Blood and haemopoietic tissue (Bone Marrow)

Lec . 8

Second year

Histology

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Learning Objectives

1. Be able to recognize all of the formed elements found in peripheral blood by light and electron microscopy.
2. Know the approximate abundance and life span of the formed elements.
3. Understand the functions of major plasma proteins and all of the formed elements.
4. Be familiar with the general process of hematopoeisis.
5. Describe the organization of the bone marrow.
6. Be able to recognize megakaryocytes in the bone marrow and understand their function in platelet production.

Functions of the Blood

1. To transport nutrients, oxygen, wastes, and carbon dioxide to and from the tissues.

2. To convey hormones, cytokines, chemokines, and other soluble regulatory molecules.

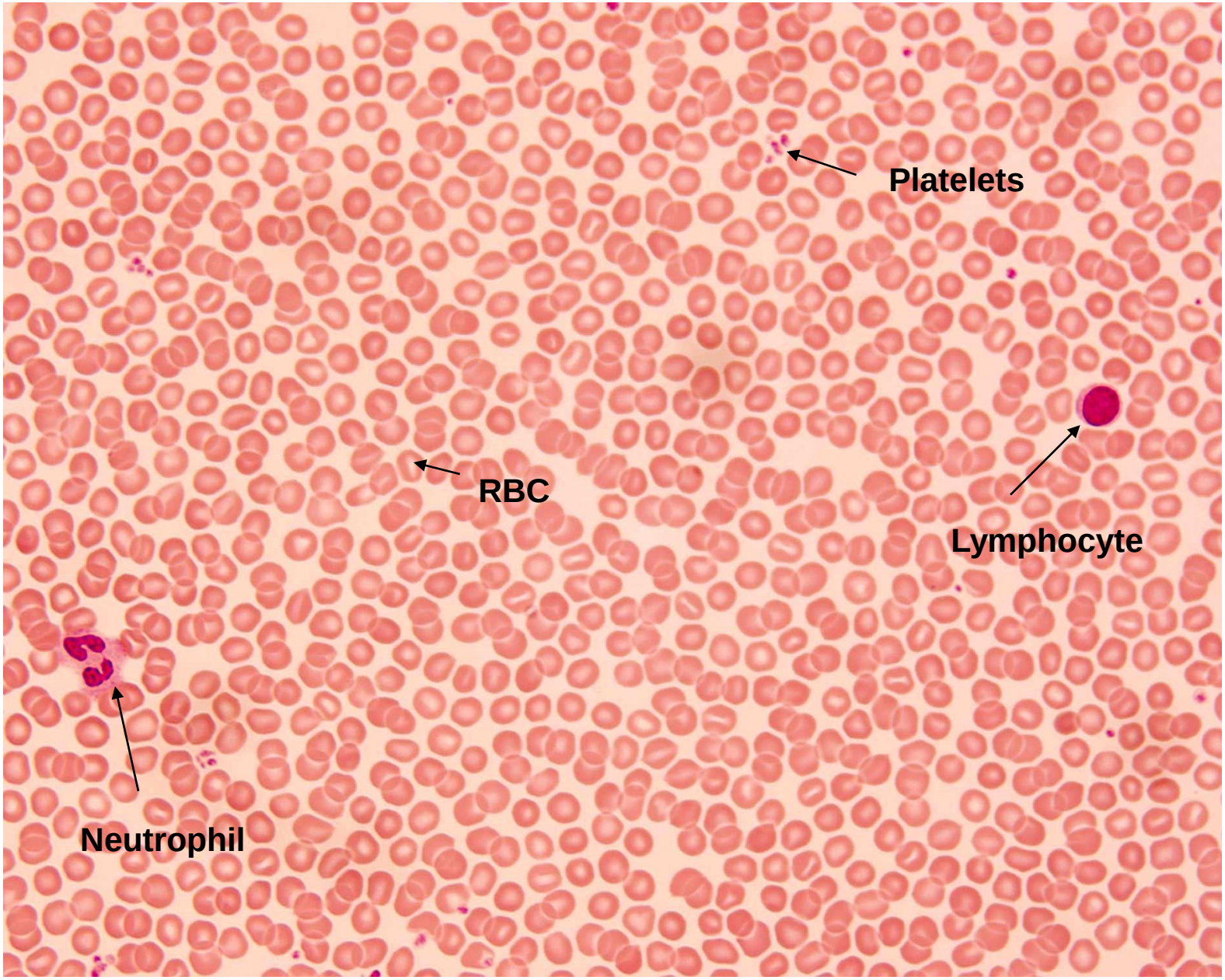
3. To transport leukocytes and antibodies through the tissues.

4. To maintain homeostasis.

Cells of the blood

- **Erythrocytes** (red blood cells, RBC)
- **Platelets** (thrombocytes)
- **Leukocytes** (white blood cells, WBC)
  - **Granulocytes** (with specific granules)
    - **Neutrophil (~60% of WBC)**
    - **Eosinophil (~4% of WBC)**
    - **Basophil (<1% of WBC)**
  - **Agranulocytes** (without specific granules)
    - **Lymphocyte (B-cell, T-cell) (~27% of WBC)**
    - **Monocyte (~8% of WBC)**

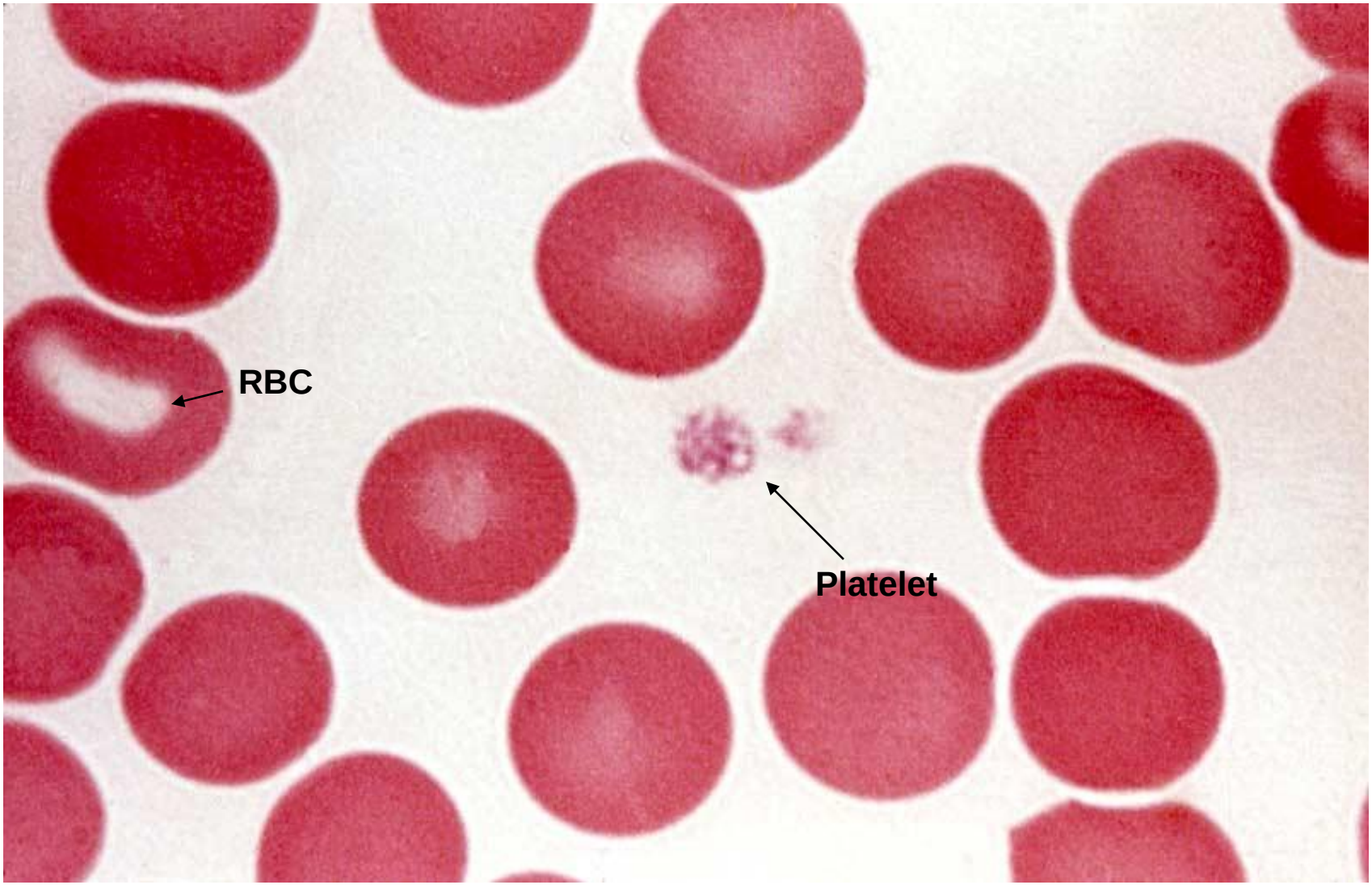
Human blood smear, with RBCs, WBCs and platelets



Erythrocyte (red blood cell, RBC)

1. **Life span in blood:** **About 120 days.**
2. **Size and shape:**
   - **biconcave disk, 8 μm diameter, 2μm at thickest point, 1 μm at thinnest**
   - **shape maintained by a cytoskeletal complex inside the plasma membrane .**
   - **flexible: RBC's normally bend to pass through small capillaries**
3. **LM appearance in smear:** **Pink circle with light center (center is thinner because of the biconcave shape). No nucleus.**
4. **TEM appearance:** **Solid dark gray cytoplasm, because of highly concentrated hemoglobin.**
5. **Function:**
   - **Transport of oxygen and carbon dioxide**
   - **pH homeostasis**

Red blood cells in a blood smear



Platelets (thrombocytes)

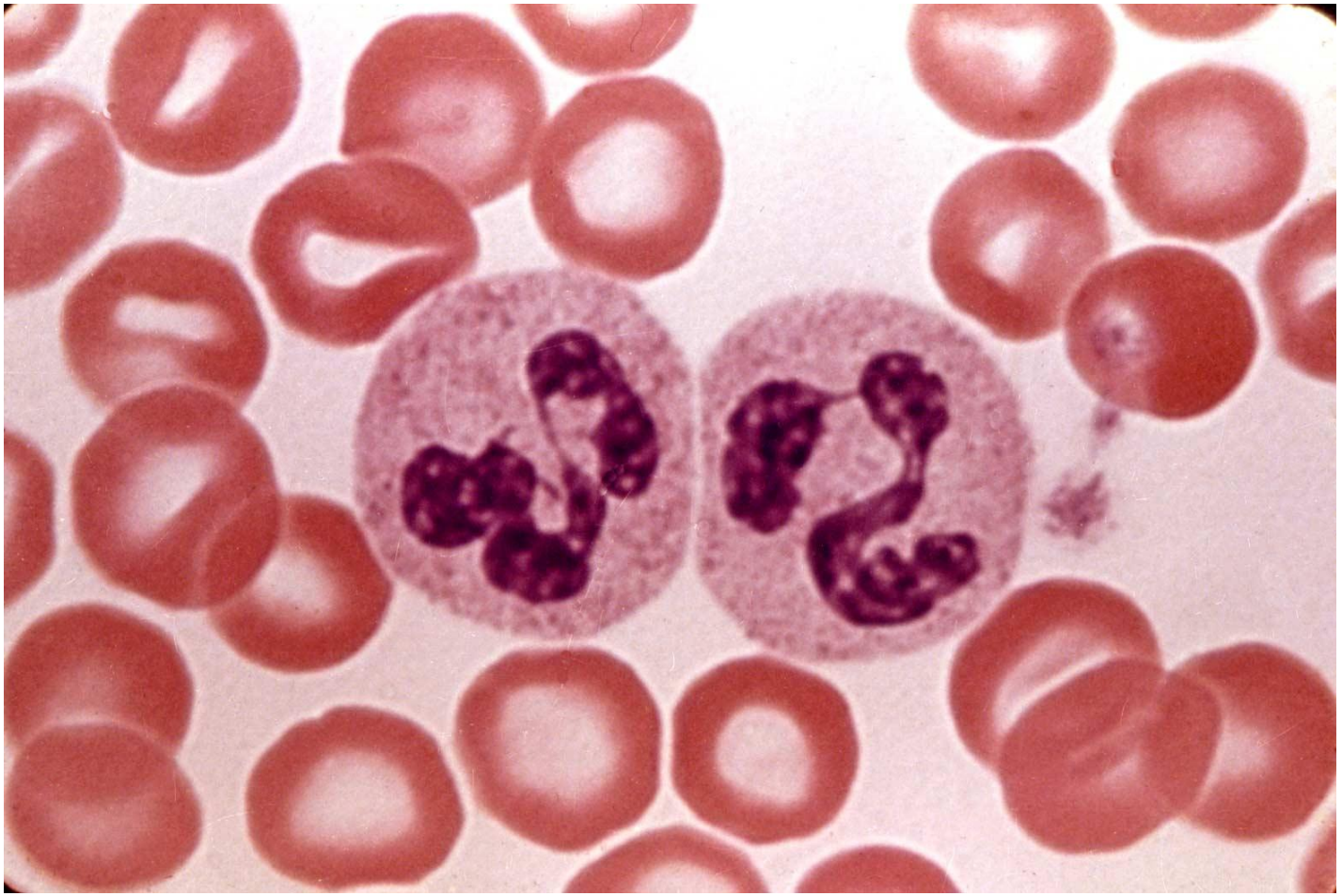
1. **Life Span:** **about 10 days**
2. **Shape, size, and origin:** **Small, biconvex disks, 2-3 μm in diameter. Non-nucleated cell fragments derived from cytoplasm of a very large cell, the megakaryocyte, in bone marrow. Platelets have a life span of about 10 days.**
3. **LM appearance in smears:** **Small basophilic fragments, often appearing in clusters.**
4. **TEM appearance:** **The platelet is bounded by a plasma membrane, and has a bundle of microtubules around the margin of the disk (which maintains the disk shape). There are three types of granules, containing fibrinogen, plasminogen, thromboplastin and other factors for clotting. There are also membrane tubules and glycogen.**
5. **Function:** **Platelets initiate blood clots.**

Neutrophil (polymorphonuclear leukocyte)

1. **Life Span:** **< 1 week**
2. **Granulocyte with specific and non-specific granules**
3. **LM appearance in smear:** **About 9-12 µm in diameter (thus larger than RBC). Nucleus long and multi-lobed (usually 2-4 lobes).**
4. **Cytoplasm has small, neutrally stained specific granules. Non-specific granules are azurophilic.**
5. **TEM appearance:** **Multi-lobed nucleus and numerous specific granules and lysosomes.**
6. **Function:** **Primarily antibacterial**

Neutrophils leave the blood and follow chemotaxic signals to sites of wounding or other inflammation, and phagocytose foreign agents such as bacteria. Pus is composed largely of dead neutrophils.

Two neutrophils in a blood smear

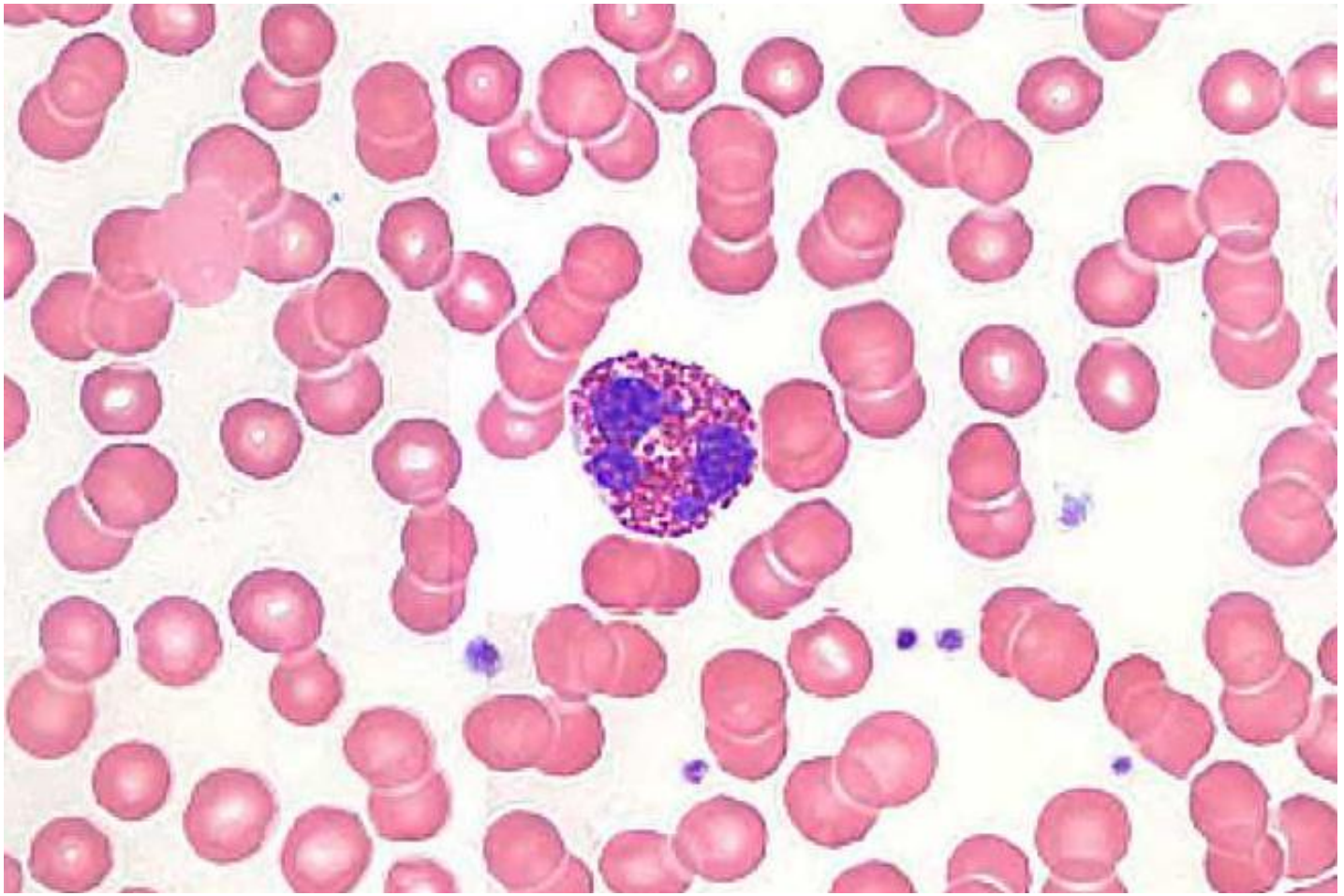


LM appearance in smear: **About 9-12 µm in diameter (thus larger than RBC). Nucleus long and multi-lobed (usually 2-4 lobes). Cytoplasm has small, neutrally stained specific granules. Non-specific granules are azurophilic.**

Eosinophil

1. Life Span: < 2 weeks
2. Granulocyte with specific and non-specific granules
3. LM appearance in smear: About 10-14 μm in diameter. Bilobed nucleus. The cytoplasm has prominent pink/red specific granules (stained with eosin dye).
4. TEM appearance: The specific granules are ovoid in shape, and contain a dark crystalloid body composed of major basic protein, effective against parasites. The rest of the granule contains other anti-parasitic substances. The cytoplasm also contains lysosomes.
5. Function:
   - Anti-parasitic activity
   - Mediators of inflammatory/allergic responses in tissues

Eosinophil in a human blood smear

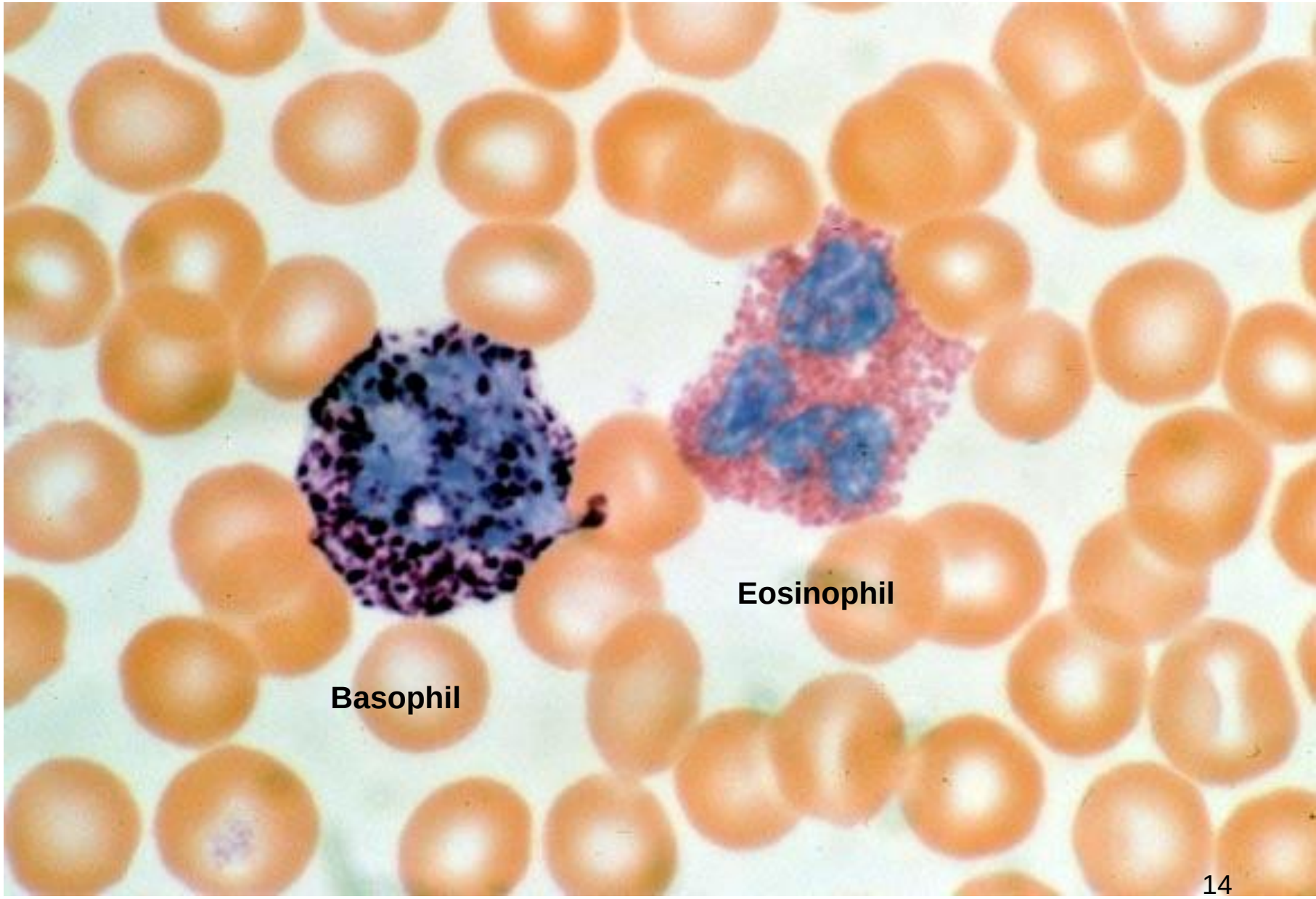


LM appearance in smear: **About 10-14 µm in diameter. Bilobed nucleus. The cytoplasm has prominent pink/red specific granules (stained with eosin dye). If the smear is not stained properly, the granules may be brownish.**

Basophil

1. Life Span: 1-2 years
2. Granulocyte with specific and non-specific granules
3. LM appearance in smear: About 8-10 μm in diameter. The cytoplasm contains large, purple/black specific granules (stained with the basic dye) that are larger but not as numerous as those of eosinophils. The nucleus is usually bilobed, but usually is partially obscured by granules, which can lie over it.
4. TEM appearance: The specific granules vary in size and shape, and have occasional myelin figures (usually formed from phospholipids). The cytoplasm also has some lysosomes (=azurophilic granules).
5. Function: Allergies and anaphylaxis (hypersensitivity reaction).
6. Similarity to tissue mast cells: Tissue mast cells also have IgE receptors and similar granule content. Mast cells and basophils have a common precursor in bone marrow.

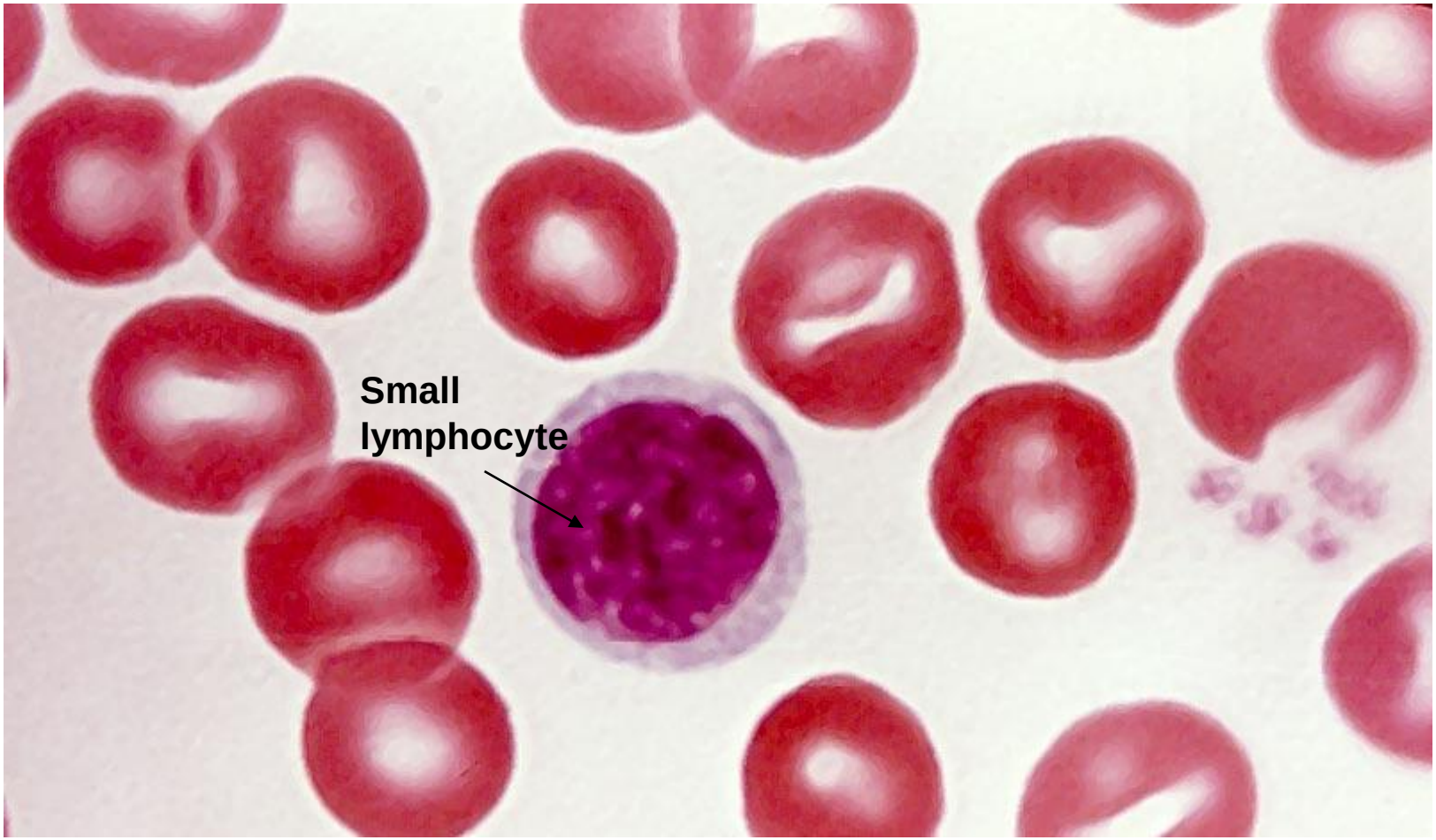
Comparison of basophil and eosinophil in a blood smear



Lymphocyte

1. **Life Span:** **variable (few days to several years)**
2. **LM appearance in smear:** **Small lymphocyte (about 90% of lymphocytes you will see) are ~8 µm in diameter, while large lymphocytes may be up to about 15 µm. Round, dense nucleus. The cytoplasm of a small lymphocyte is a narrow rim around the nucleus, and when well stained is pale blue. T-lymphocytes and B-lymphocytes cannot be distinguished in a smear.**
3. **TEM appearance:** **The cytoplasm doesn't appear to be very active, containing mainly mitochondria and free ribosomes.**
4. **Function:** **Cellular and humoral immunity.**

Small lymphocyte in a blood smear



LM appearance in smear: **Small lymphocyte (about 90% of lymphocytes you will see) are ~8 µm in diameter, while large lymphocytes may be up to about 15 µm. Round, dense nucleus (abundant heterochromatin). The cytoplasm of a small lymphocyte is a narrow rim around the nucleus, and when well-stained is pale blue.**

Monocyte

1. **Life Span:** few days in blood, several months in connective tissue
2. **LM appearance in smears:** About 16 µm in smears, thus the largest leukocyte. Large, eccentric nucleus either oval, kidney-shaped or horseshoe-shaped, with delicate chromatin that is less dense than that of lymphocytes. Pale cytoplasm, often grayish, may contain occasional stained granules (lysosomes = azurophilic granules). Large lymphocytes may resemble monocytes, but the lymphocyte nucleus is usually more dense.
3. **TEM appearance:** Cytoplasm contains mitochondria and some small lysosomes.
4. **Function**
   - Migrate into tissues and constitute mononuclear phagocyte system that help destroy foreign bodies and maintain or remodel tissues

     Tissue macrophages — Kupfer cells (liver) — Osteoclasts (bone)

     Dust cells (lungs) — Microglia (brain)
   - Mediate inflammatory response
   - Antigen presenting cells: Dendritic Cells, Langerhans cells

Monocyte in a blood smear

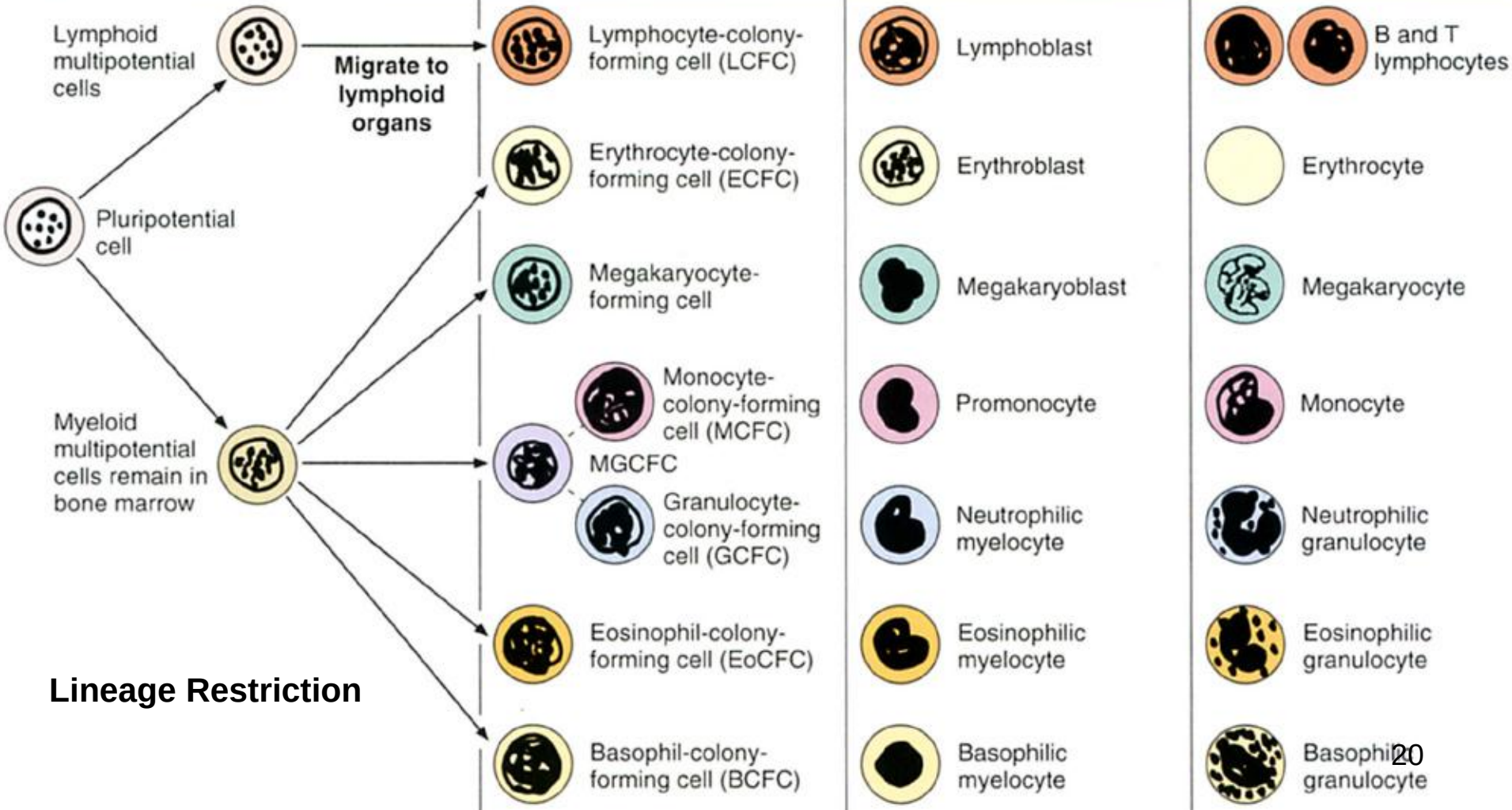
LM appearance in smears: **About 16 µm in smears, thus the largest leukocyte. Large, eccentric nucleus either oval, kidney-shaped or horseshoe-shaped, with delicate chromatin that is less dense than that of lymphocytes. Pale cytoplasm, often grayish, may contain occasional stained granules (lysosomes = azurophilic granules). Large lymphocytes may resemble monocytes, but the lymphocyte nucleus is usually more dense.**

Blood cell development (hematopoiesis = hemopoiesis)

1. Normally occurs in red bone marrow in adult (also spleen & liver, if necessary)
2. Mitotic stem and progenitor cells undergo increasing lineage restriction to produce committed precursors.
3. Precursors undergo cell division and differentiation into mature cells.
4. Maturation involves (note exceptions for megakaryocytes below):
   - **decrease in cell size***
   - **shutting down transcription (nucleoli disappear and chromatin condenses)***
   - **adoption of morphological characteristics specific to that lineage.**
   - **Future granulocytes produce specific and non-specific granules, and then shape their nucleus.**
   - **Future monocytes produce non-specific granules and shape their nucleus.**
   - **Future small lymphocytes decrease their size and enter the blood, but then undergo extensive further maturation at another site (T-cells in the thymus, and B-cells in the "bursa equivalent" –to be discussed in immune system lecture).**
   - **Future erythrocytes fill cytoplasm with hemoglobin, synthesized on free polysomes (ribosomes on mRNA), and eventually extrude their nucleus.**
5. Mature cells enter marrow sinus; immature cells in peripheral blood typically indicates disease.

*** Megakaryocytes develop into large polyploid cells that remain transcriptionally active and extrude platelets as cytoplasmic fragments directly into marrow sinus.**

Phase	Stem Cells	Progenitor Cells	Precursor Cells (Blasts)	Mature Cells
Early morphologic	Not morphologically distinguishable; have the general aspect of lymphocytes		Beginning of morphologic differentiation	Clear morphologic differentiation
Mitotic activity	Low mitotic activity; self-renewing; scarce in bone marrow	High mitotic activity; self-renewing; common in marrow and lymphoid organs; mono- or bipotential	High mitotic activity; not self-renewing; common in marrow and lymphoid organs; monopotential	No mitotic activity; abundant in blood and hematopoietic organs



Lineage Restriction

Cellular Changes during Myeloid Differentiation

Mitotic Cell

Postmitotic Cells

Myelocyte | Metamyelocyte | Band Cell | Mature Neutrophil

Nuclear Changes-

Large, euchromatic, transcriptionally active nucleus → Smaller, euchromatic, less transcriptionally active nucleus → Condensed, heterochromatic, transcriptionally inactive nucleus

Cytoplasmic Changes-

Basophilic cytoplasm, active synthesis of specific and non-specific granules → Reduced basophilia, granule maturation → Pale bluish-pink cytoplasm, mature granules

Changes during Erythroblast Differentiation

Mitotic Cells | *Postmitotic Cells*

Proerythroblast | Early Erythroblast | Middle Erythroblast | Late Erythroblast | Erythrocyte (Reticulocyte)

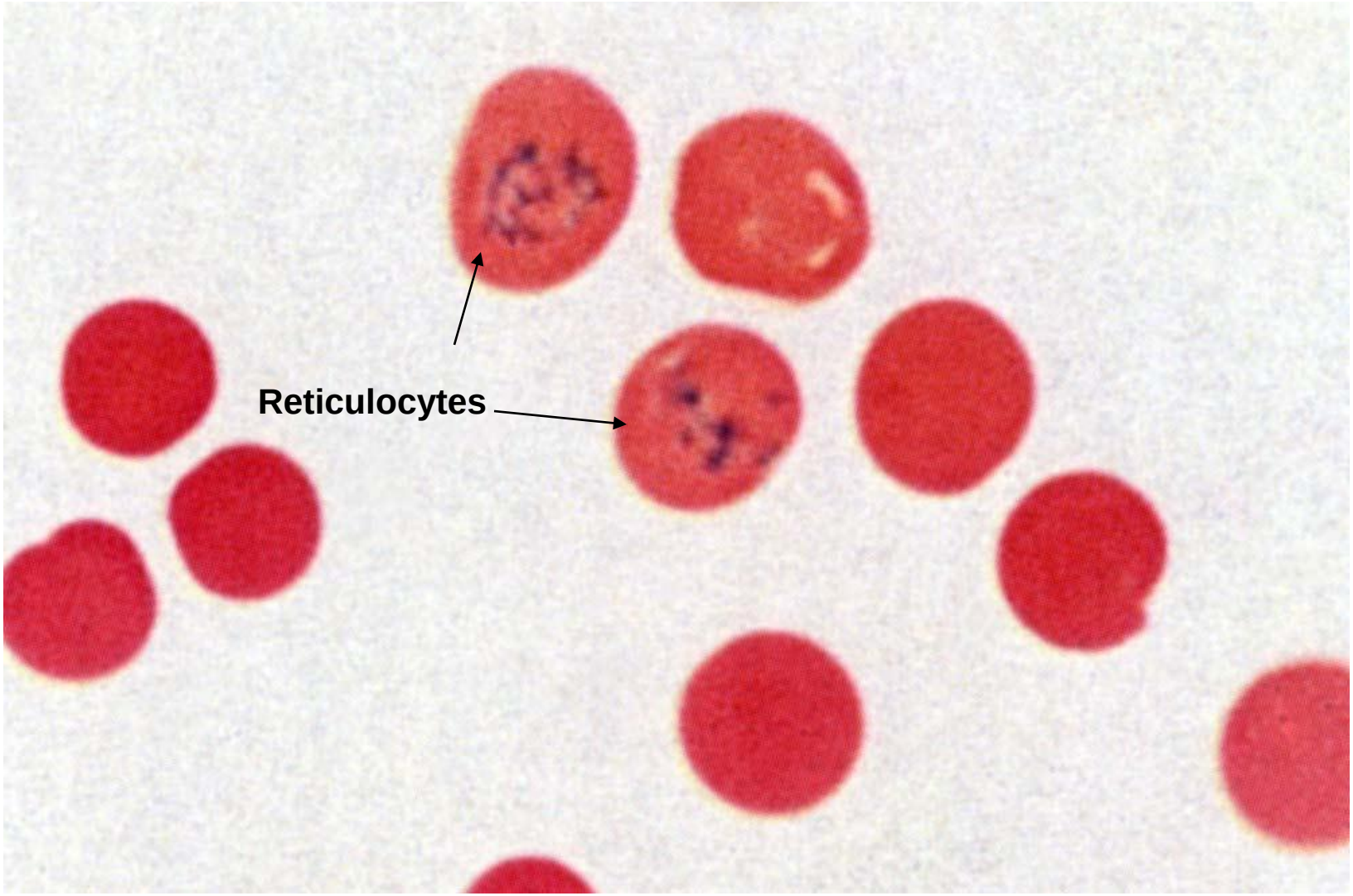
Nuclear Changes-

Large, euchromatic, and transcriptionally active → Smaller, heterochromatic, and transcriptionally inactive → Absent

Cytoplasmic Changes-

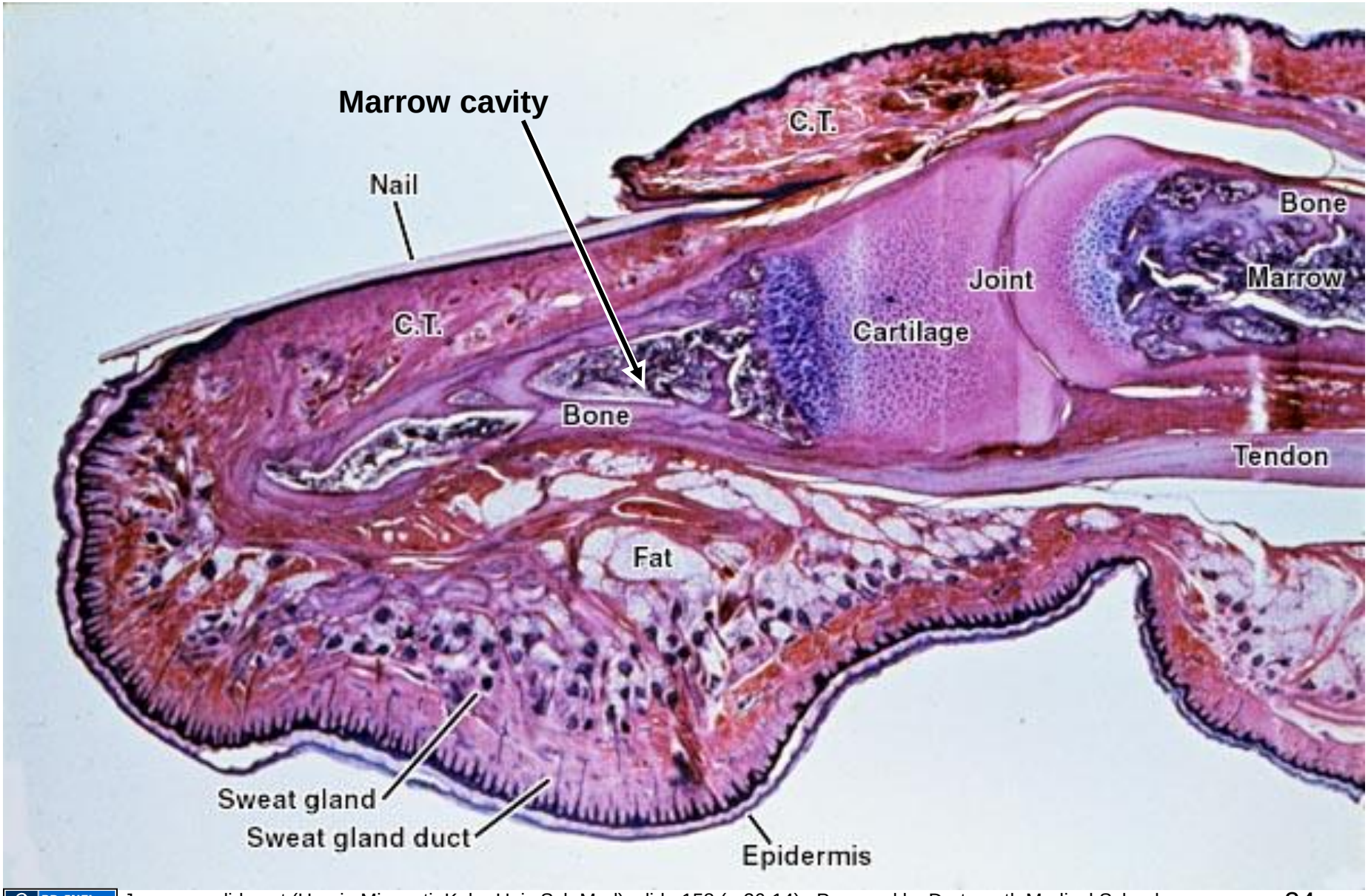
Basophilic with abundant RER → Polychromatic (basophilic and acidophilic) with abundant RER and hemoglobin → Acidophilic, with abundant hemoglobin

Reticulocytes (somewhat immature RBCs) in blood smear, cresyl blue stain



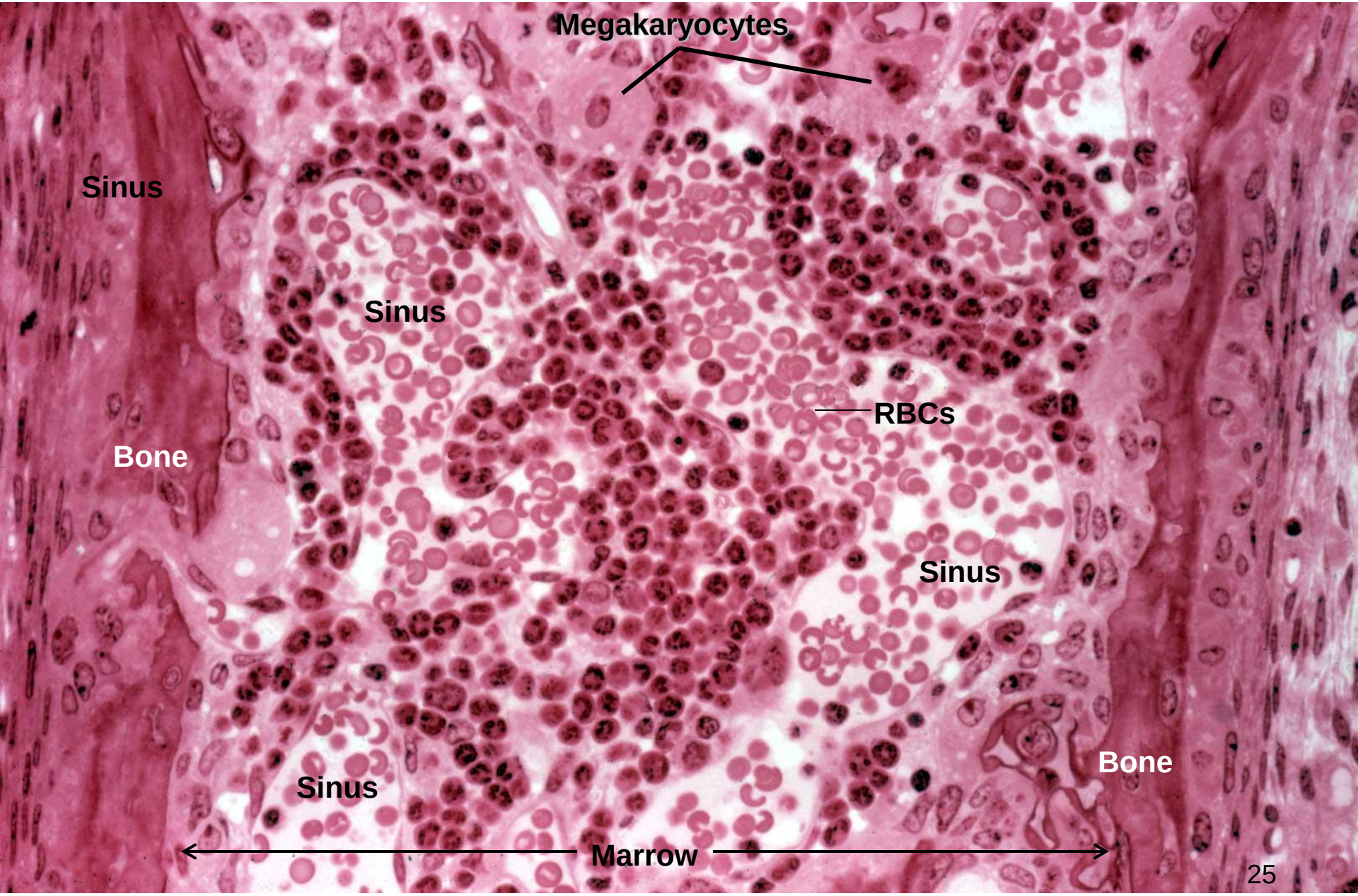
Residual ribosomes in cytoplasm are basophilic. Number of reticulocytes in peripheral blood reflects status of erythropoesis –generally increased by anemia and hypoxia.

Finger, bone marrow in phalanges



 Japanese slide set (Humio Mizoguti, Kobe Univ Sch Med), slide 158 (= 26-14). Prepared by Dartmouth Medical School.

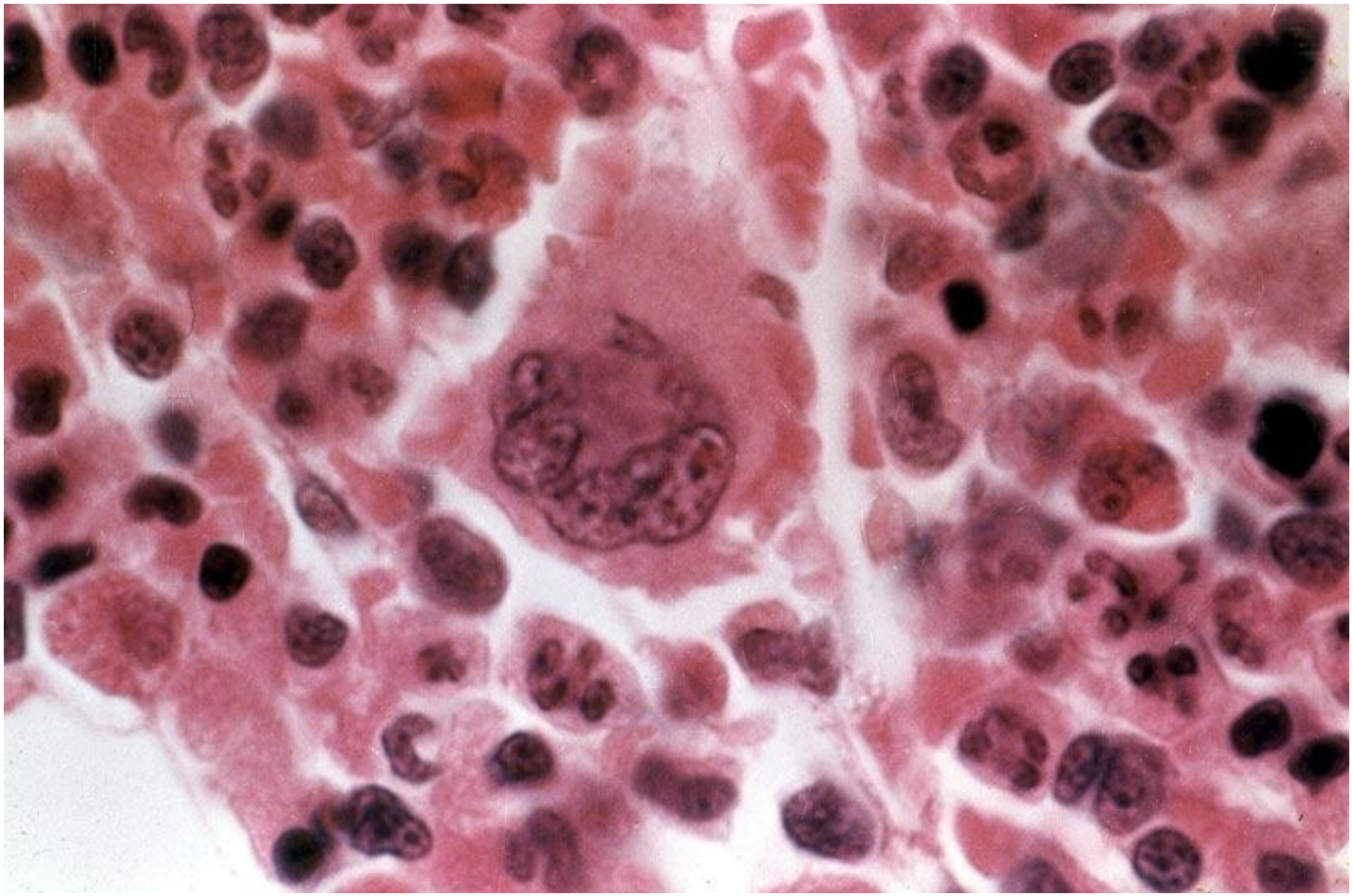
Section of bone marrow, LM



Megakaryocytes in bone marrow produce blood platelets

- **LM appearance:** **A huge cell, up to 50 µm in diameter. Its long nucleus has several lobes. The cytoplasm is pale pink/red, without visible granules. In bone marrow, megakaryocytes are situated adjacent to a marrow sinus (large capillary), although this may not be obvious in tissue sections.**
- **TEM appearance:** **Particularly striking in the cytoplasm are many curved white lines that are the platelet demarcation channels, membrane-bound spaces forming the boundaries between future platelets. The cytoplasm also contains granules of various sizes, that will be in the platelets.**
- **Function:** **Megakaryocytes produce blood platelets by fragmentation of their cytoplasm, extending cell processes through the endothelium of a marrow sinus, and releasing clusters of immature platelets into the blood, to become mature platelets.**

Megakaryocyte, LM section



LM appearance: **A huge cell, up to 50 µm in diameter. Its long nucleus has several lobes (the nucleus is polyploid and can be up to 64N). The cytoplasm is pale pink/red, without visible granules. In bone marrow, megakaryocytes are situated adjacent to a marrow sinus (large capillary), although this may not be obvious in tissue sections.**