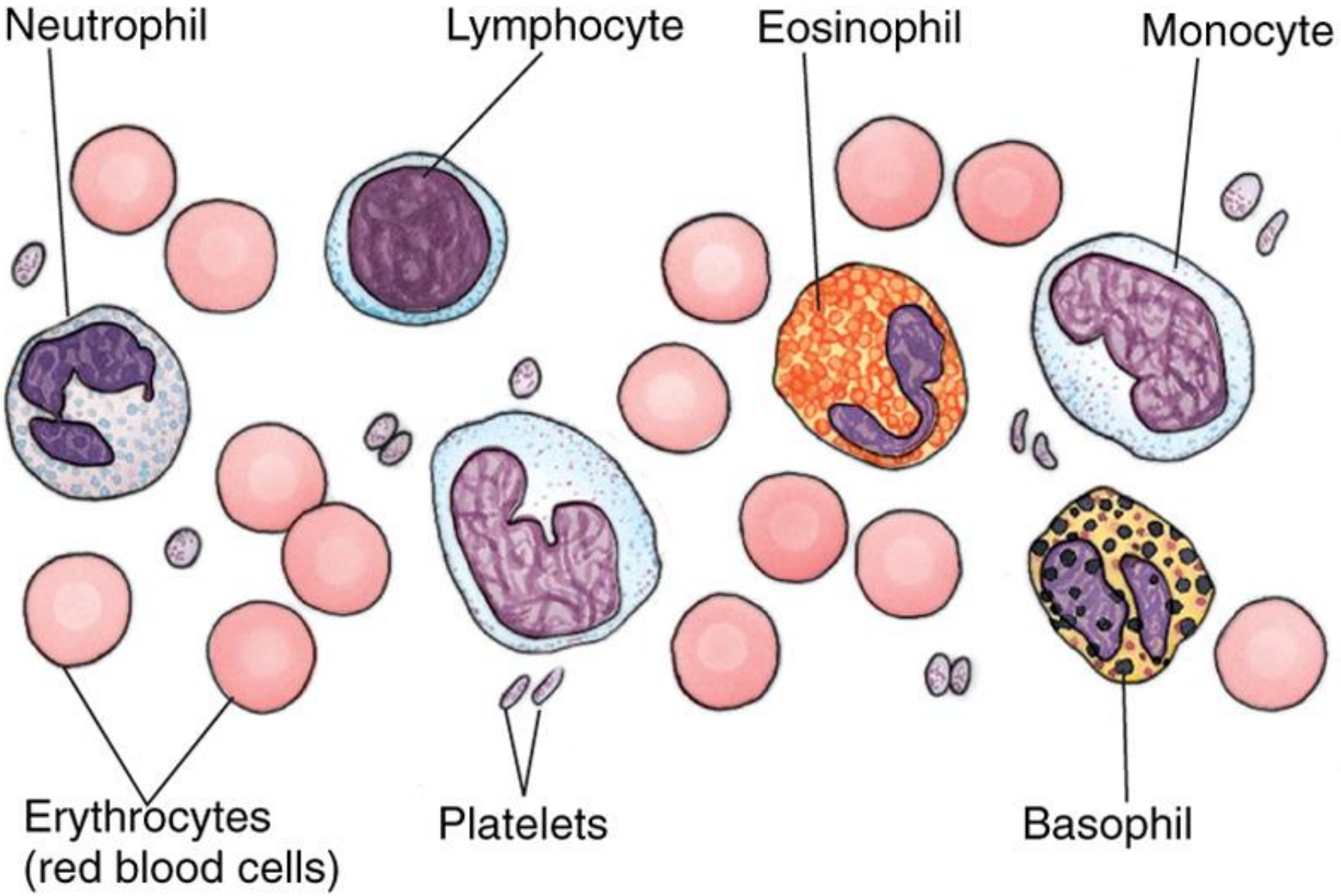
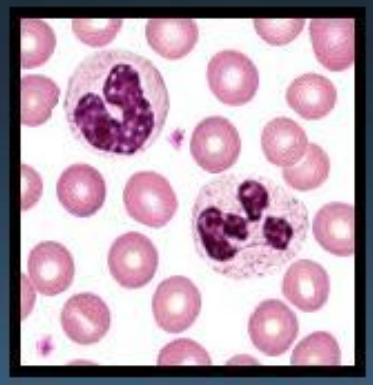


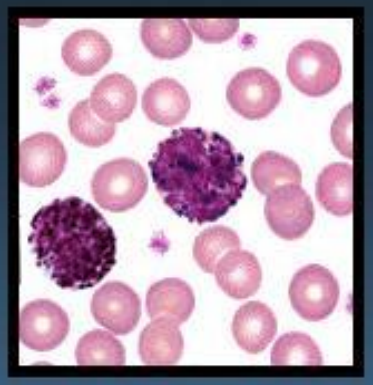
Figure 9.19. **EM of a ortochromatophilic erythroblast (normoblast).** X10,000.

Leucocytes - WBCs

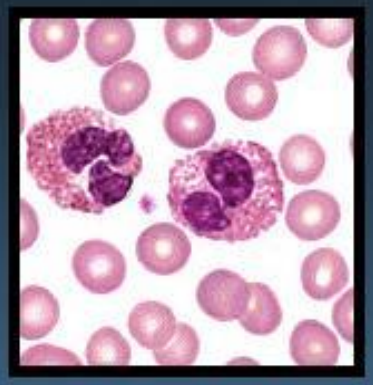




Neutrophils -
Engulf bacteria and cellular debris
37-77%



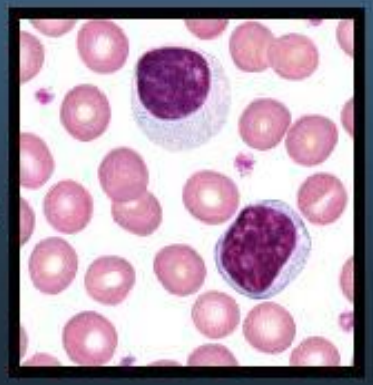
Basophils -
Hypersensitivity, Release histamine
0-1.6%

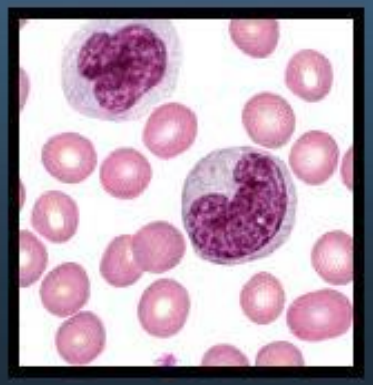


Eosinophils -
Parasitic infections, Allergic response
1-7%

White Blood Cells

Lymphocytes -
Produce antibodies, regulate the immune response
10-44%





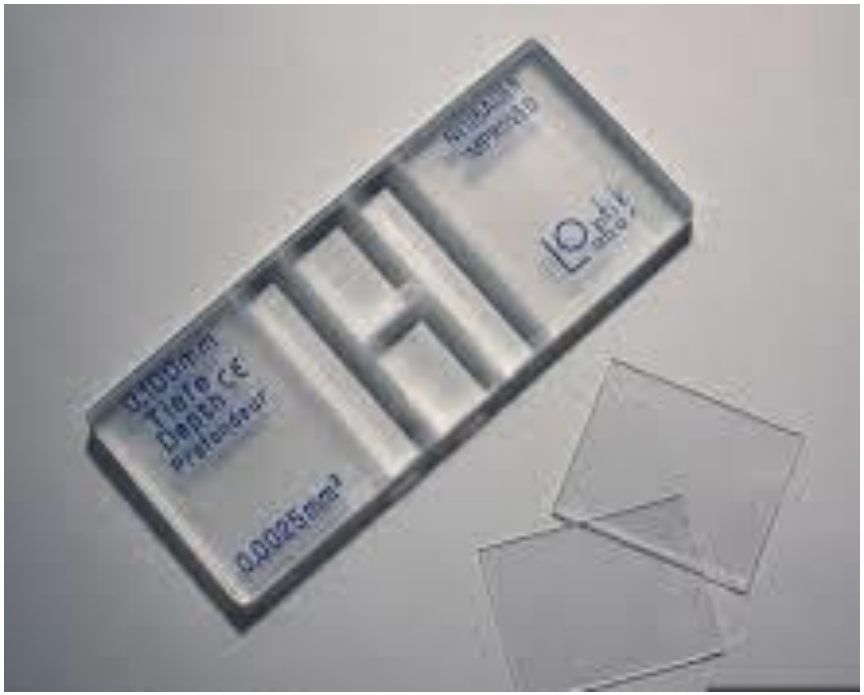
Monocytes -
Engulf cellular debris, antigen processing
2-10%

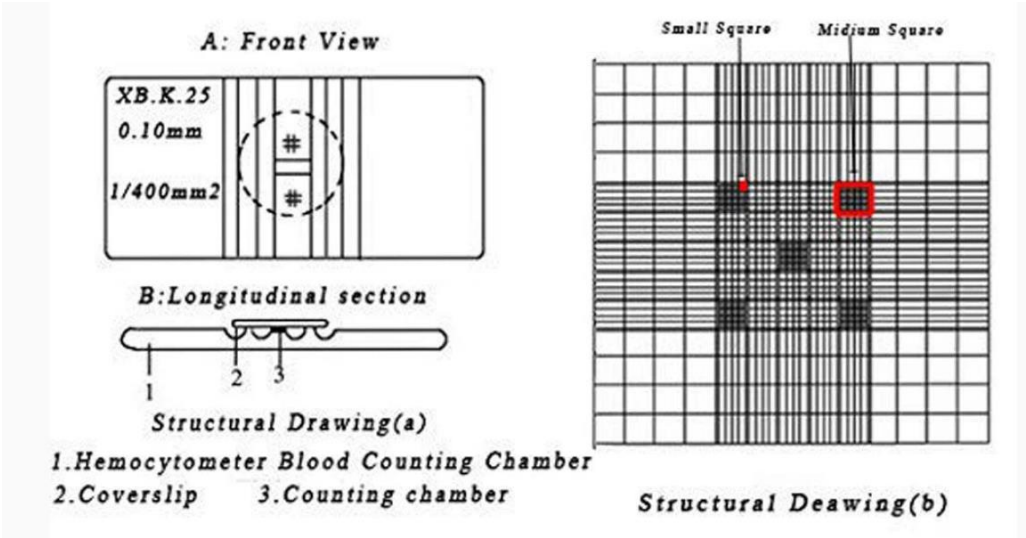
1µl
5 million rbc
8000 wbc
300,000 pl

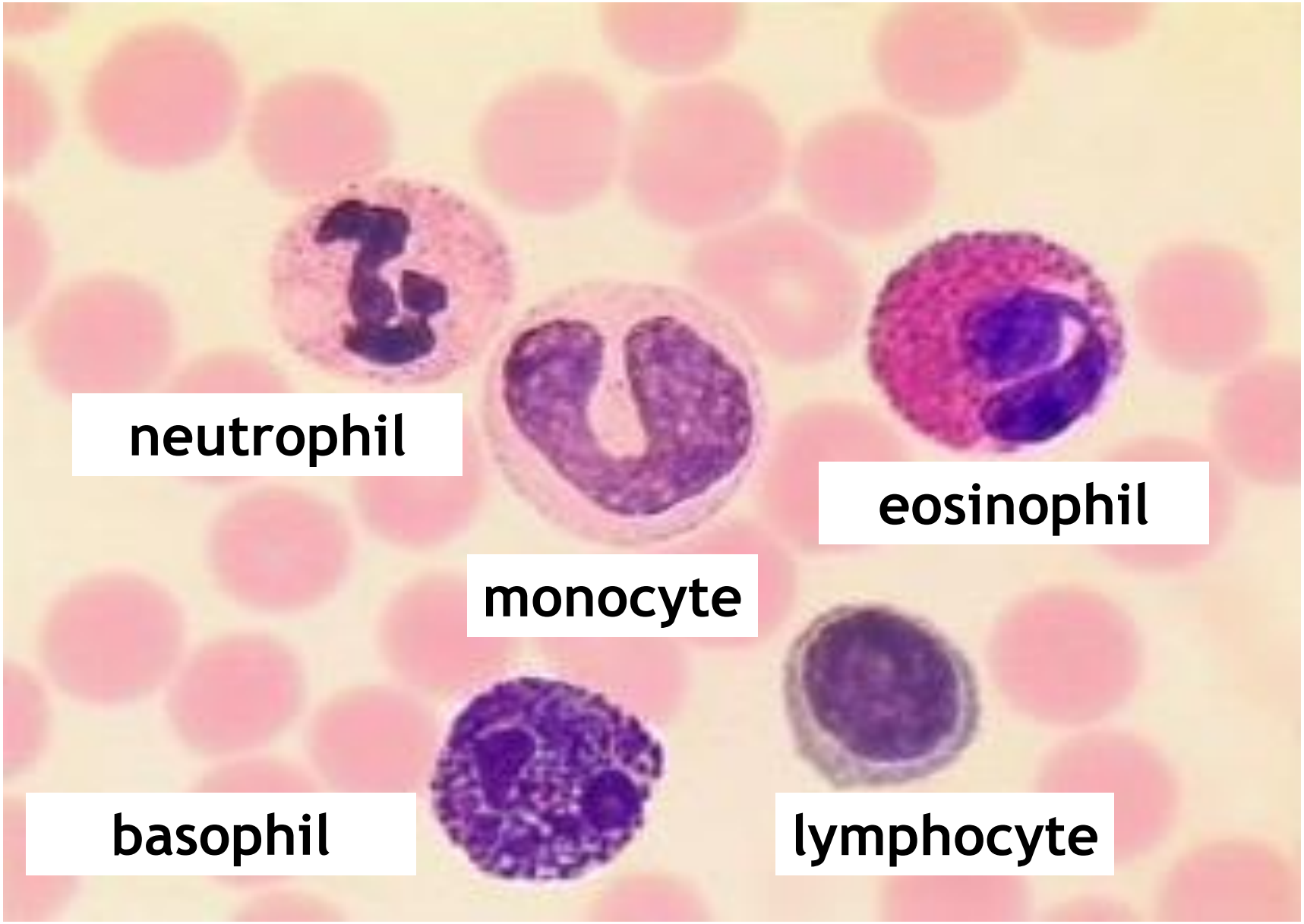
WBCs
N 60%
L 30%
M 5%
E 3%
B 1%
? 1%

Haemocytometer Slide

Differential white cell count



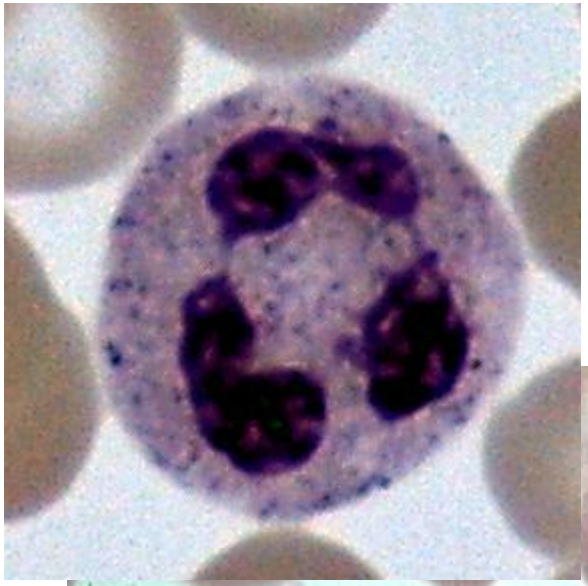




White Blood Cells

Granular Leucocytes

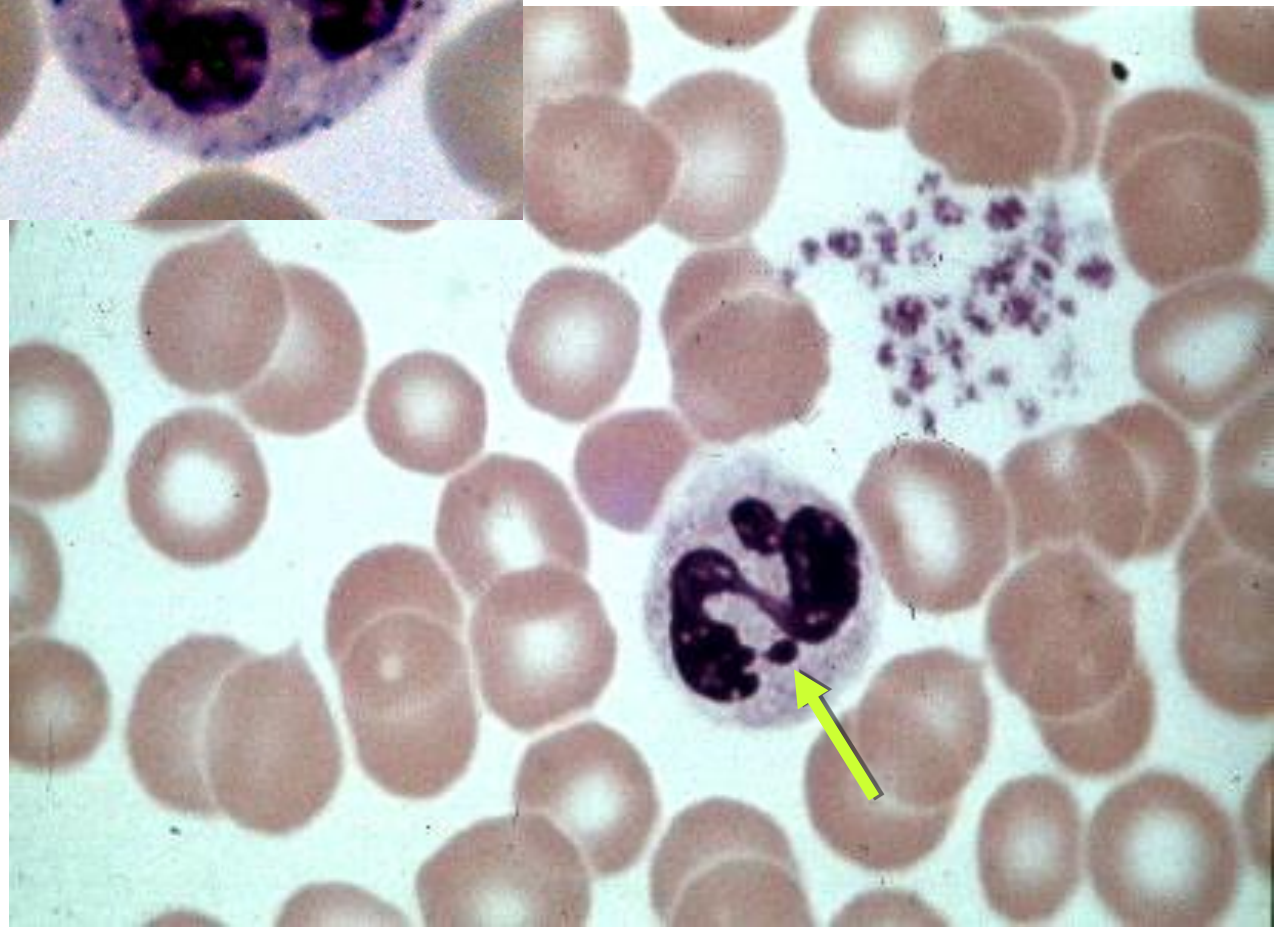
- Neutrophils
- Eosinophils
- Basophils



Neutrophil

Granules unstained

Multilobed nuclei
(polymorphonuclear)



Functions

Motility

Phagocytosis

Number

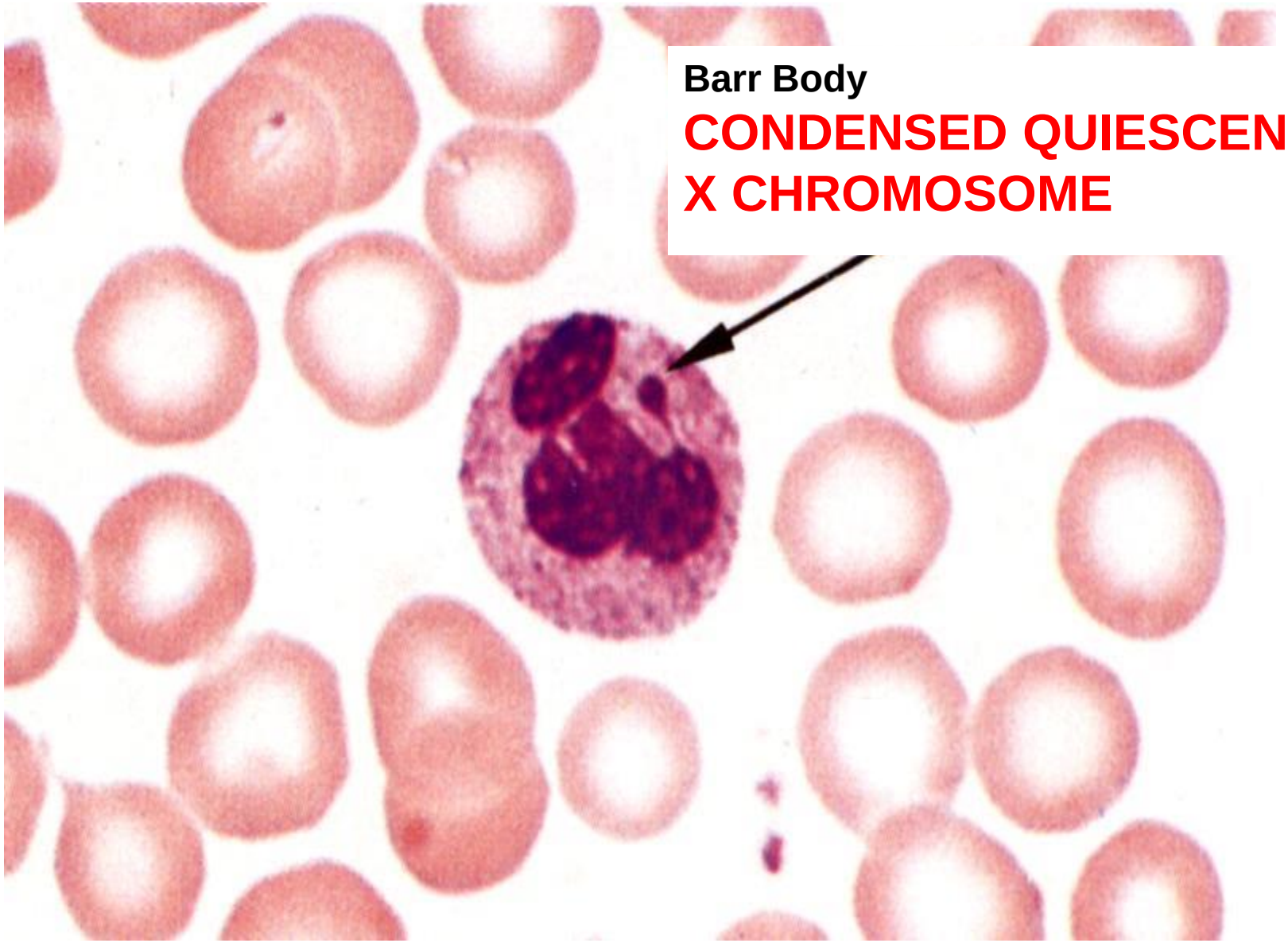
60% WBCs

Size

10-12 µm

Sex

Drumsticks



Neutrophil **(~4% in Females show Barr Body)**

TEM

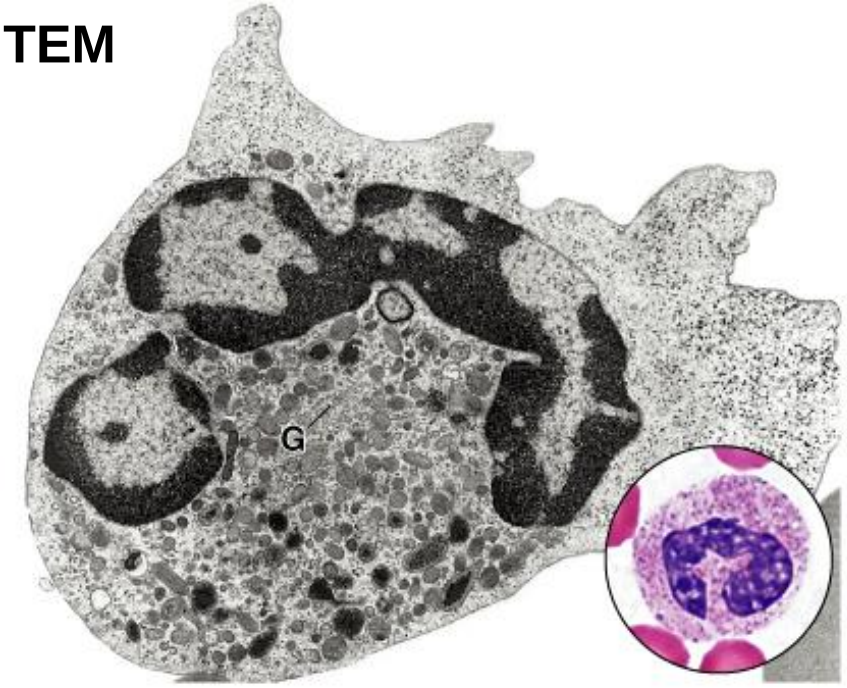
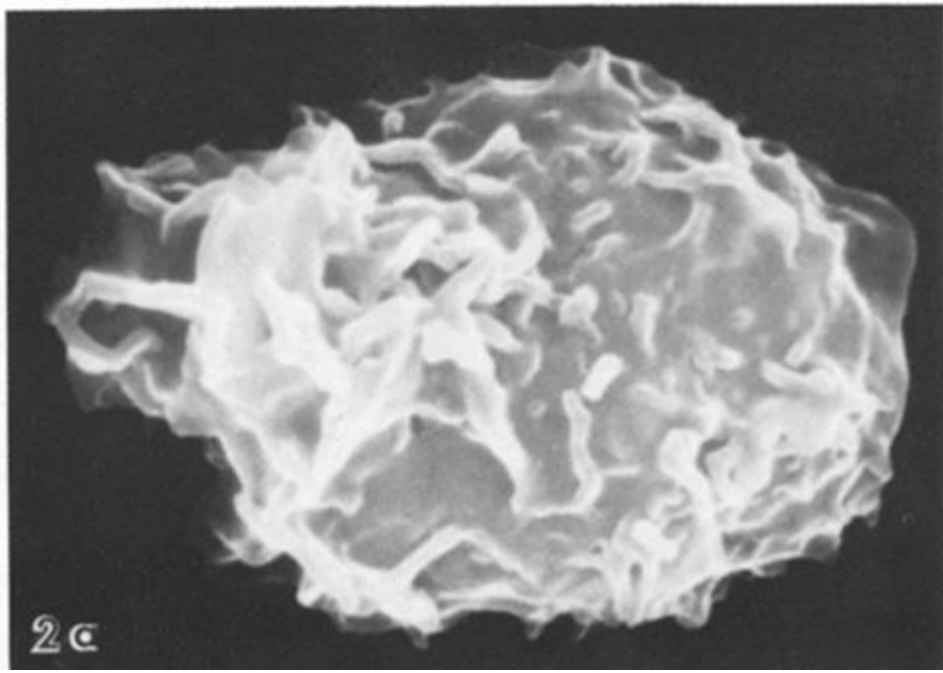


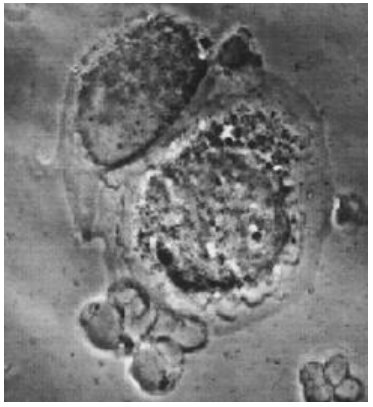
Figure 9.7. **EM of a human mature neutrophil.** *G*, Golgi apparatus. X22,000. **Inset.** Neutrophil from a blood smear observed in the light microscope. X1,800.

Granules

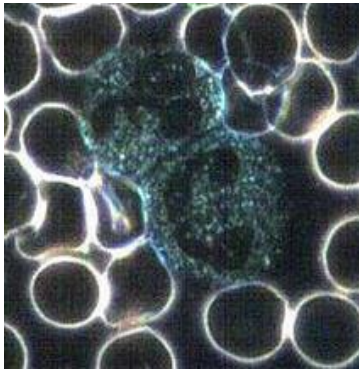
- Specific – enzymes
- Azurophilic – lysosomes
- Tertiary – phosphatases, metalloptoteinases

SEM



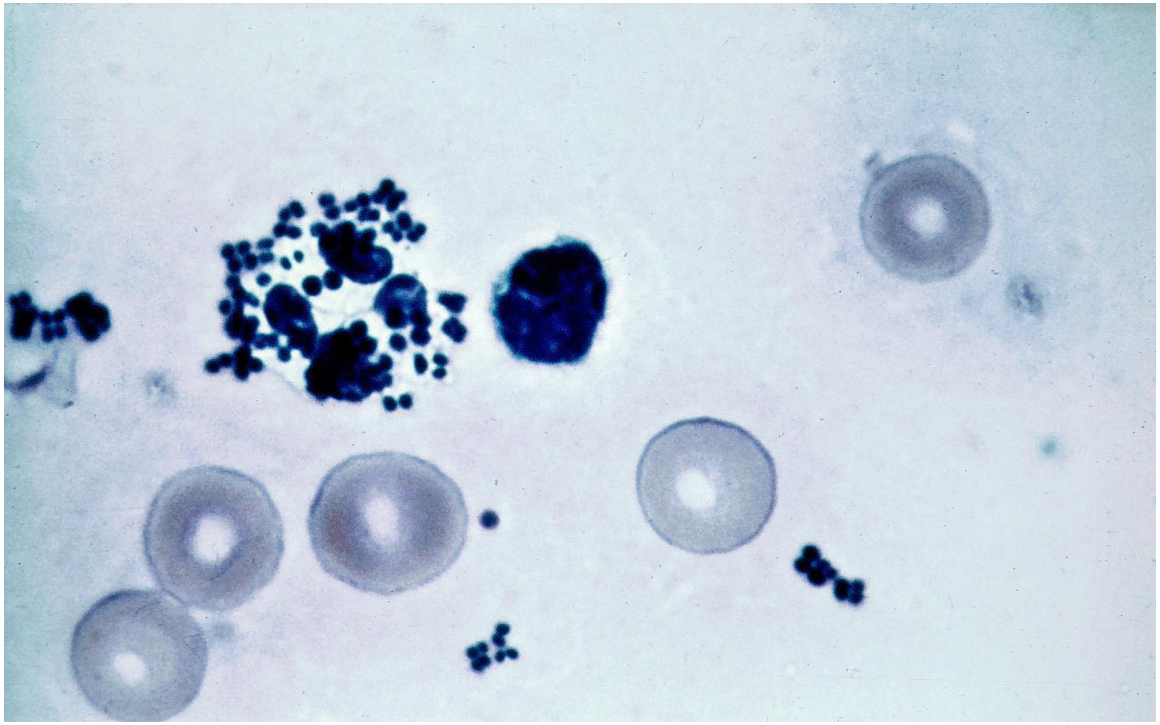


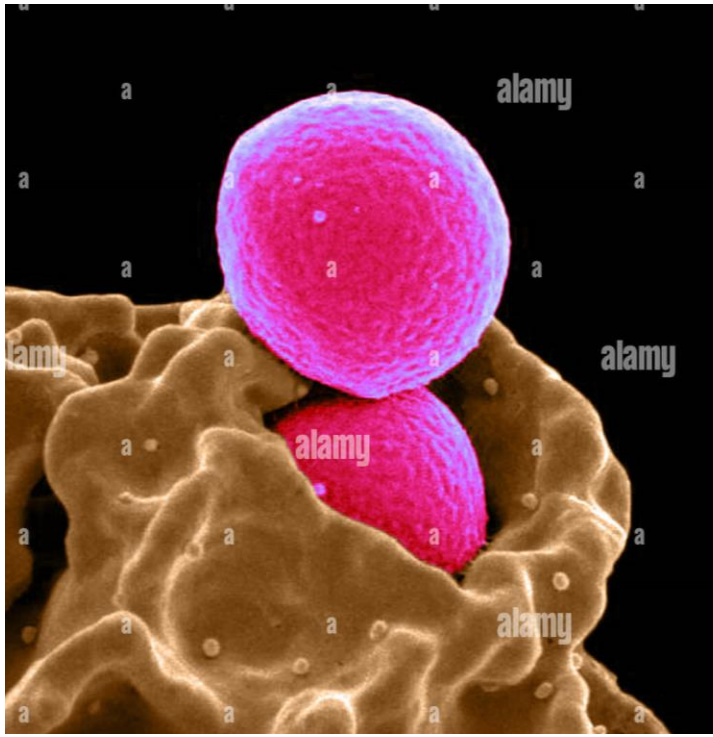
Phase Contrast



Darkfield

Neutrophil Phagocytosing Bacteria





Neutrophils escaping into other tissues to cause inflammatory response and pus

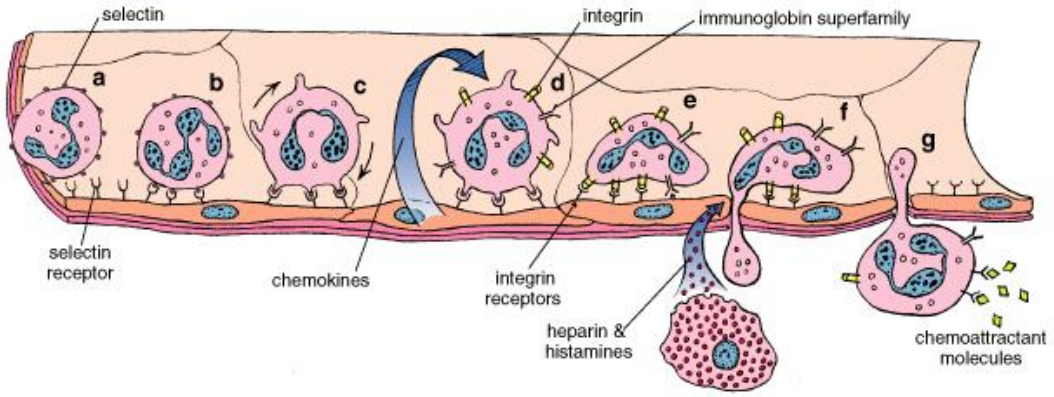
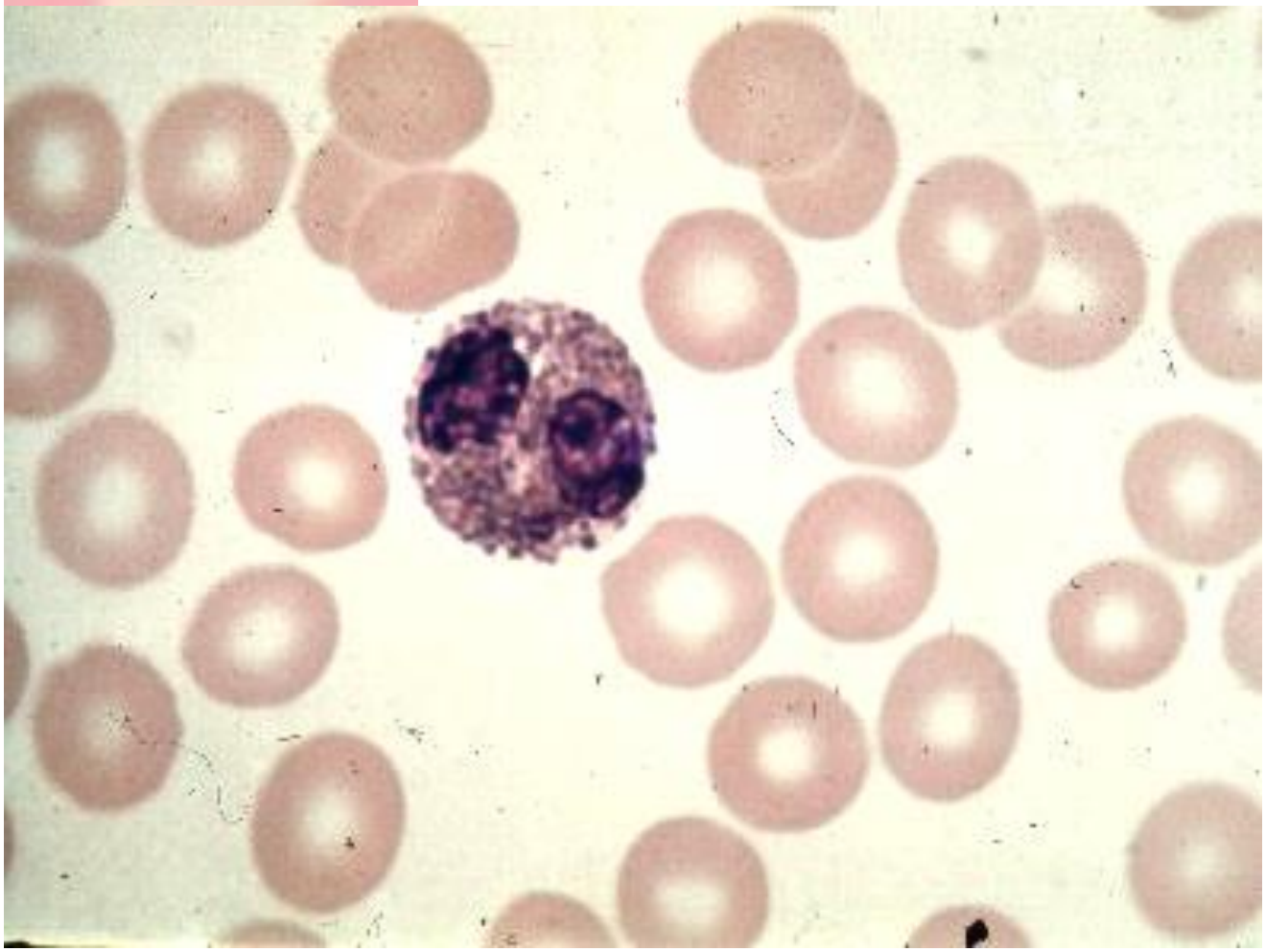


Figure 9.8. **Diagram of events in the migration of a neutrophil from a postcapillary venule into the connective tissue.** See text for description of steps.

Eosinophil

Granules stained red

Bilobed nucleus



Functions

Allergic response

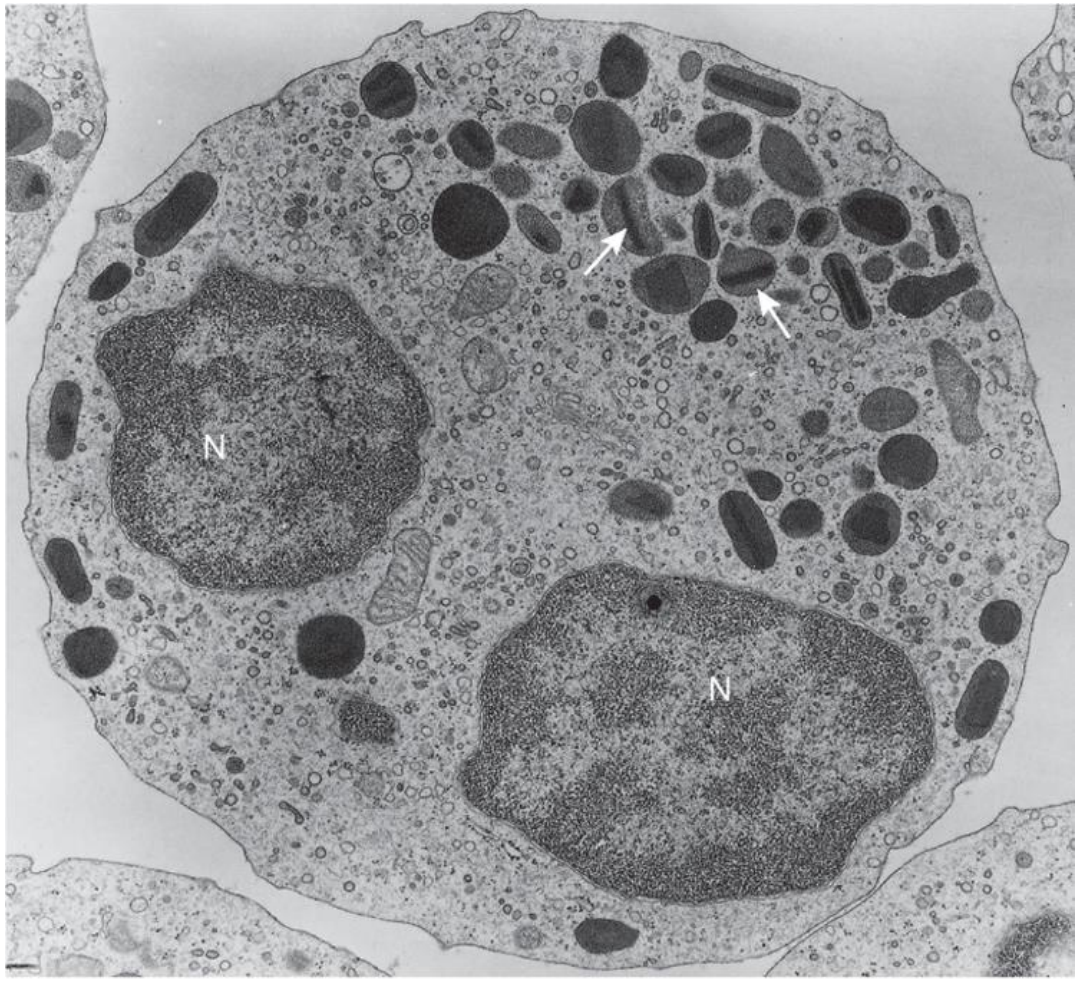
Number

3% WBCs

Size

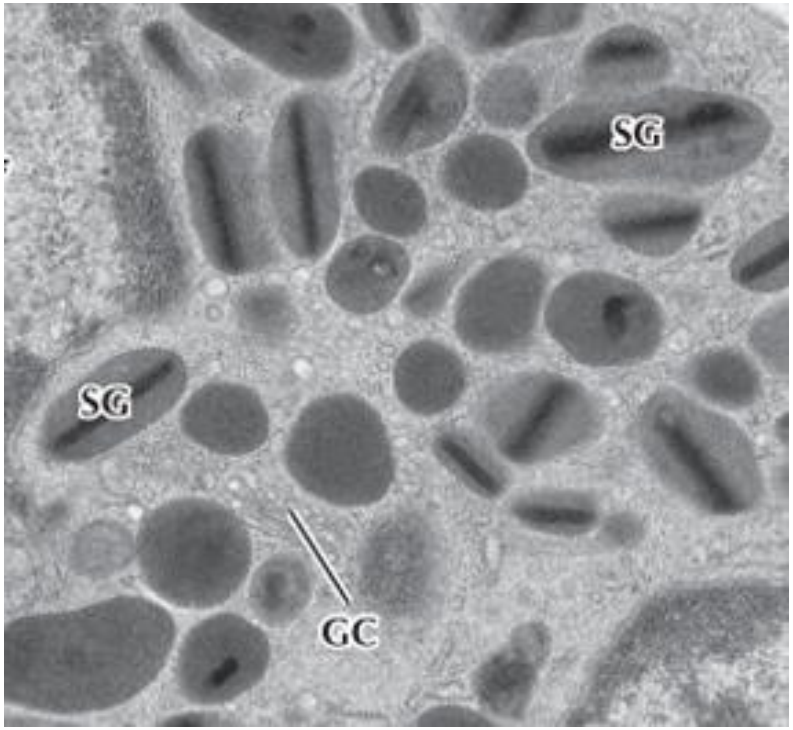
12-14 µm

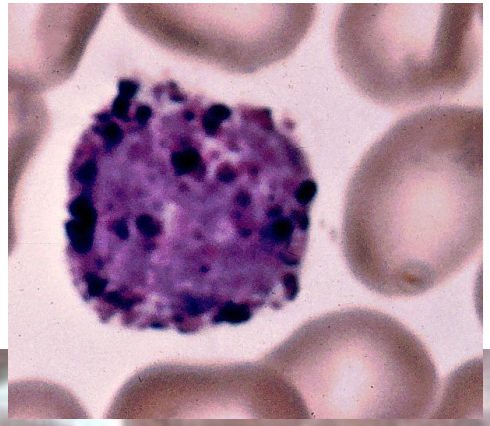
TEM



Granules

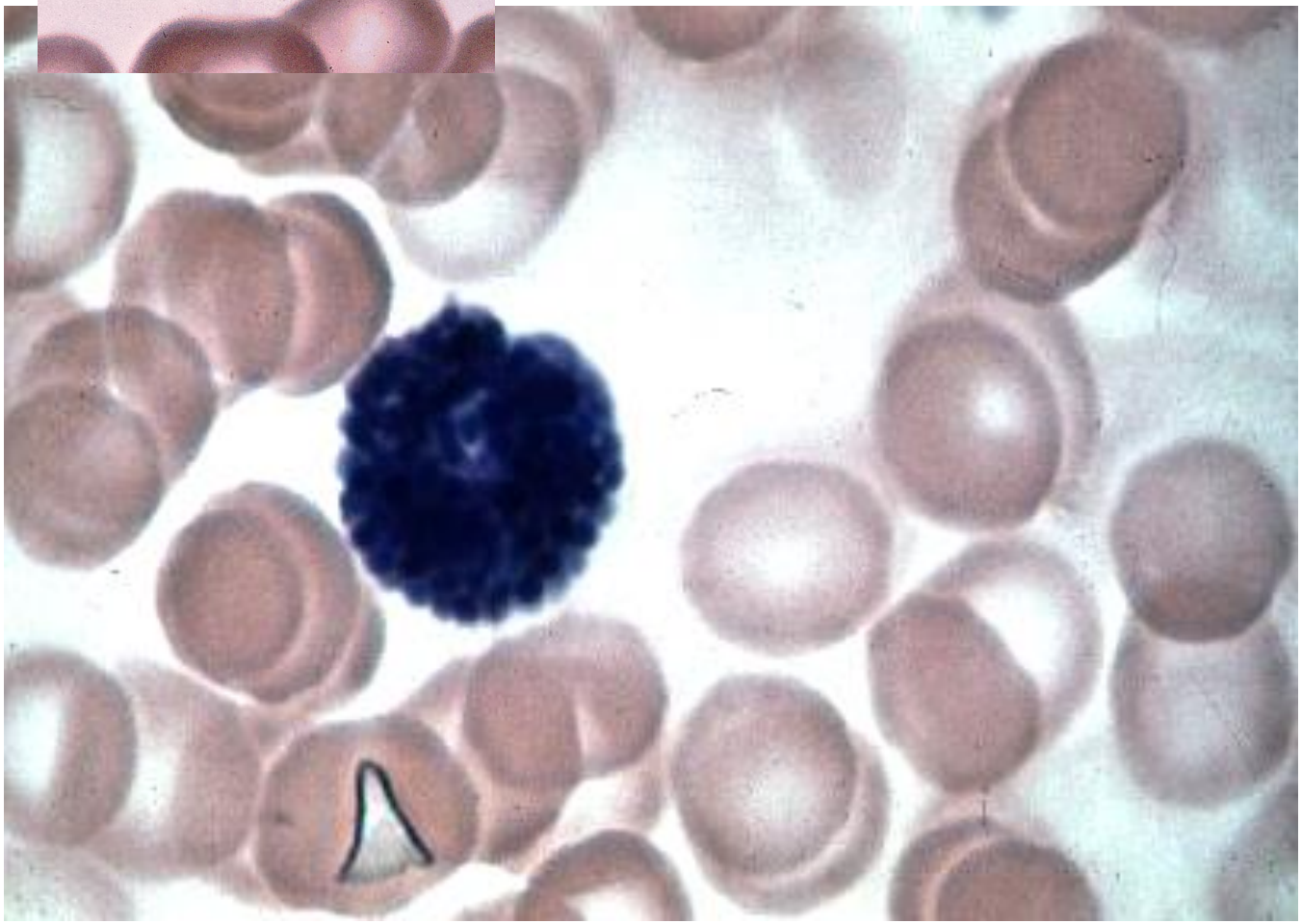
- crystalloid bodies – cytotoxic to protozoans & helminths
- lysosomes





Basophil

Granules – large, dark blue (‘Blackberry’) Nucleus multilobed



Functions

hypersensitivity

≡ mast cell

bind IgE, vasoactive

Number

<1%

Size

12 µm

TEM

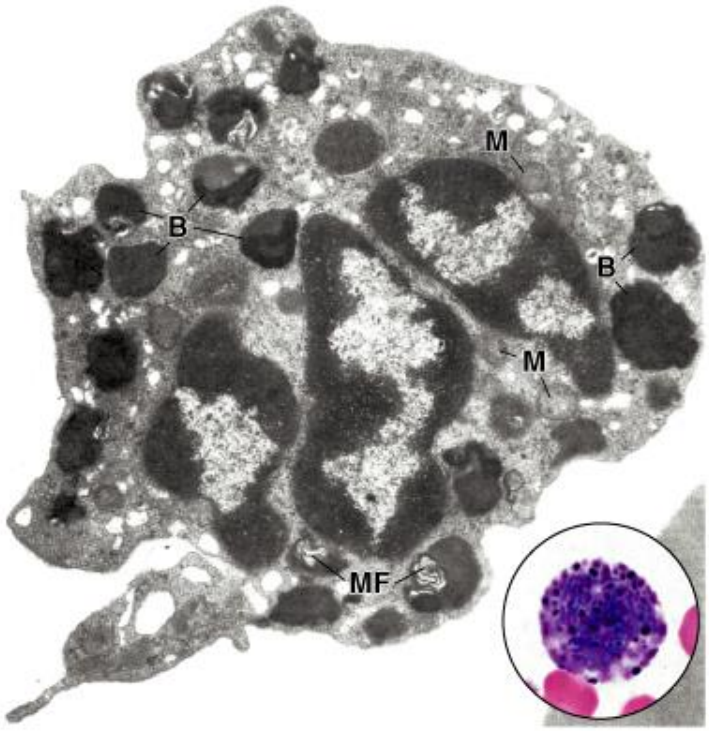
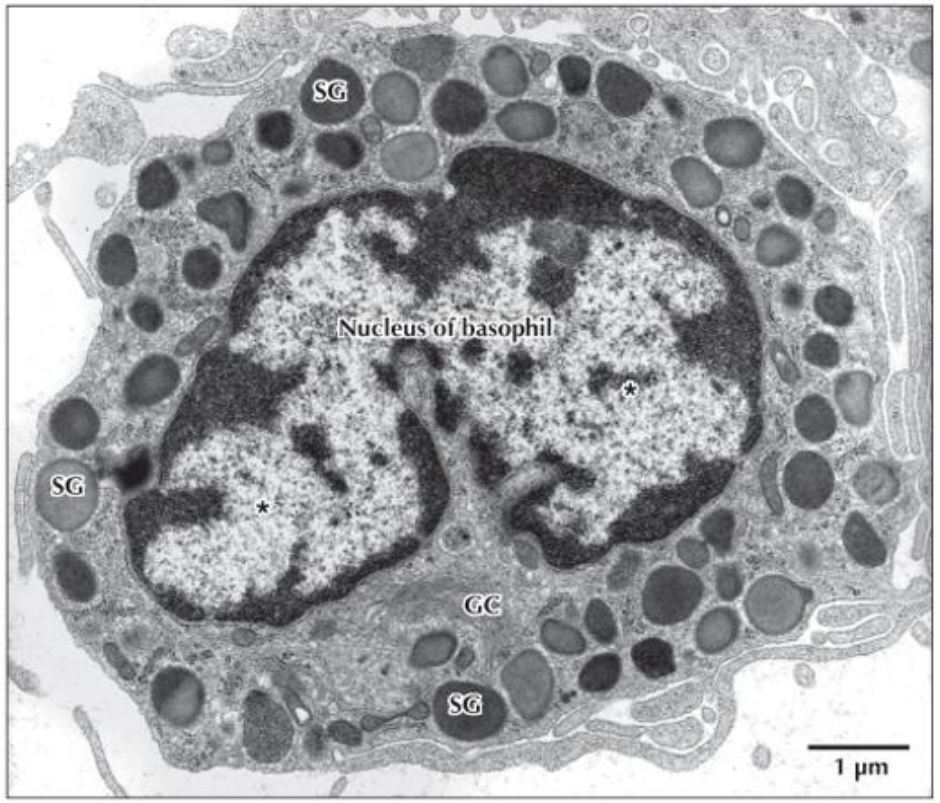
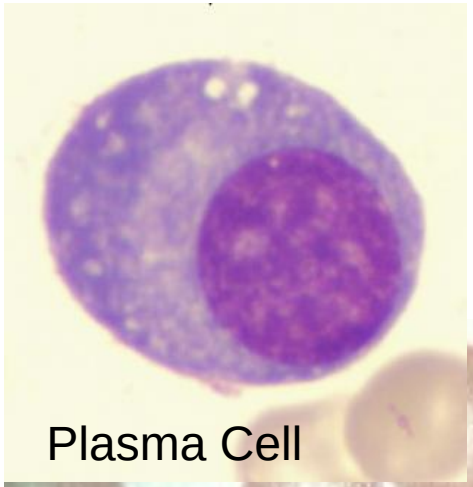


Figure 9.11. **EM of a human basophil.** *B,* basophil granules; *Mf,* myelin figures. X26,000 **Inset.** A blood smear demonstrating the light microscopic appearance of a basophil. X1,800

► **EM of a basophil.** Its nucleus is bilobed (*). A peripheral rim of heterochromatin surrounds central euchromatin. The cytoplasm has many prominent, closely packed specific granules (**SG**) that are derived from the Golgi complex (**GC**). These membrane-bound granules vary in size and density. 11,300×.

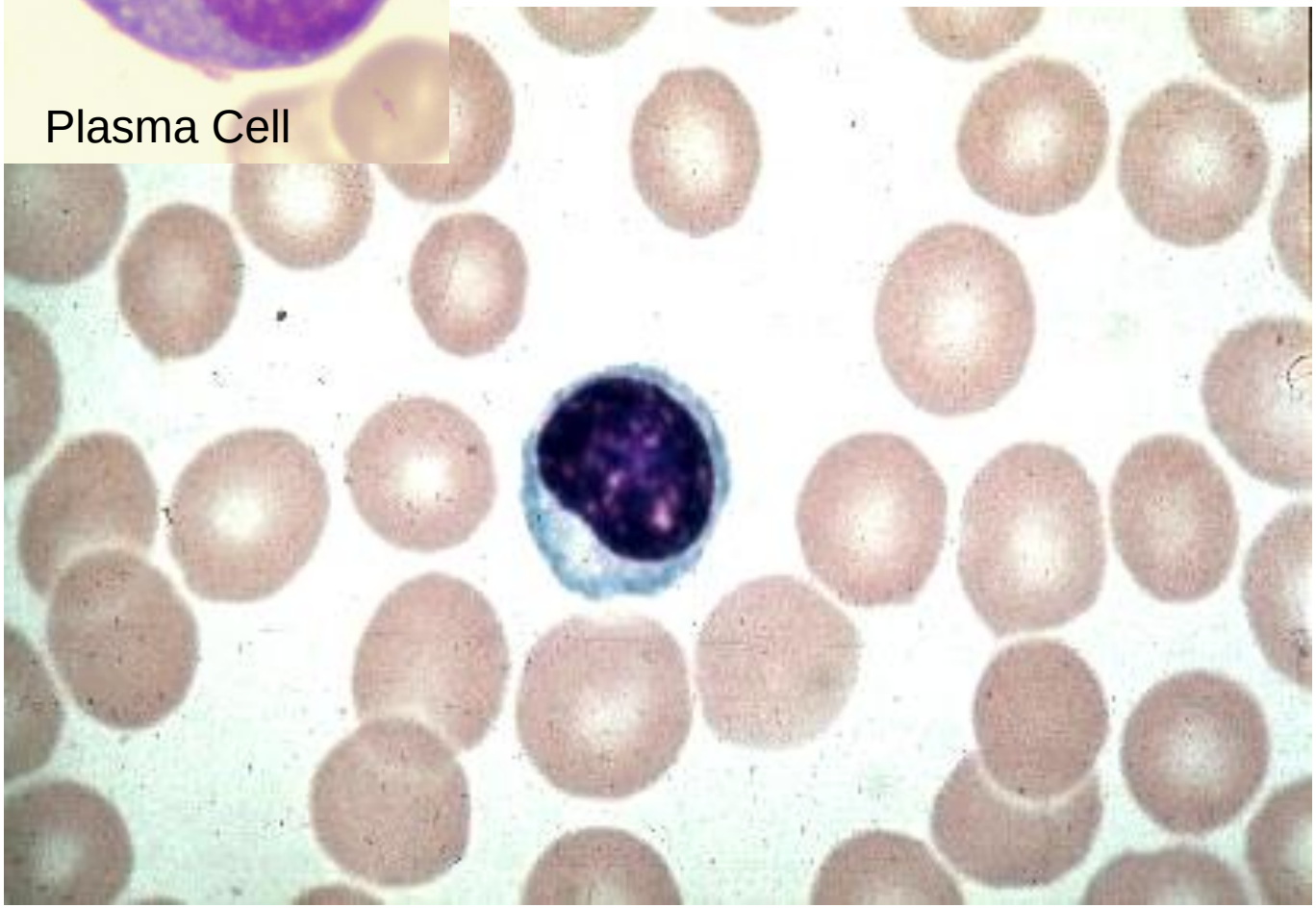


Lymphocyte



Plasma Cell

Agranular 'Spherical' nucleus



Functions

Immunity
Recirculate

Number

30% WBCs

Size

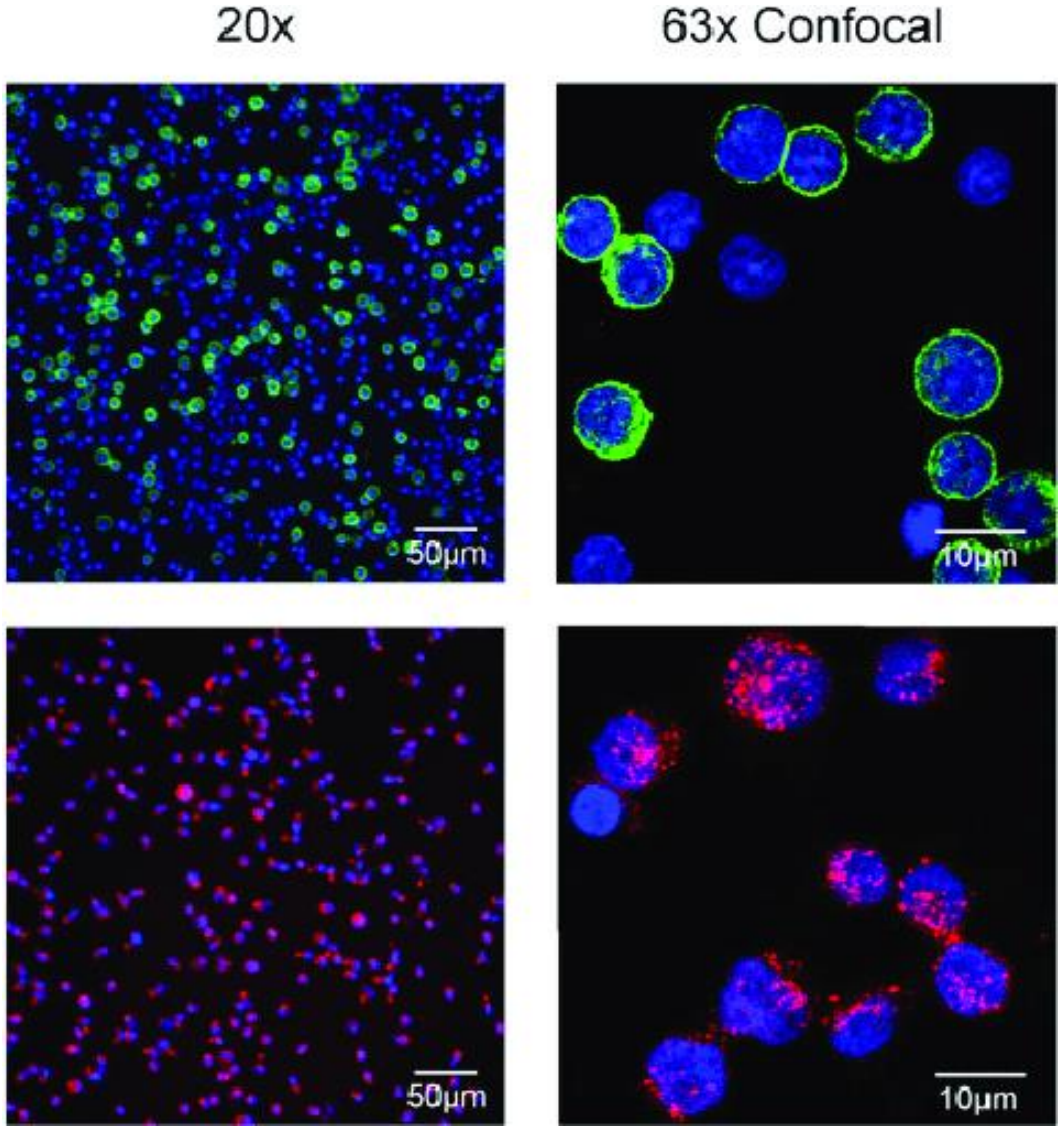
6-15 µm
S, M, L

(T, B, NK)

Recognise antigens, memory, produce antibodies, kill cells

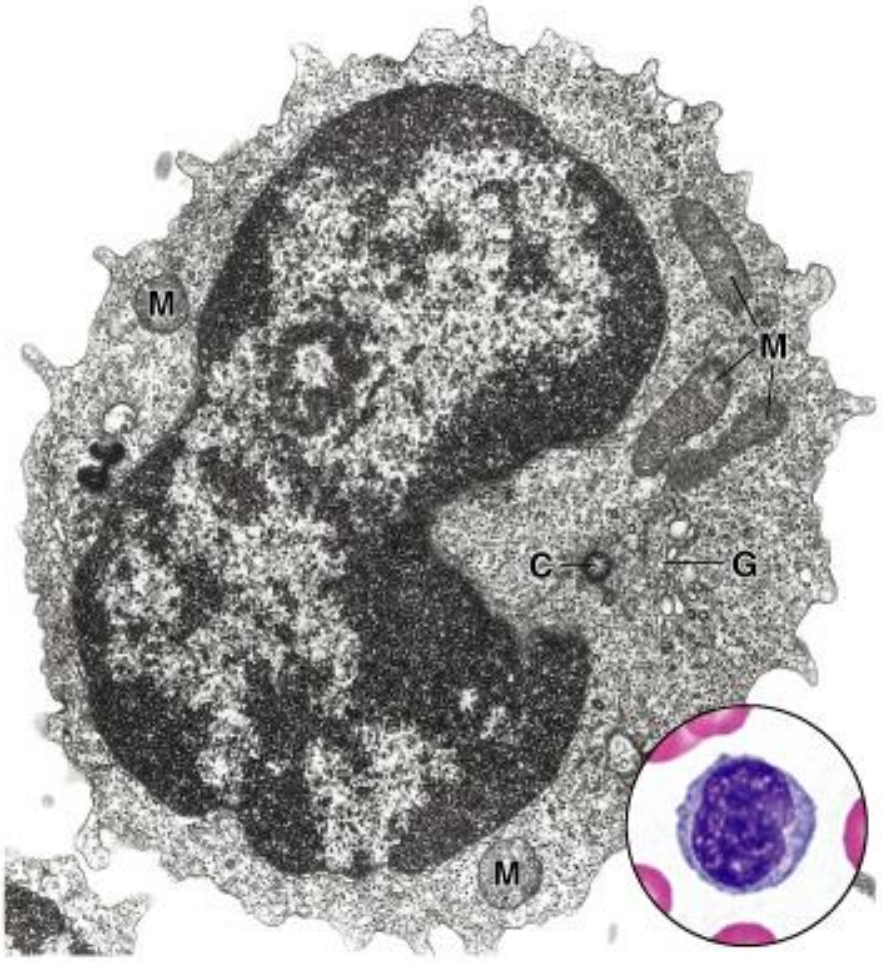
Fluorescence Microscopy

Immunostaining with fluorescent antibodies used to identify specific lymphocyte types



TEM

Ribosomes
Golgi
Centriole



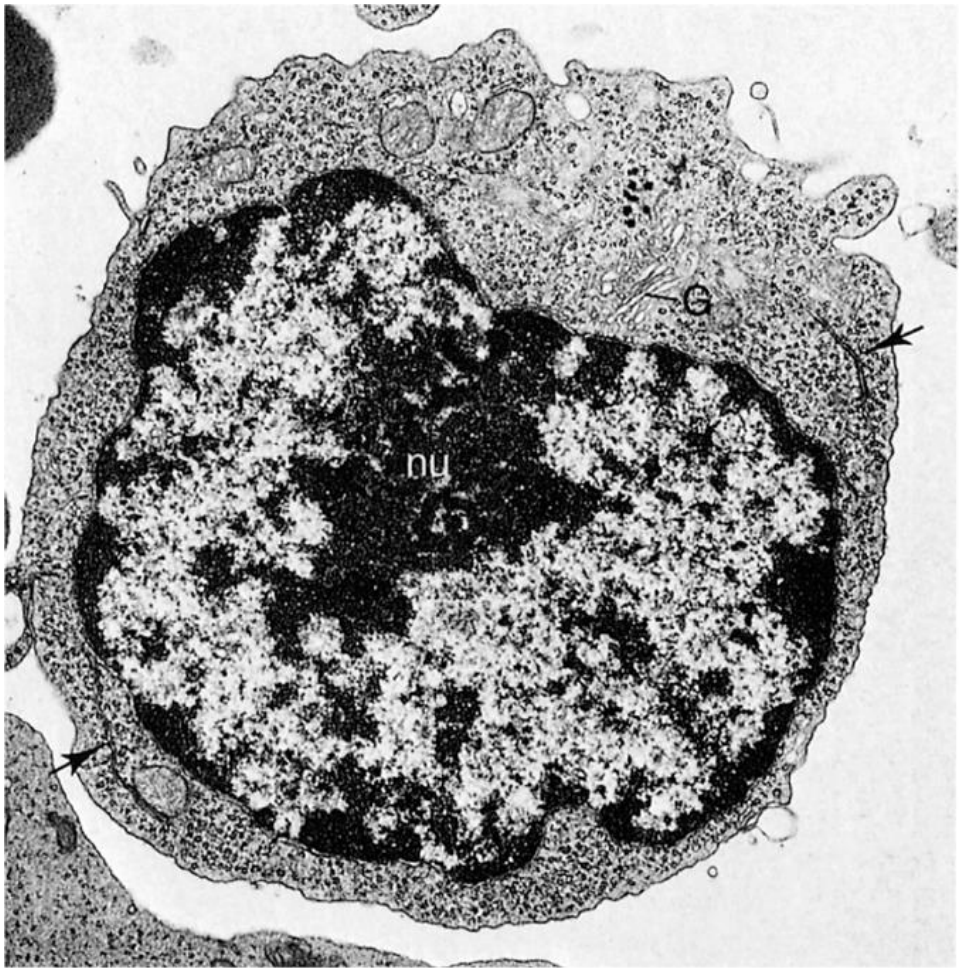
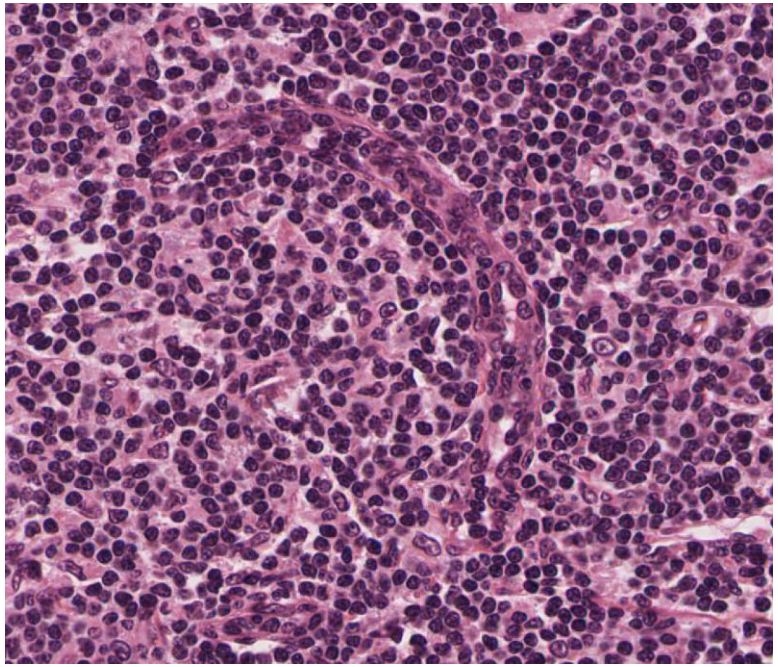
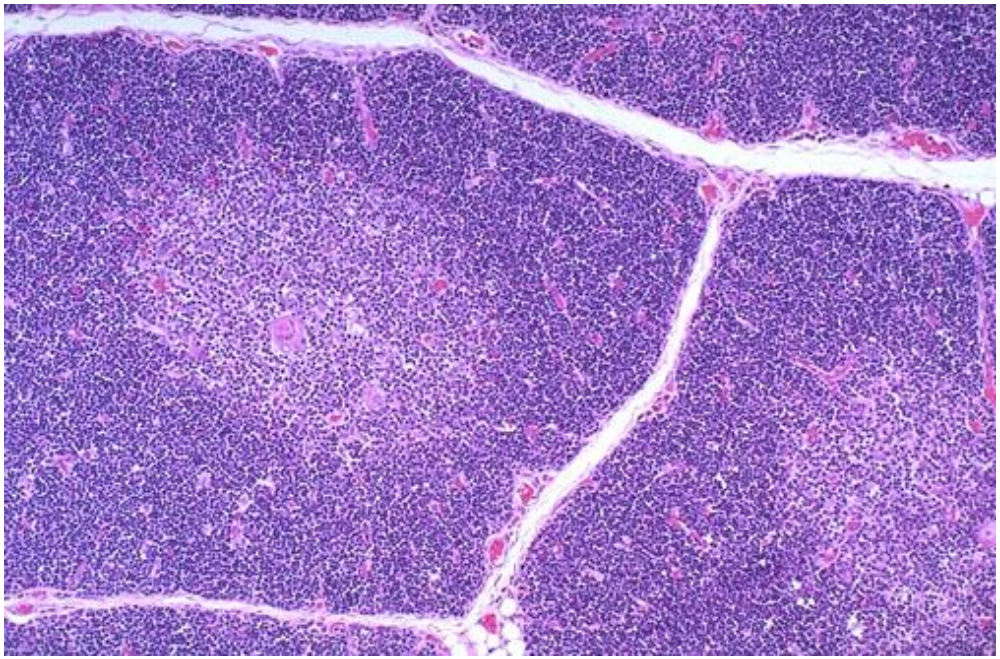


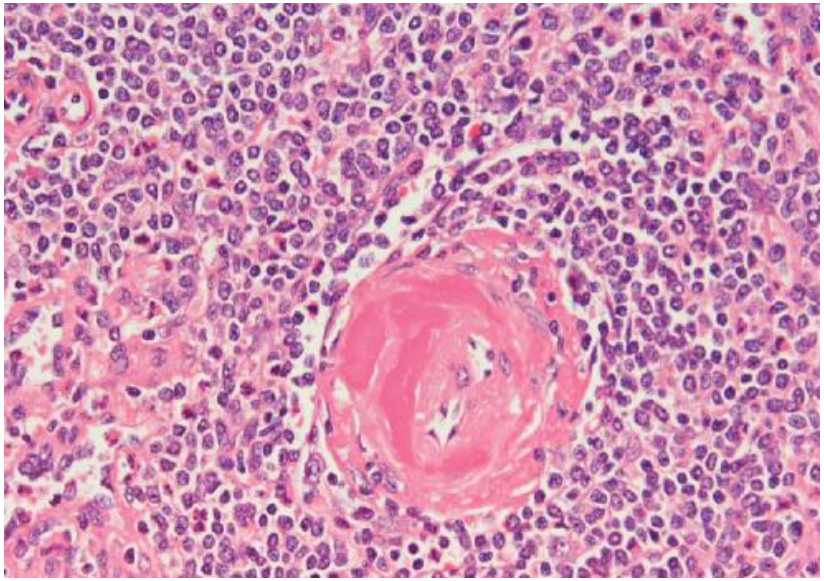
Figure 9.12. **EM of a medium-sized lymphocyte.** *M*, mitochondria; *G*, Golgi apparatus; *C*, centriole.. X26,000 **Inset**. Light microscopic appearance of a medium-sized lymphocyte from a blood smear. X1,800.

Lymph Node



Thymus

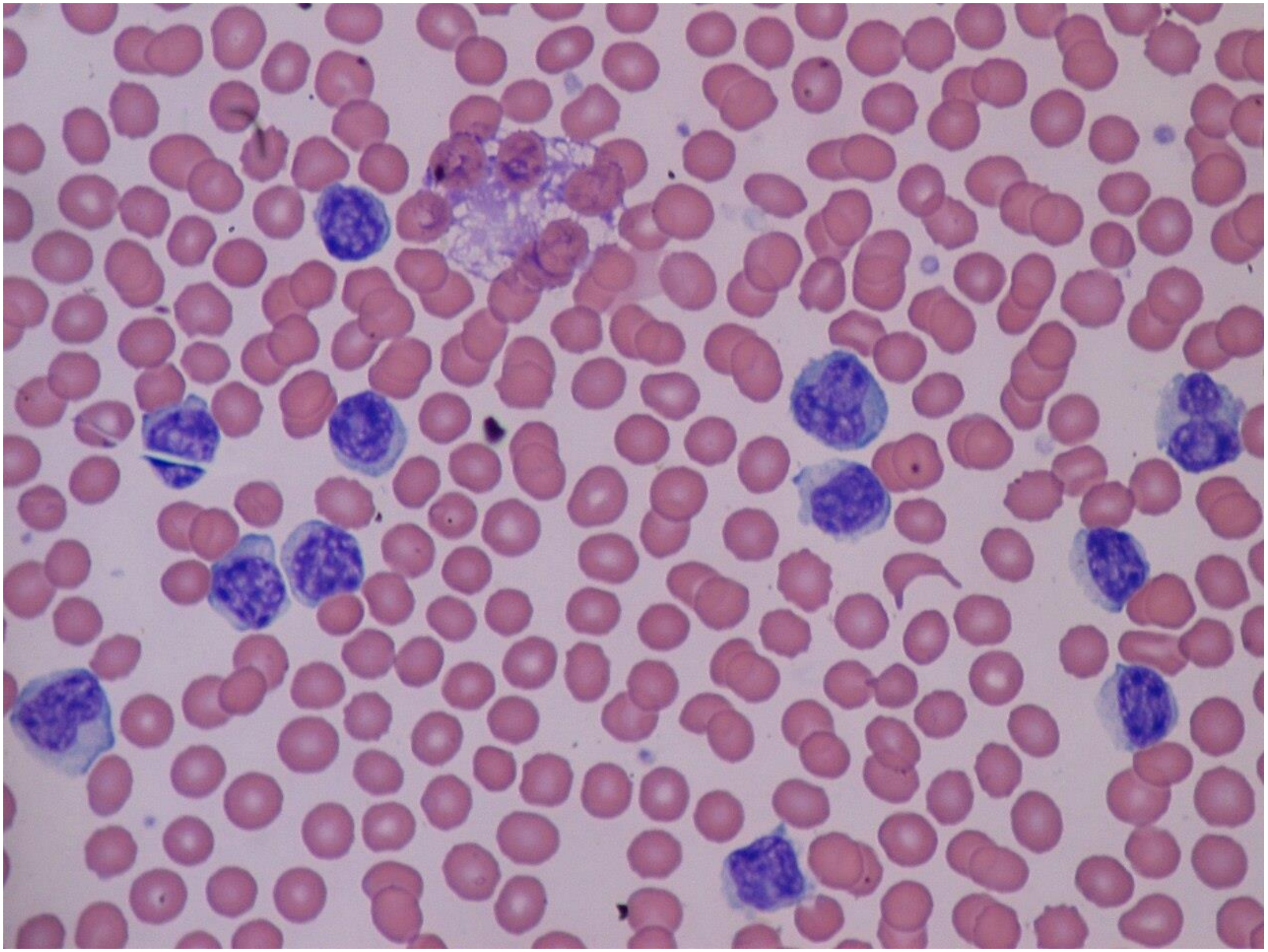




Spleen

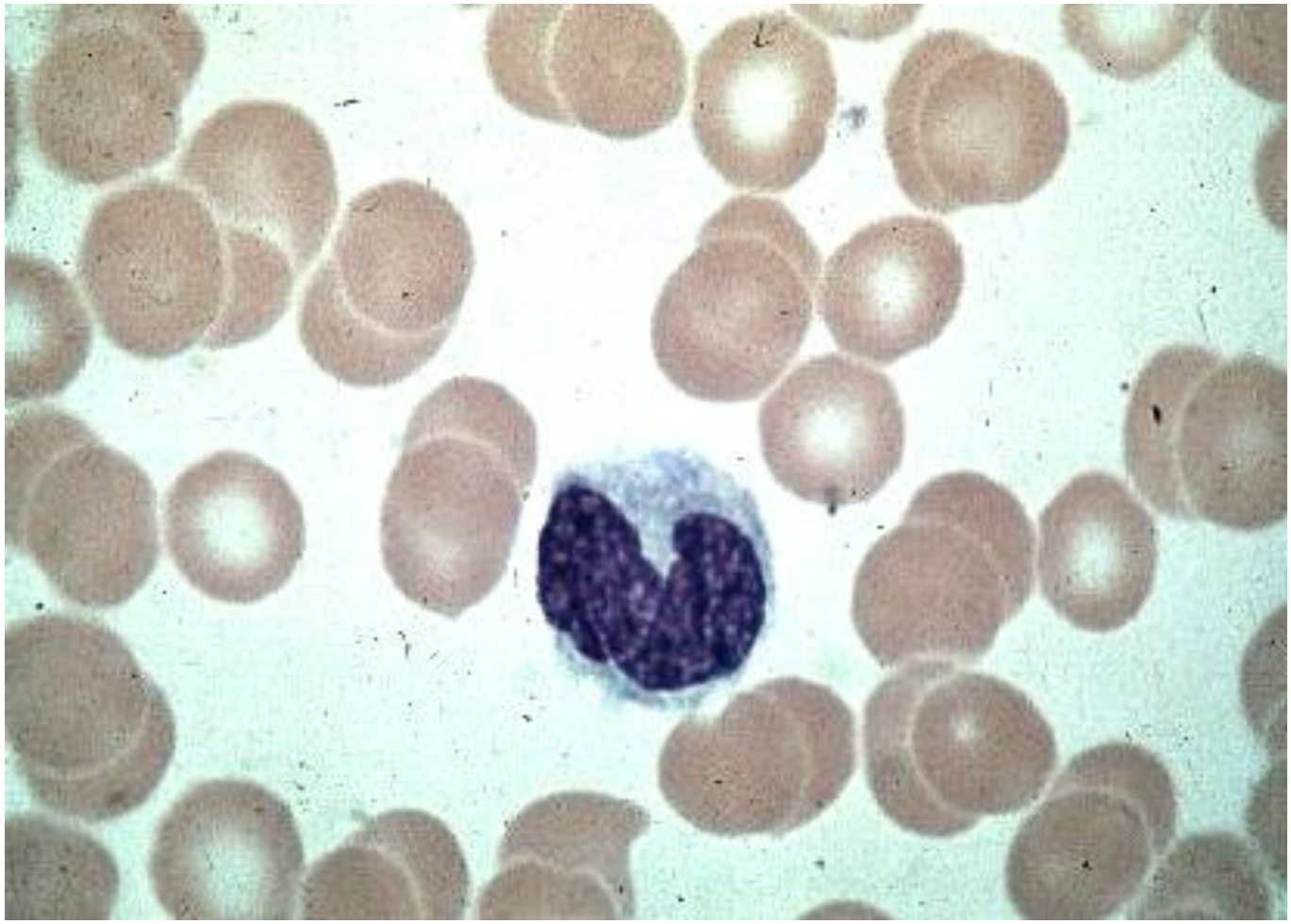
Most lymphocytes are located in the Lymphoid Organs

Lymphocytosis (>40% WBCs are Lymphocytes) due to infection, leukemia, …, <2y



Monocyte

Agranular Deeply indented nucleus



Functions

Motility

Phagocytosis

≡ macrophages

Number

5% WBCs

Size

18 µm

TEM

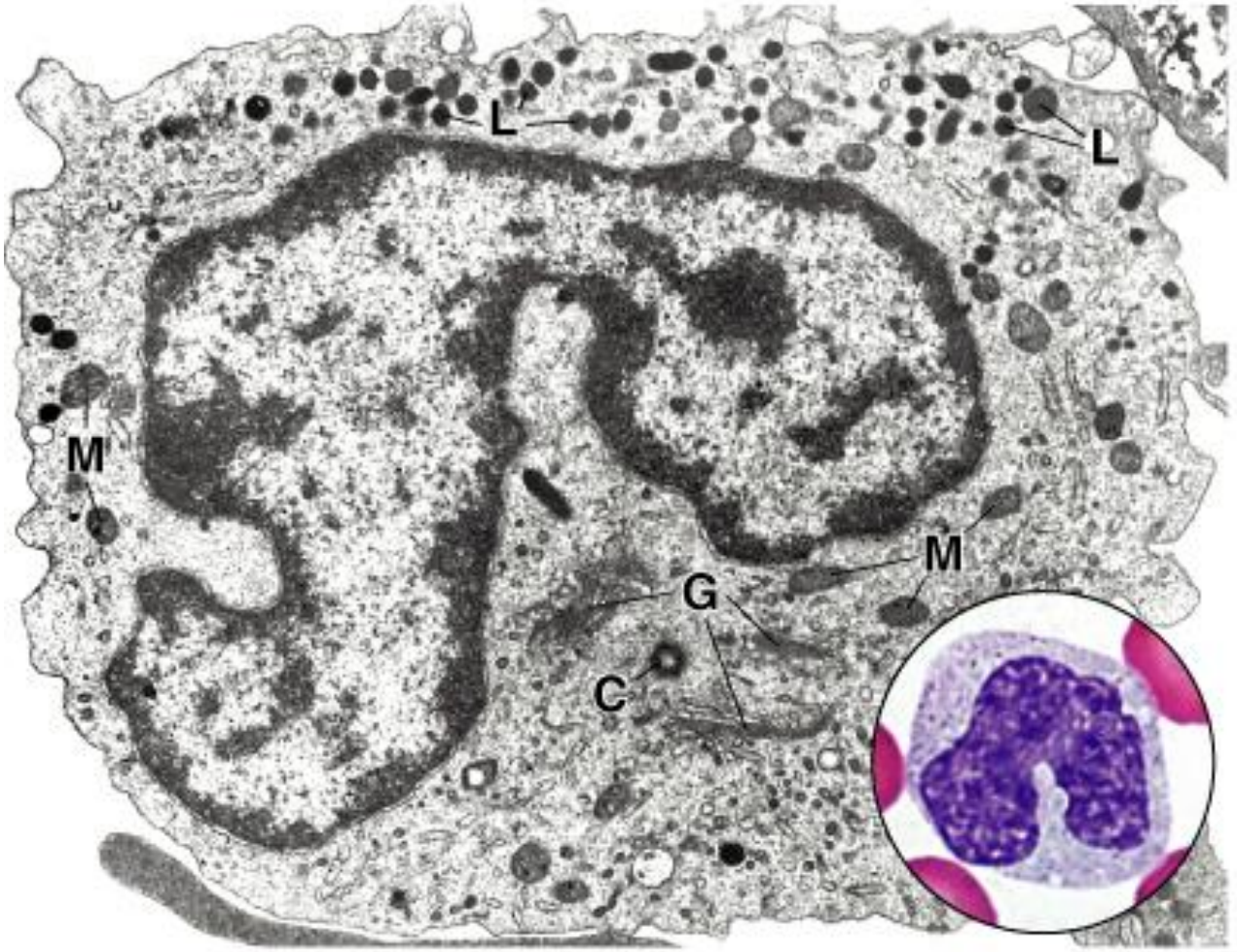
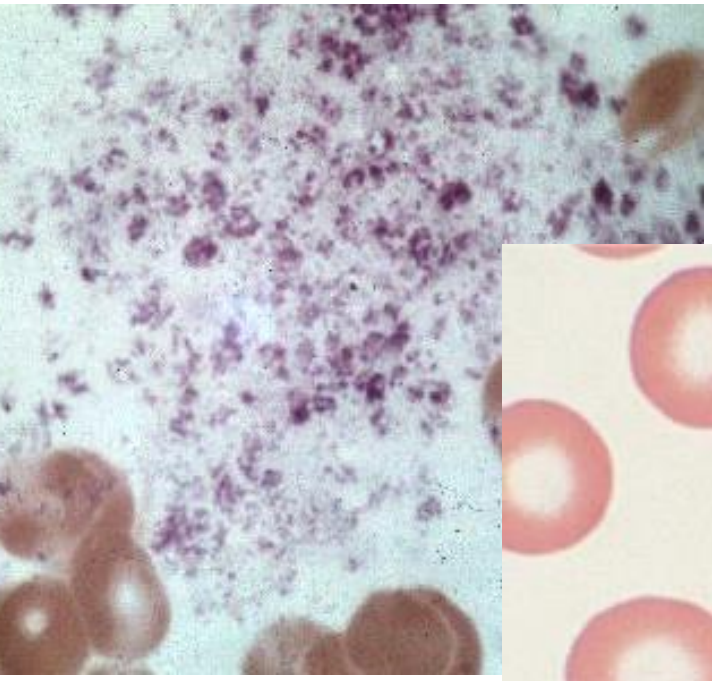
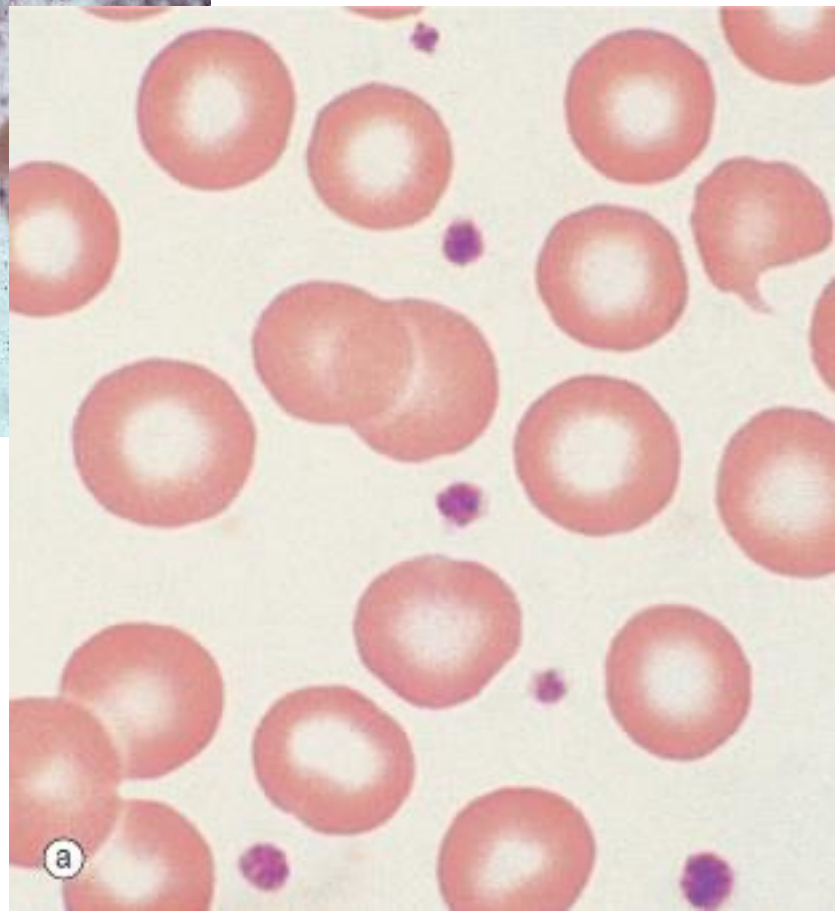


Figure 9.13. **EM of a human mature monocyte.** *C,* centriole; *G,* Golgi profiles; *L,* lysosomes; *M,* mitochondria. X22,000 **Inset.** Light microscopic appearance of a monocyte from a blood smear. X1,800.

Platelets (Thrombocytes)





Cell fragments

No nucleus

Functions

Hemostasis

Clotting

Injury repair

Number

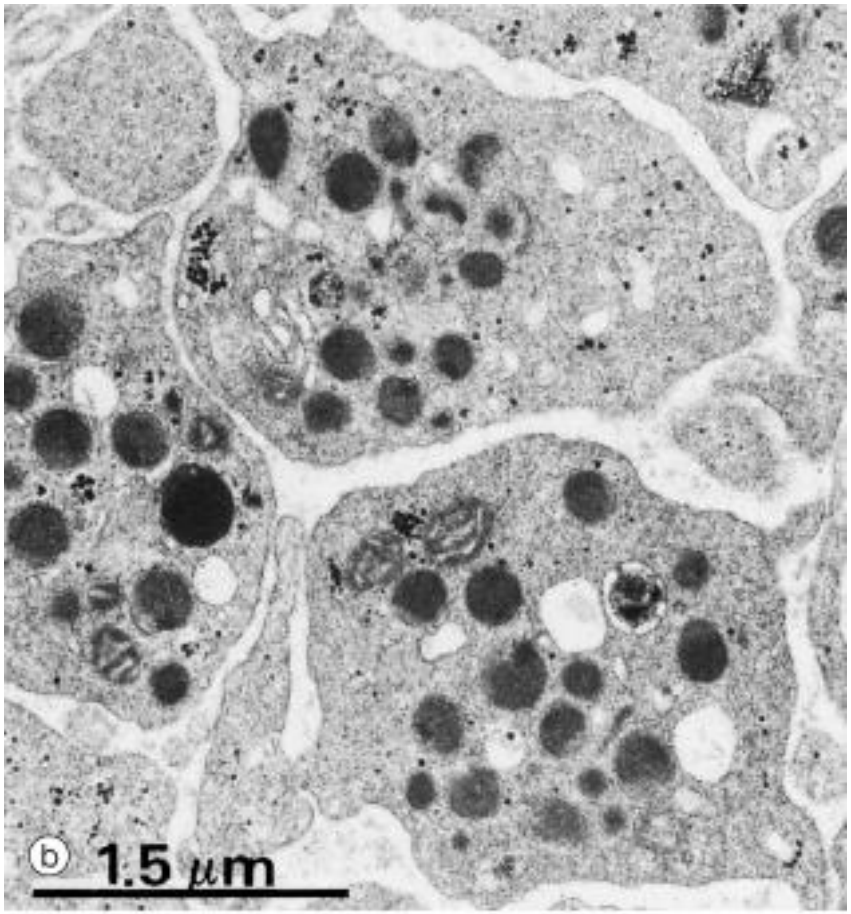
300,000/µl

Size

2 µm

Lifespan ~10 days

TEM



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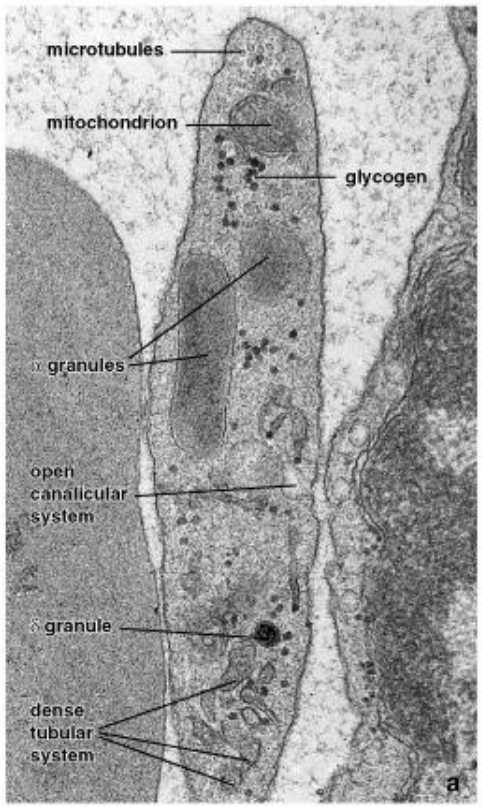


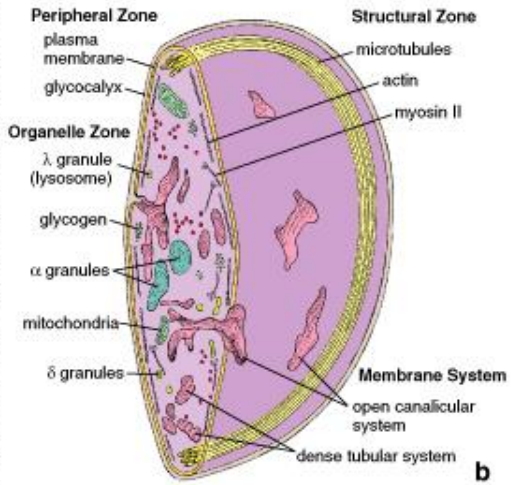
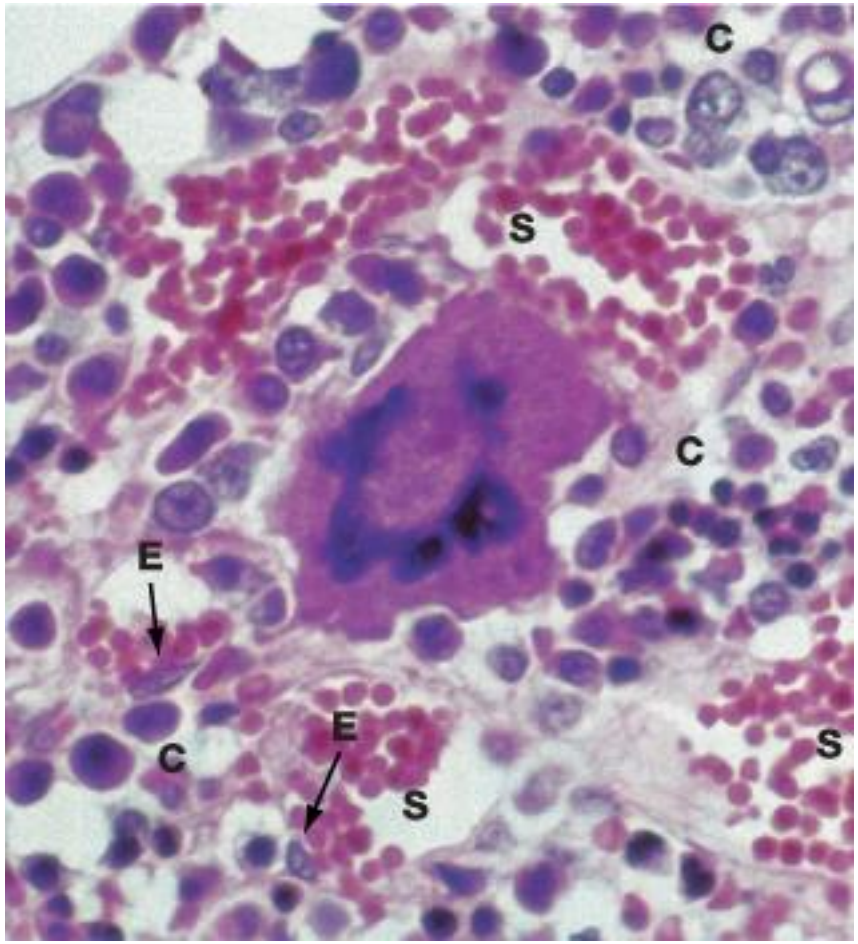
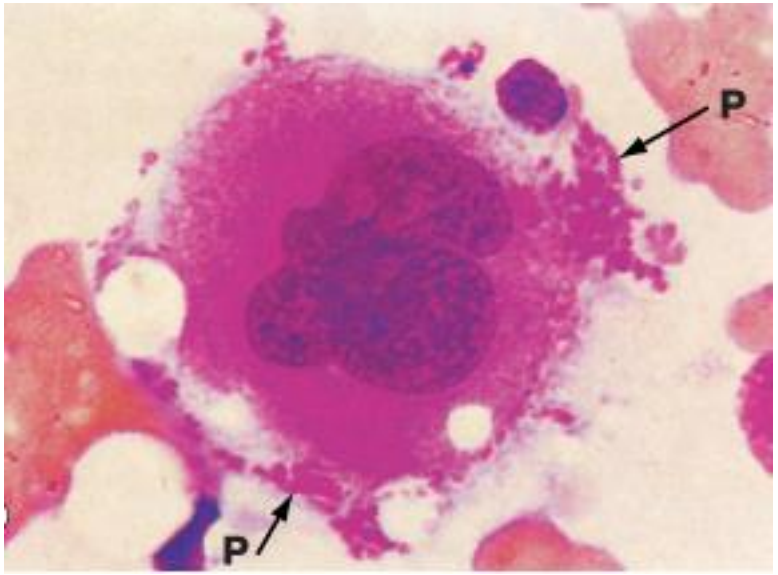


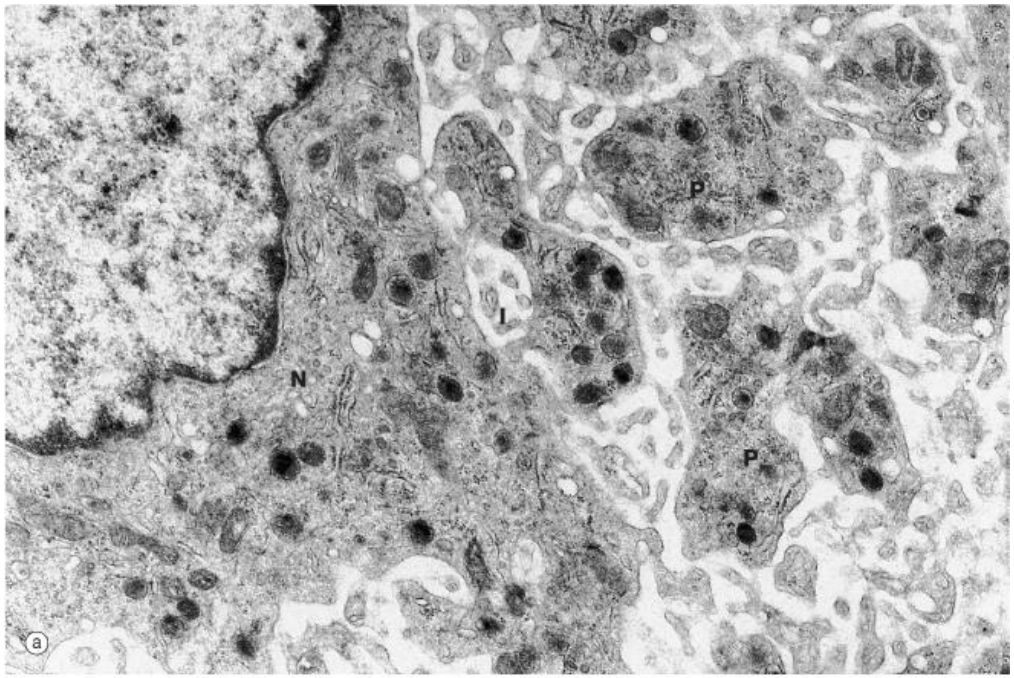
Figure 9.15a and b. **Platelet electron micrograph and diagram.**

Contain Serotonin, plasminogen, myosin, PDGF, …

Platelets are derived from large multinucleated **Megakarocytes** in bone marrow







Bone Marrow

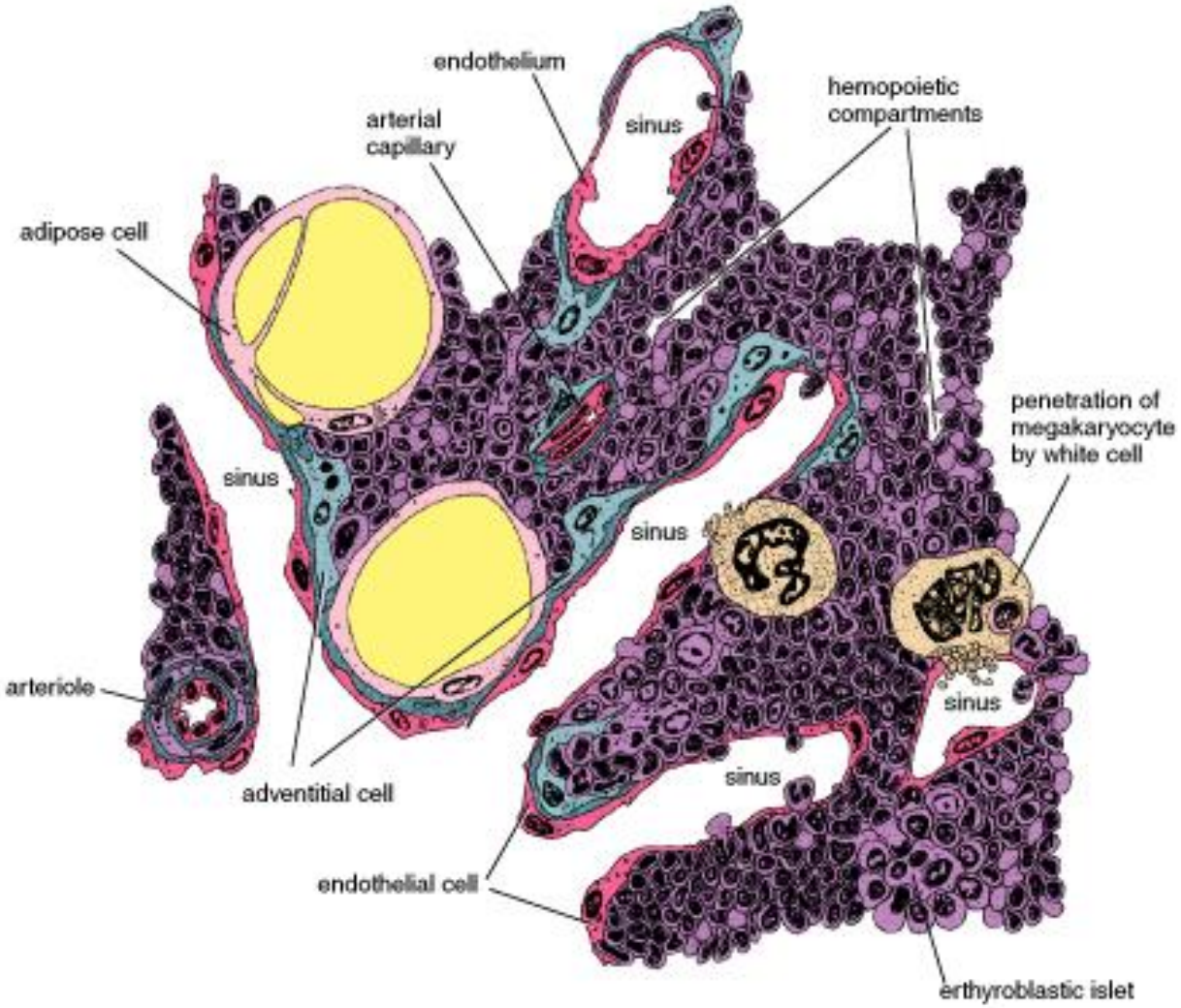
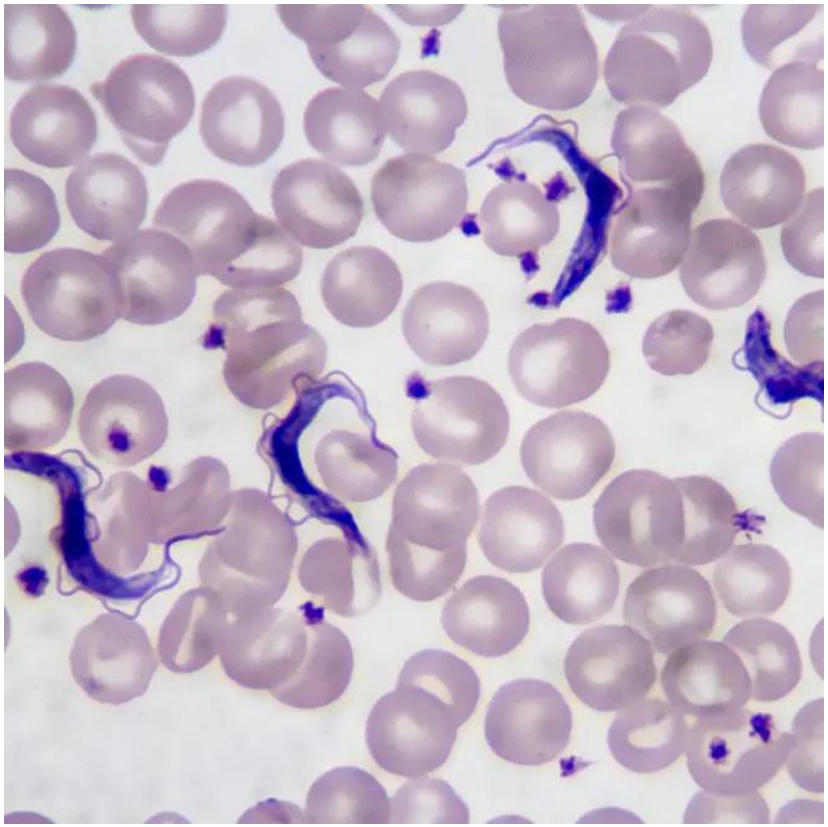
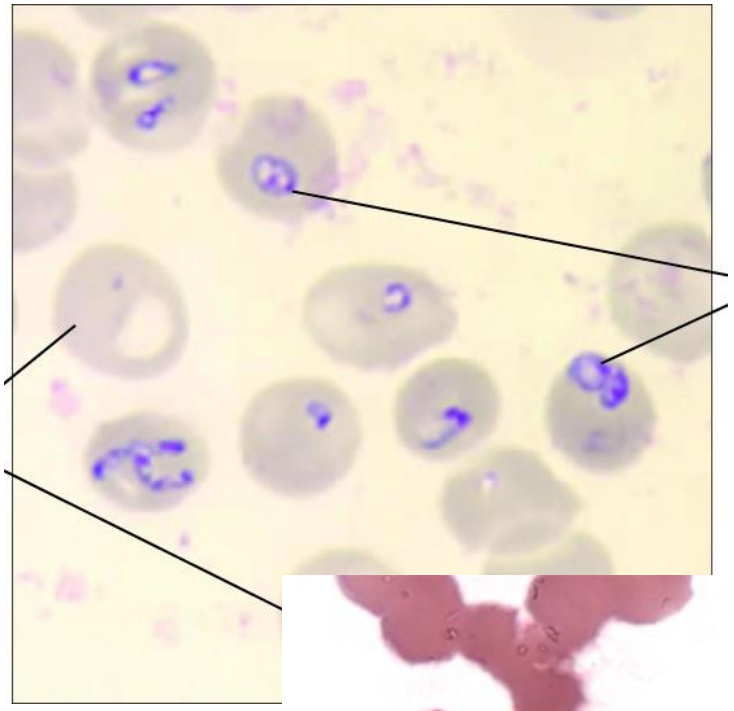


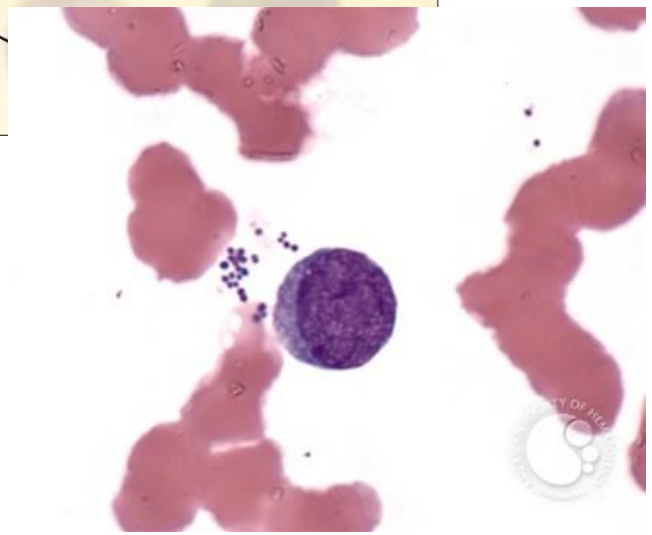
Figure 9.21. **Diagram of the marrow with active hemopoiesis.**

Other Inclusions:

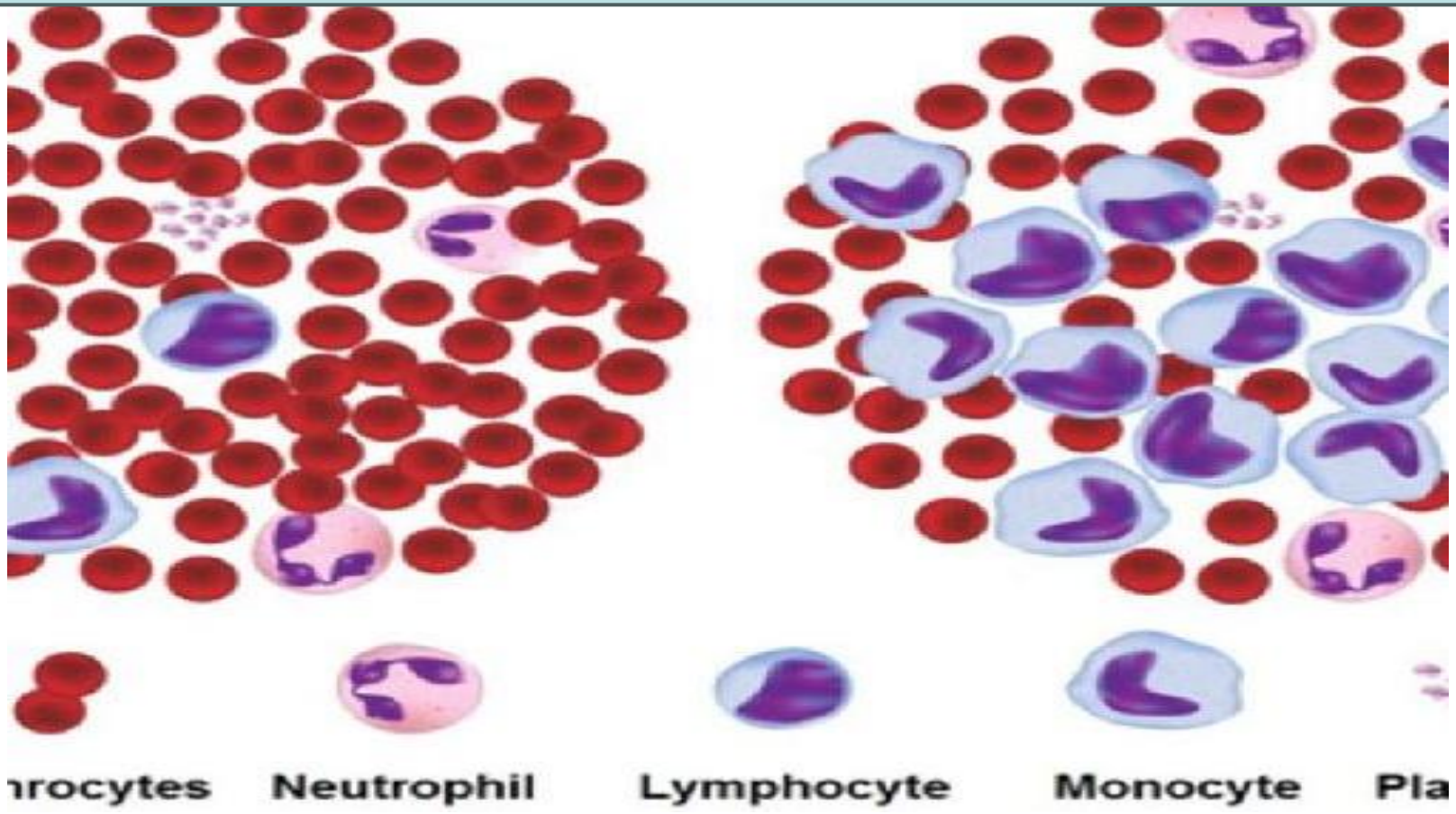
Parasites, Fungi, Bacteria, Viruses, …



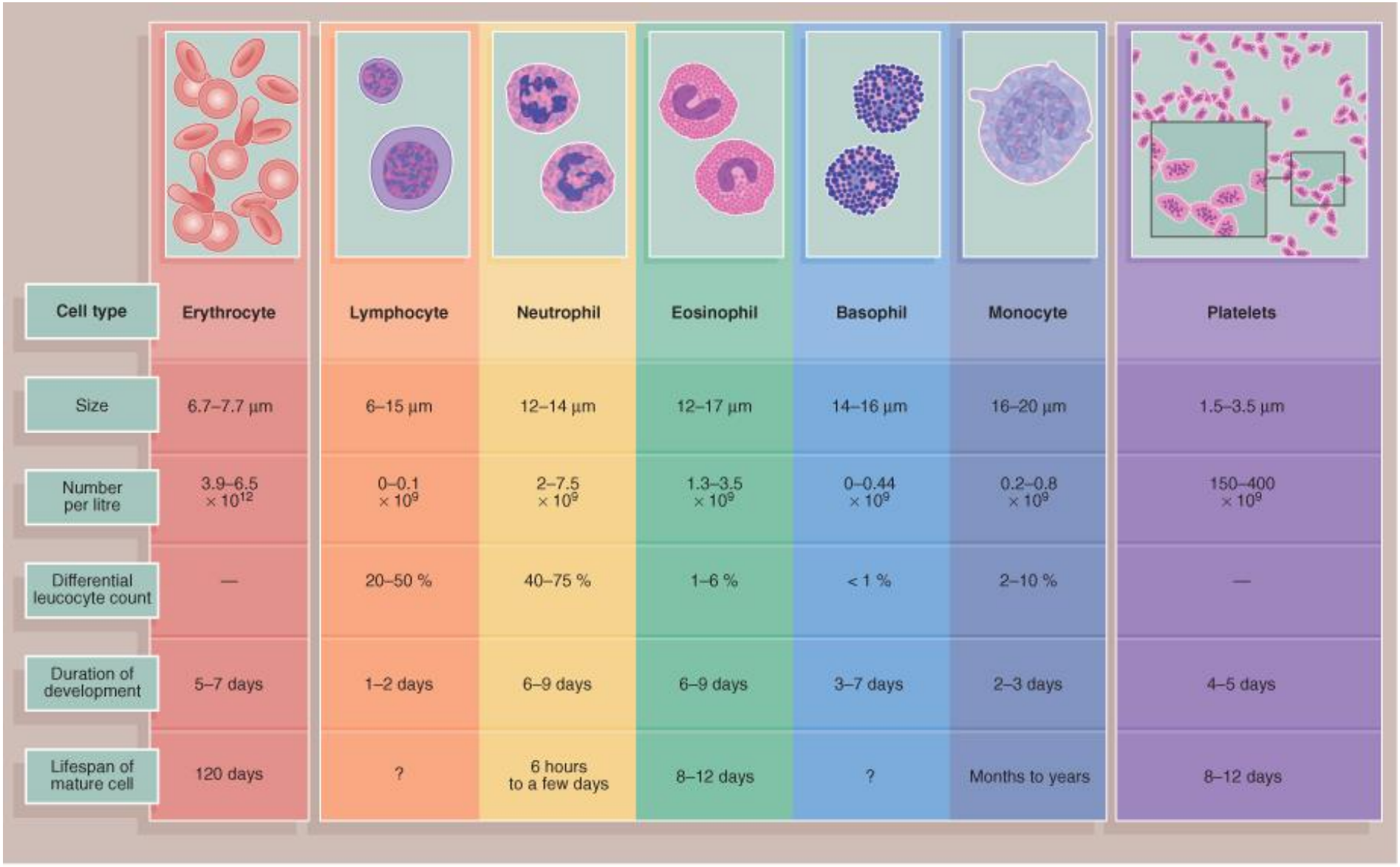




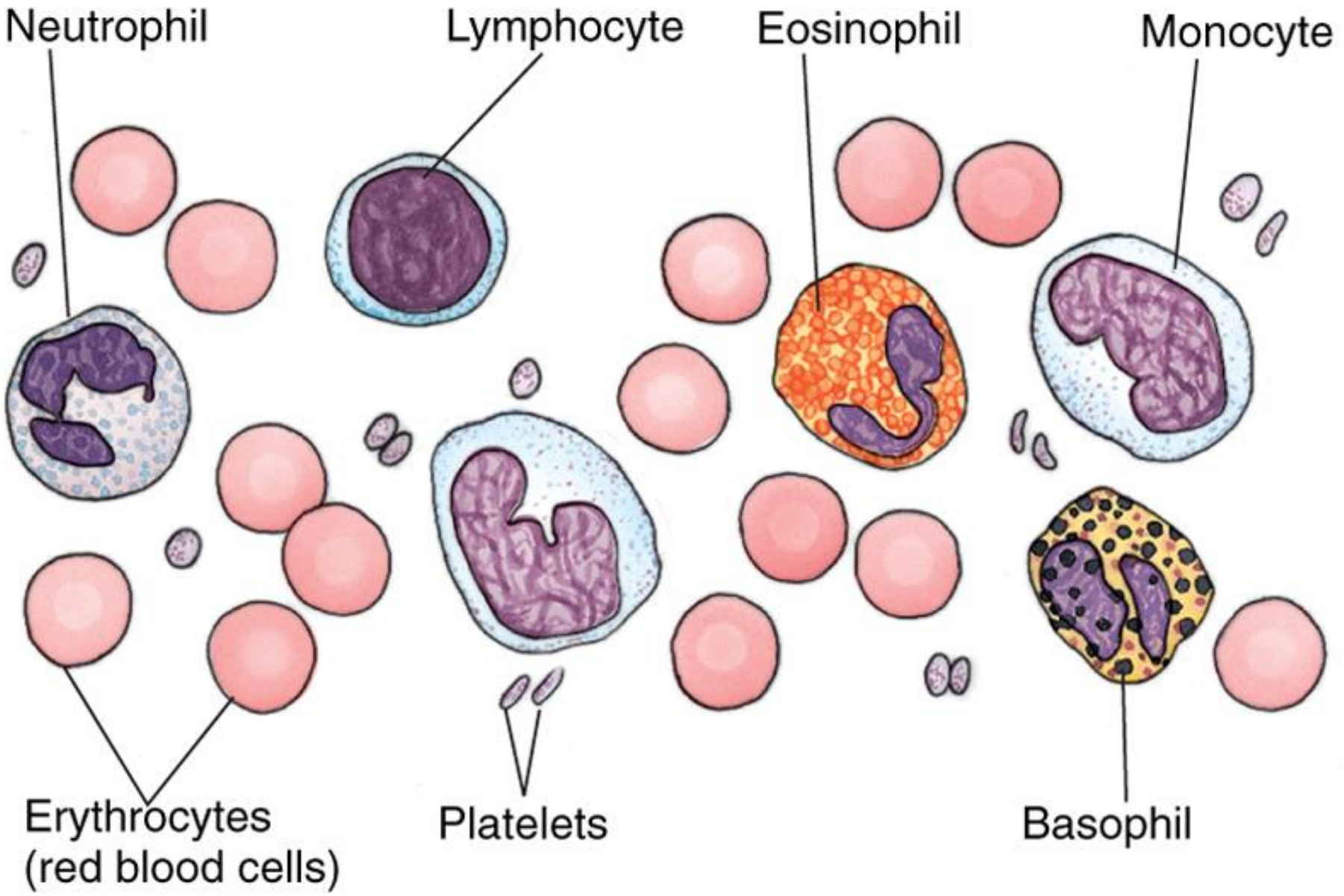
Normal Blood **Leukaemia**



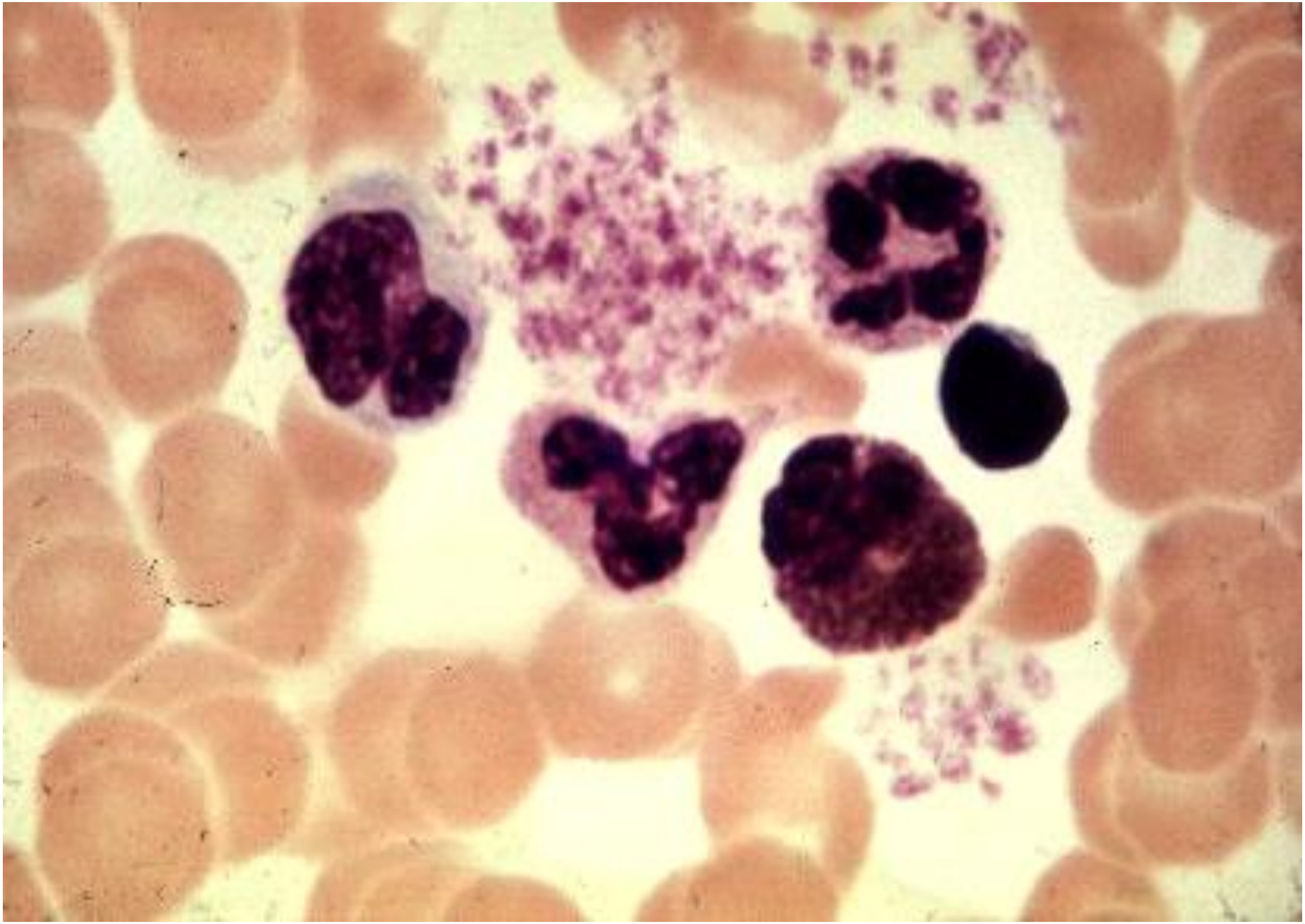
Summary Data

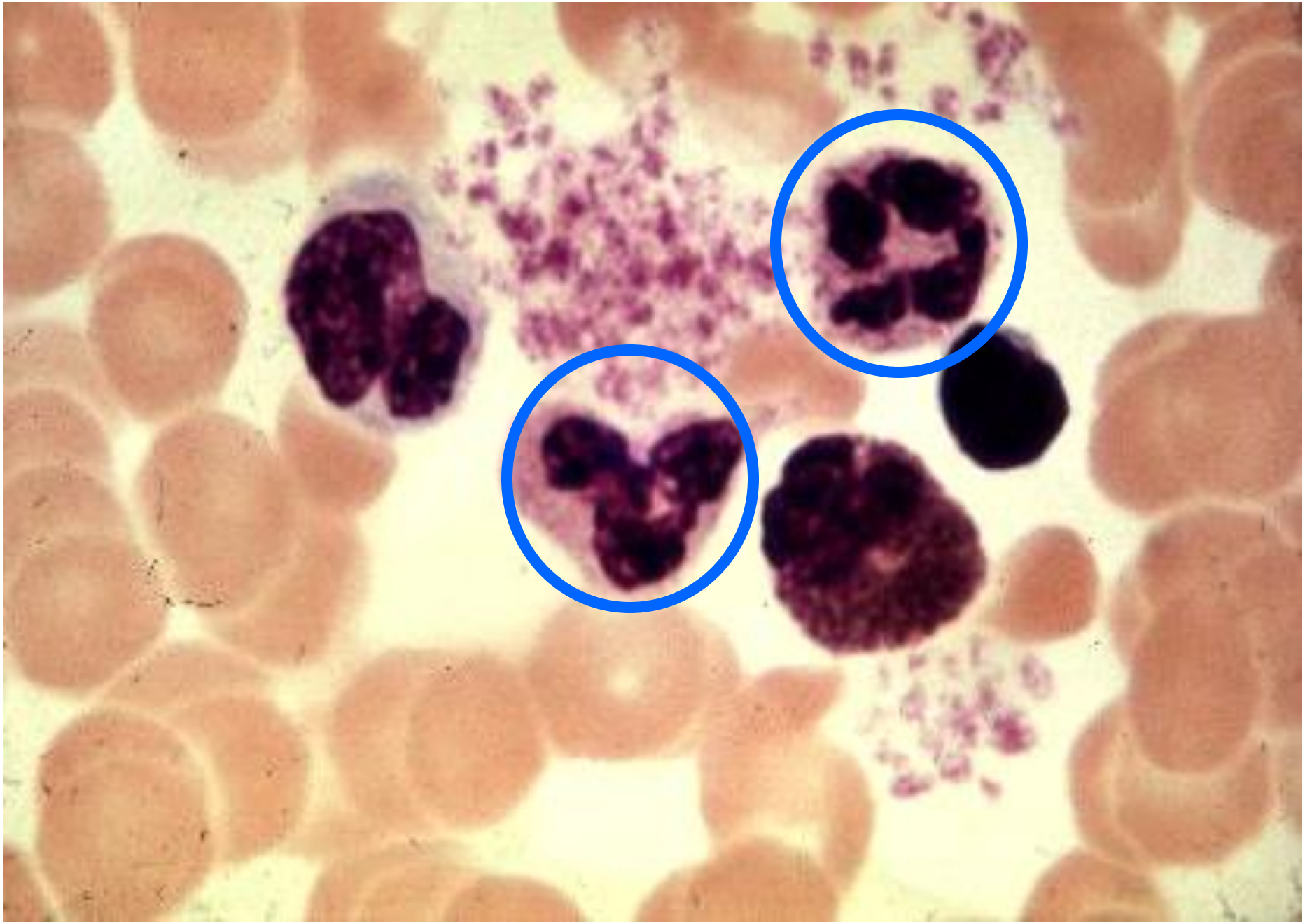


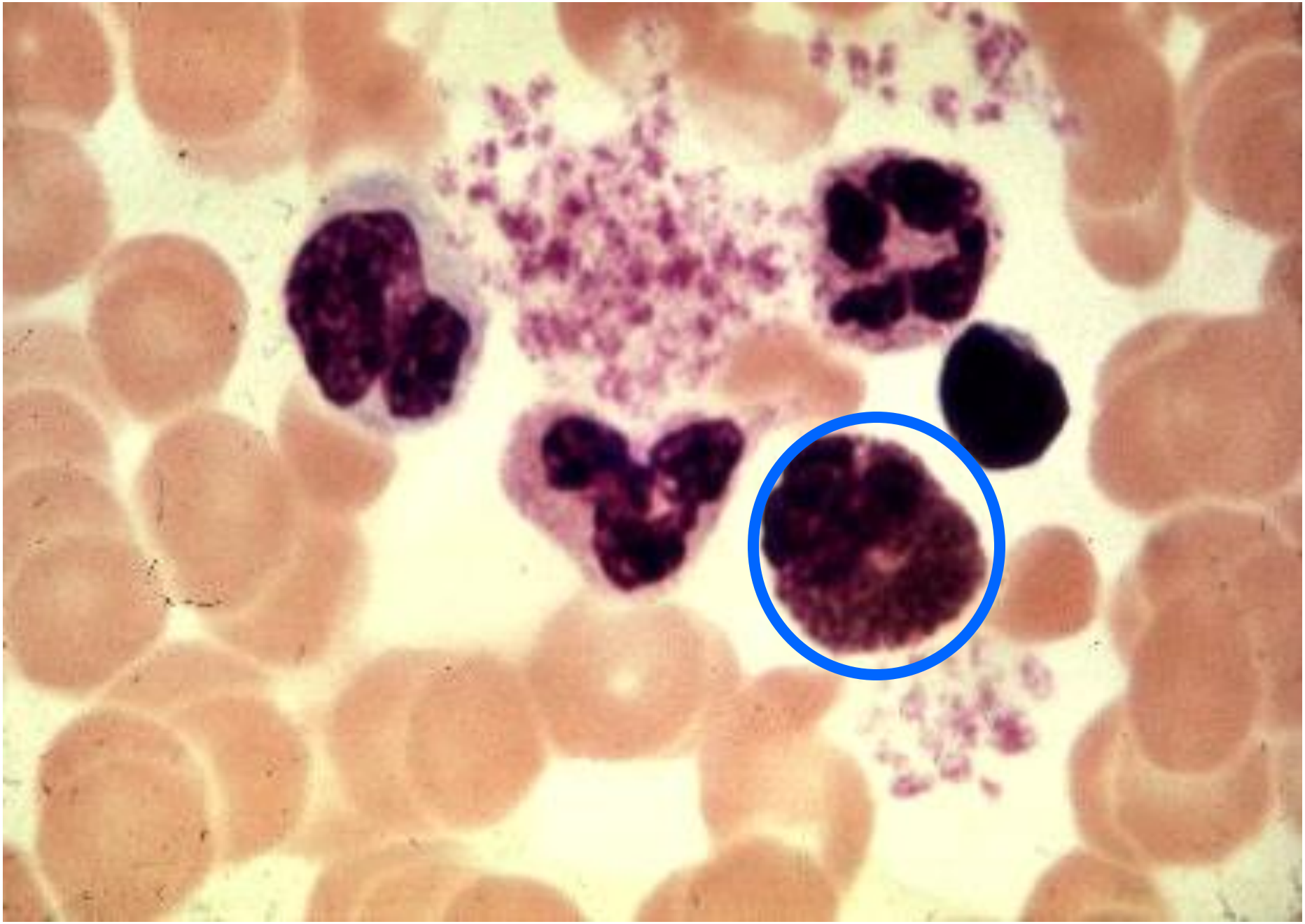
Cell type	Erythrocyte	Lymphocyte	Neutrophil	Eosinophil	Basophil	Monocyte	Platelets
Size	6.7–7.7 µm	6–15 µm	12–14 µm	12–17 µm	14–16 µm	16–20 µm	1.5–3.5 µm
Number per litre	3.9–6.5 $\times 10^{12}$	0–0.1 $\times 10^{9}$	2–7.5 $\times 10^{9}$	1.3–3.5 $\times 10^{9}$	0–0.44 $\times 10^{9}$	0.2–0.8 $\times 10^{9}$	150–400 $\times 10^{9}$
Differential leucocyte count	—	20–50 %	40–75 %	1–6 %	< 1 %	2–10 %	—
Duration of development	5–7 days	1–2 days	6–9 days	6–9 days	3–7 days	2–3 days	4–5 days
Lifespan of mature cell	120 days	?	6 hours to a few days	8–12 days	?	Months to years	8–12 days

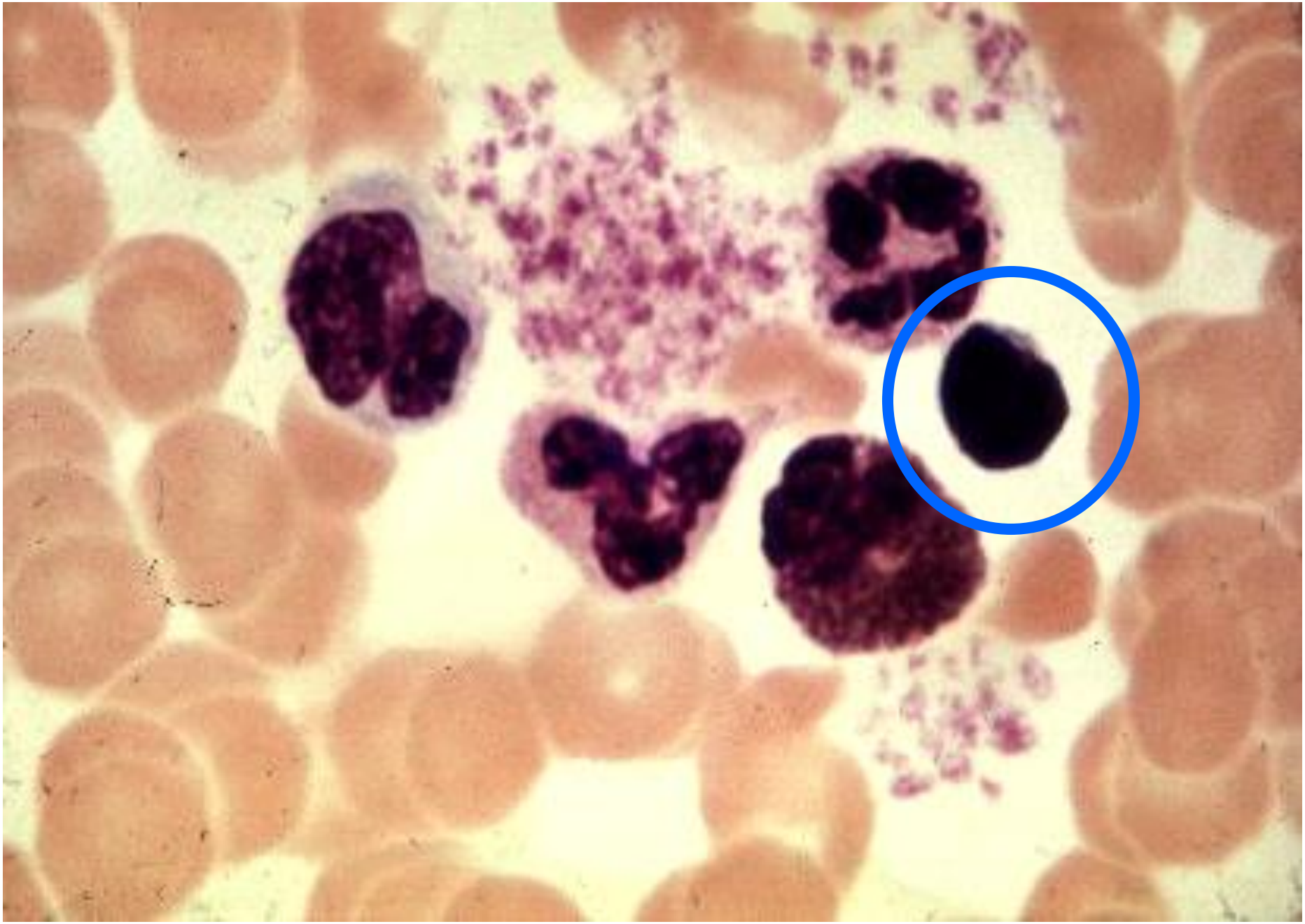


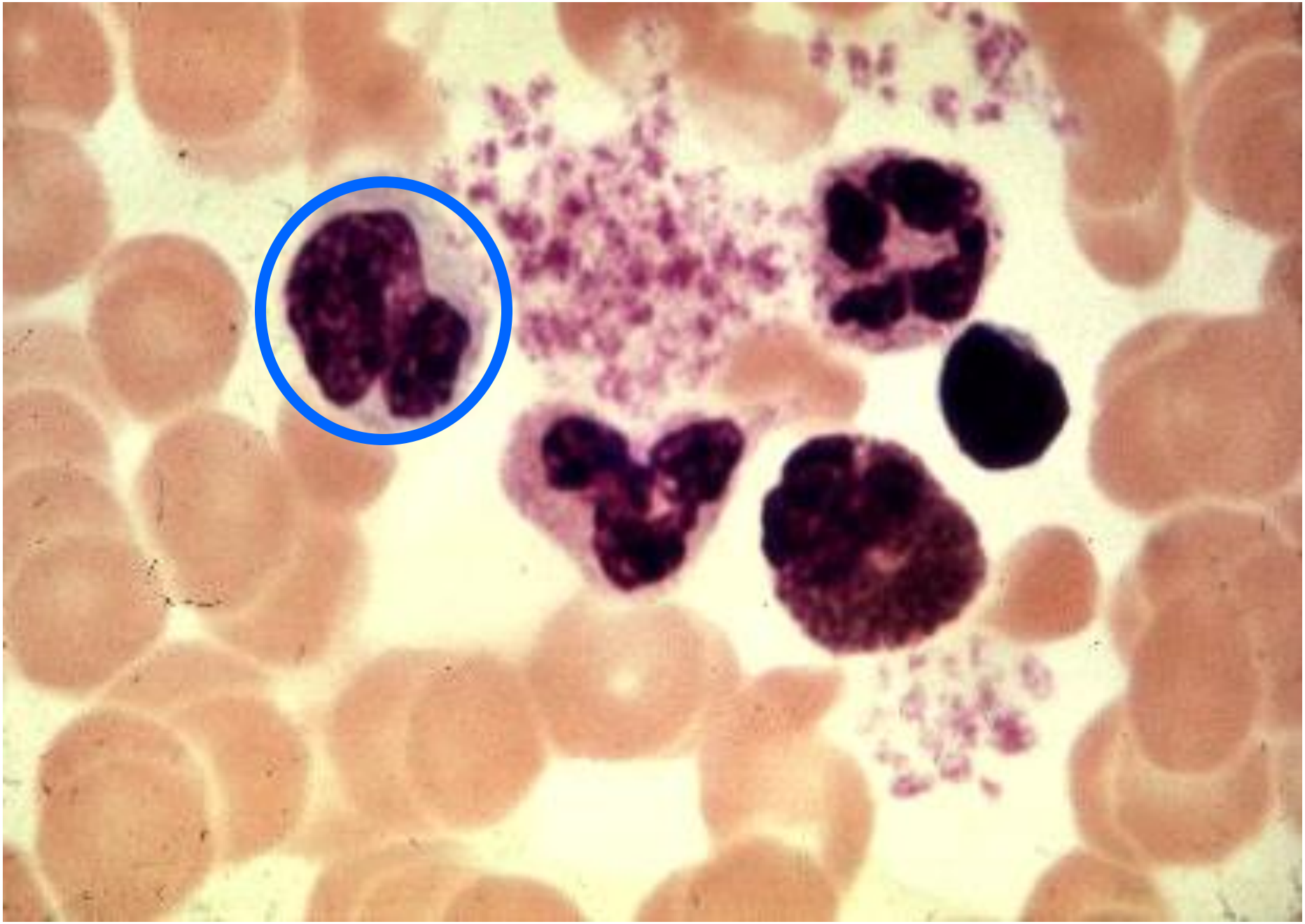
Over to you ….. !!











Practical - 7 slides

- **Normal Blood (HLV1)**
  - Identify Red Blood cells
    - Also - Look out for reticulocytes, abnormal shapes or sizes
  - Identify Neutrophils, Lymphocytes, Eosinophils, Basophils, Platelets
    - Perform WBC count %
- **Anaemia (HLV2)**
  - Reticulocytes

- **Blood Pathologies**
  - **HLV13** Neutrophil leucocytosis
  - **HLV14** Eosinophilia
  - **HLV15** Chronic Granulocytic Leukaemia
  - **HLV16** Chronic Lymphocytic Leukaemia
  - **HLV 17** Acute Myeloid Leukaemia

- **Prepare own Blood Smears** (….. at home !!)

End