

Schedule: Week 1

1. TA Introduction: Rachel Wilson, Lindsay Holden
2. Welcome
3. Syllabus
4. Week 1 Lecture
 - Chapter 1: Histology and Its Methods of Study
 - Chapter 2: The Cytoplasm
 - Chapter 3: The Nucleus



Welcome

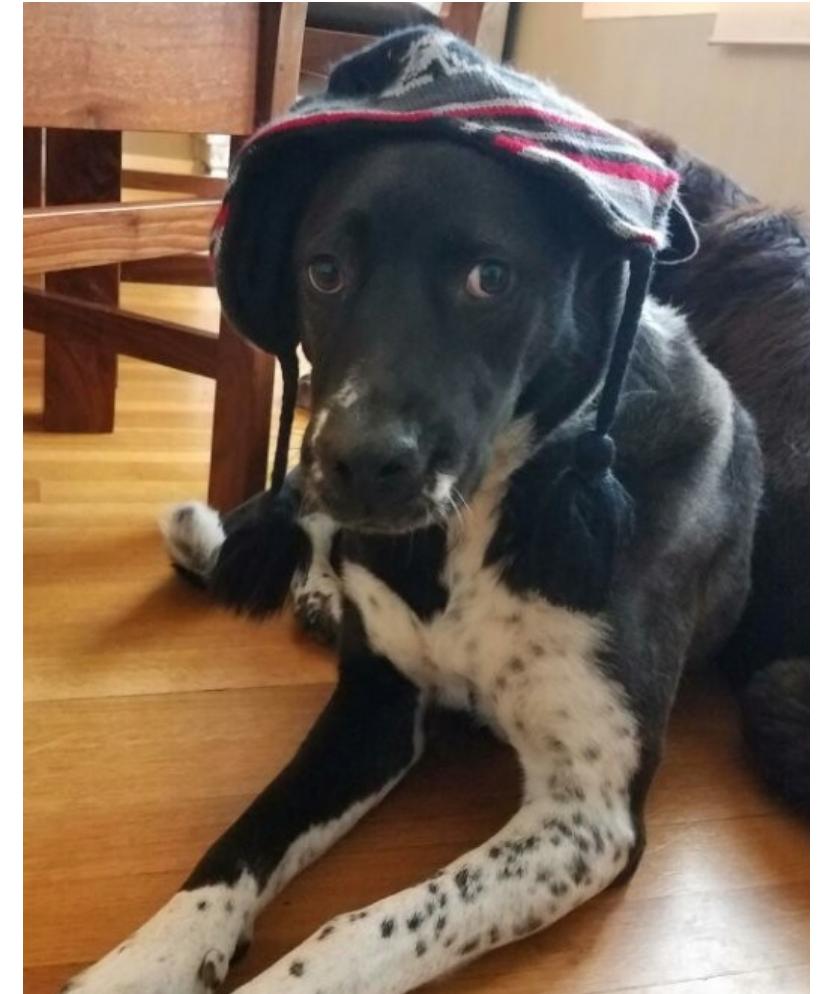
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- Post Doctoral Fellow New York University
- Peace Corp Volunteer
- Senior Research Associate Oregon Health & Science University
- Instructor PSU present

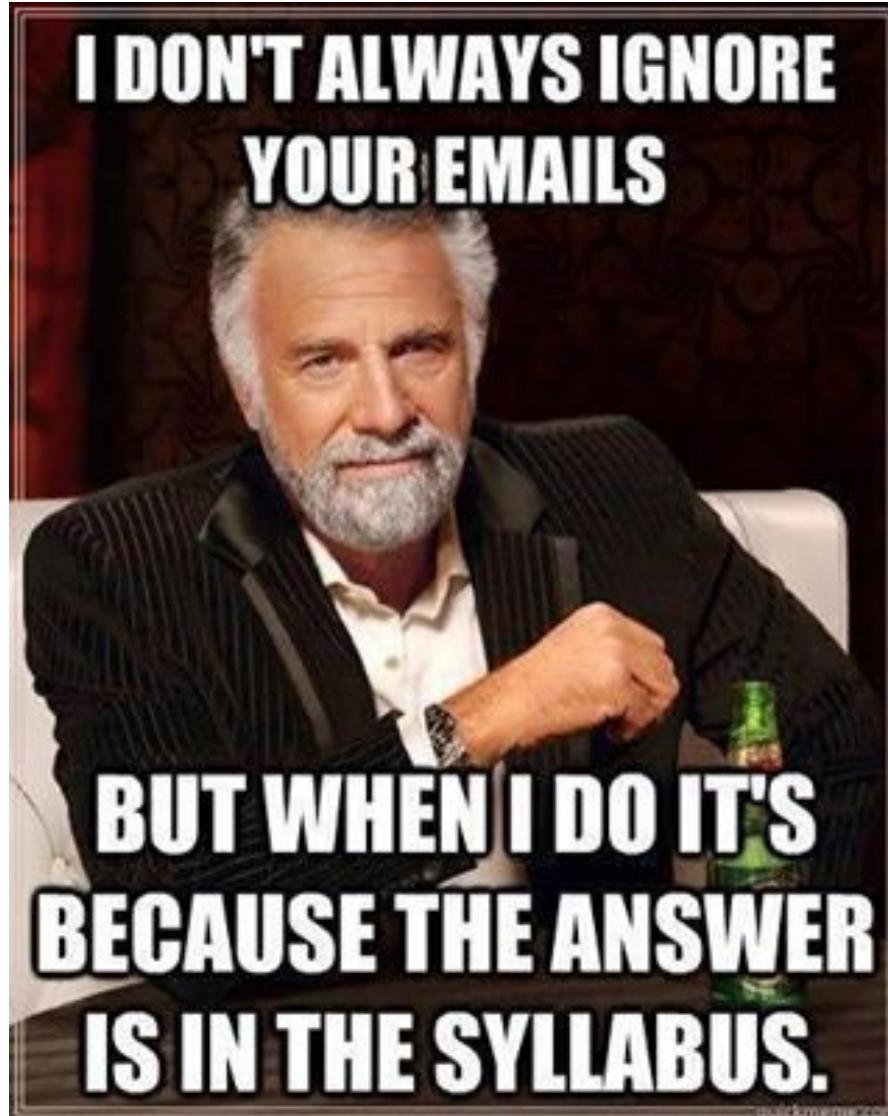
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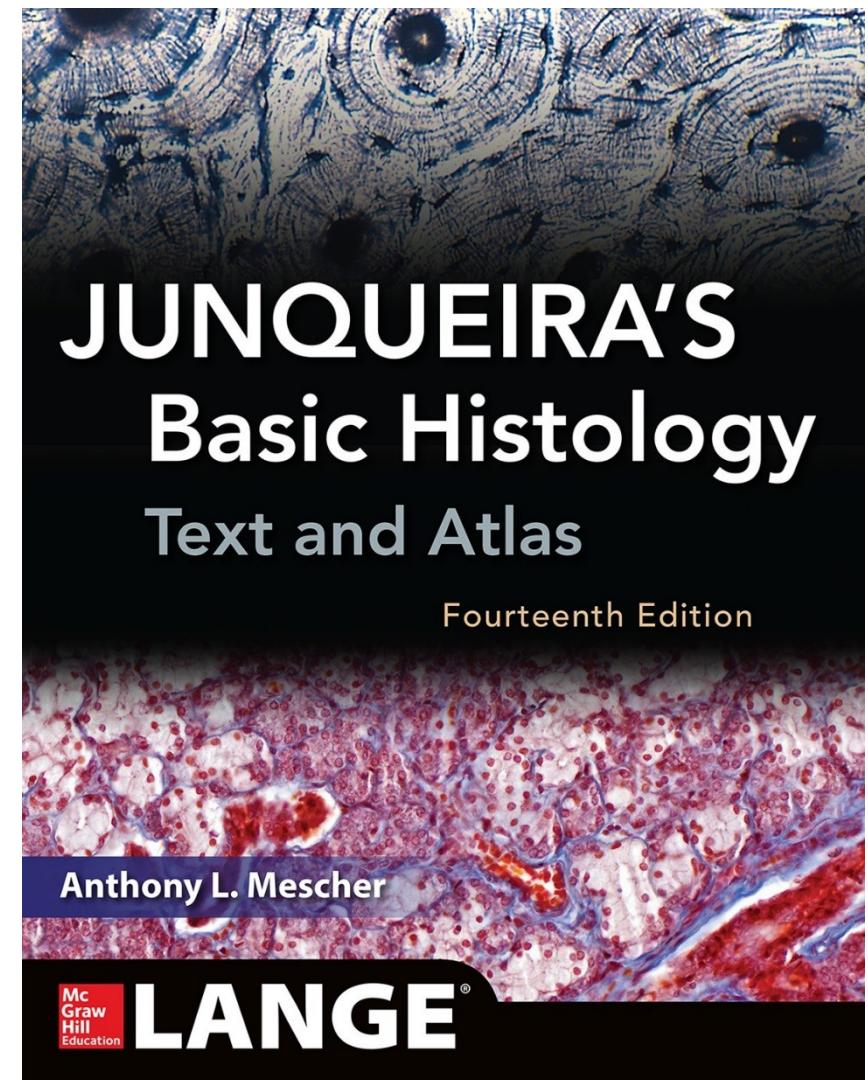
Syllabus



The course syllabus can be found on our D2L webpage. It is your ultimate resources for navigating this course

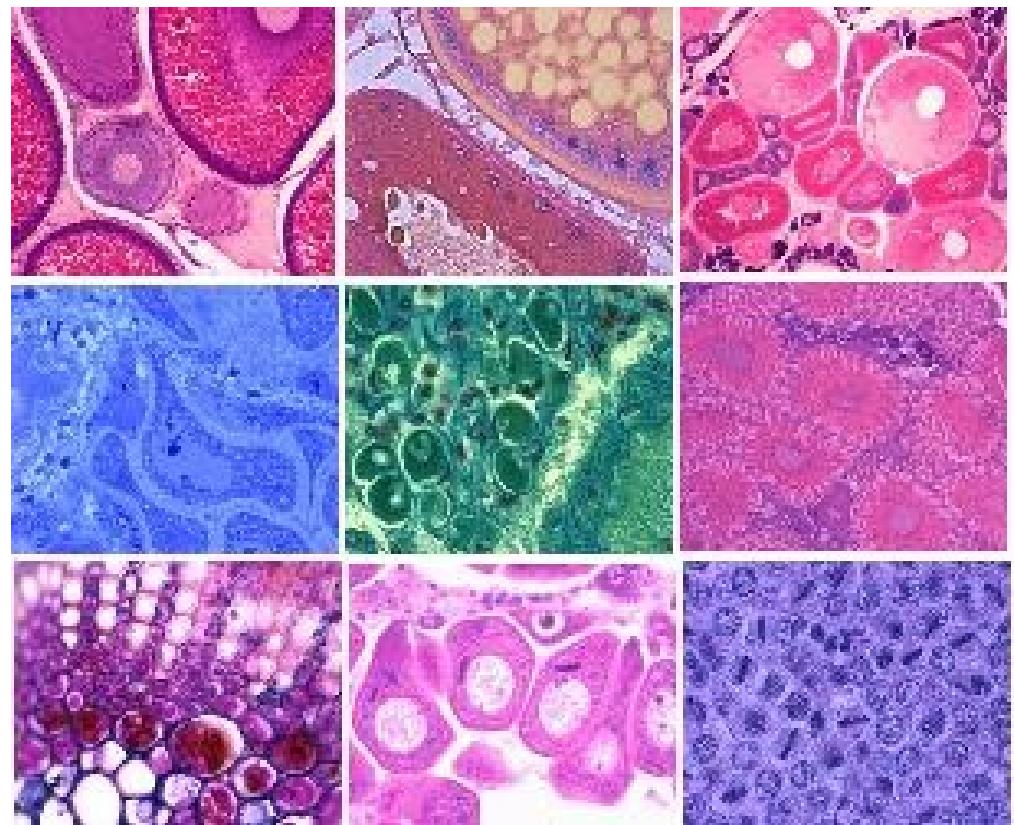
Week 1 Lab

- Always bring the text book to lab!!!
- Day 1: Complete the 1st 9 pages of the lab manual
- Day 2: Group cancer activity, complete remainder of lab.



Radhika Reddy, PhD

BI 455 CHAPTER 1- 3



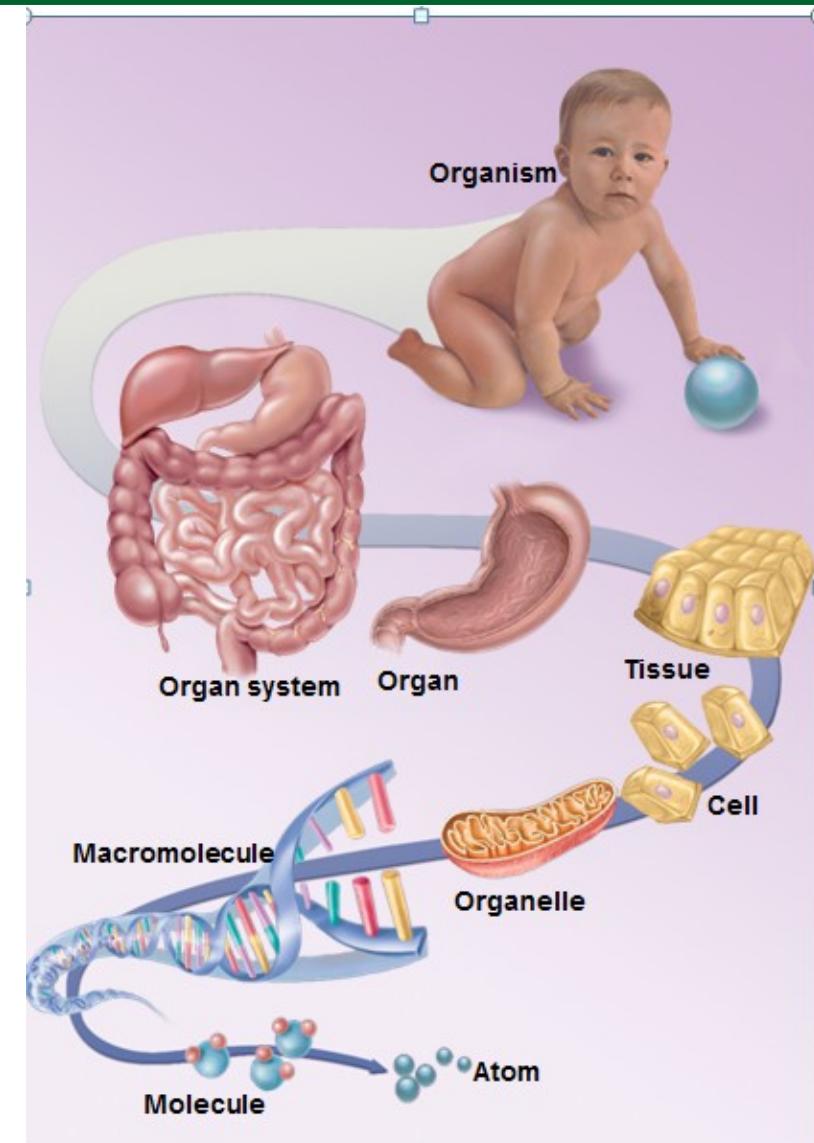


Tissue Preparation, Staining, Microscopy

CHAPTER 1: HISTOLOGY AND ITS METHODS OF STUDY

The Structural Basis of Human Function: The Anatomical Sciences

- Organism is composed of **organ systems**
- Organ systems composed of **organs**
- Organs composed of **tissues**
- Tissues composed of **cells**
- Cells composed of **organelles**
- Organelles composed of **molecules**
- Molecules composed of **atoms**



The Structural Basis of Human Function: The Anatomical Sciences

■ Gross anatomy

- Structure visible to the naked eye
- by surface observation or dissection

■ Histology (microscopic anatomy)

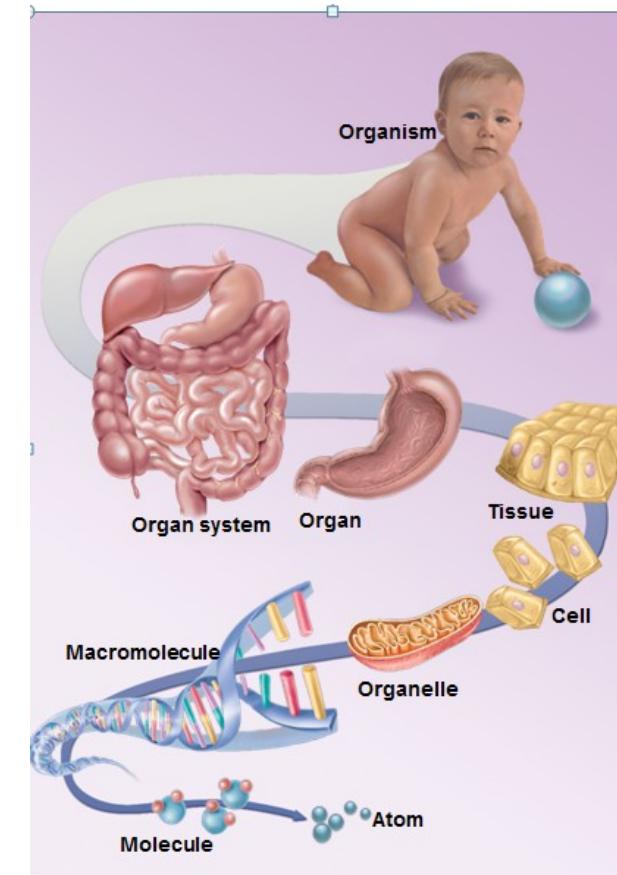
- Tissue specimens thinly sliced and stained
- Observed under a microscope
- Histopathology:** microscopic examination of tissues for disease

■ Surface anatomy

- External structure of the body
- Important in conducting physical exam

■ Systemic anatomy

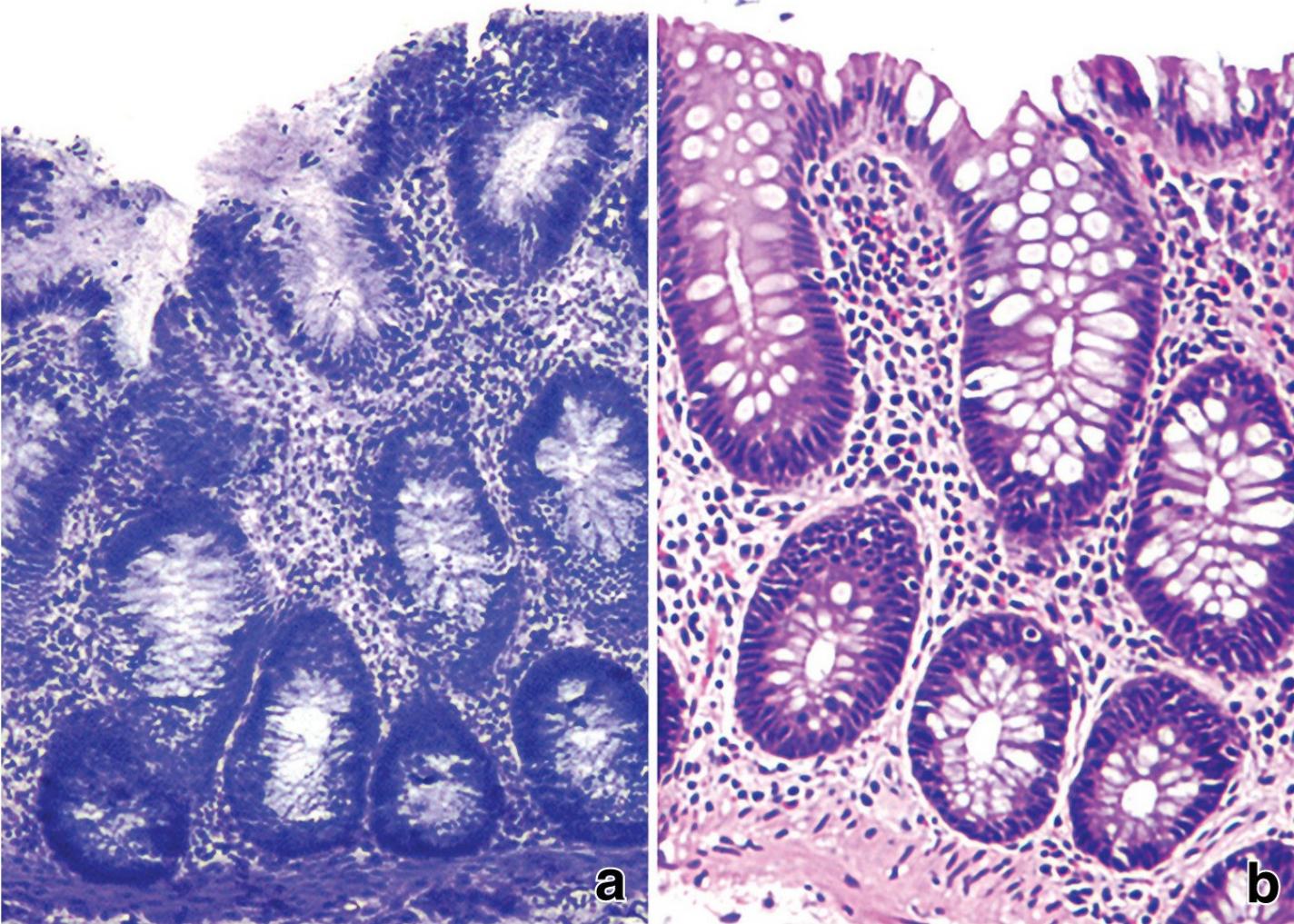
- Study of one organ system at a time



Histology at the University of Bristol:

<https://www.youtube.com/watch?v=PafHxS5bq9A&noredirect=1>

Clinical Correlation: Frozen sections and stained tissue



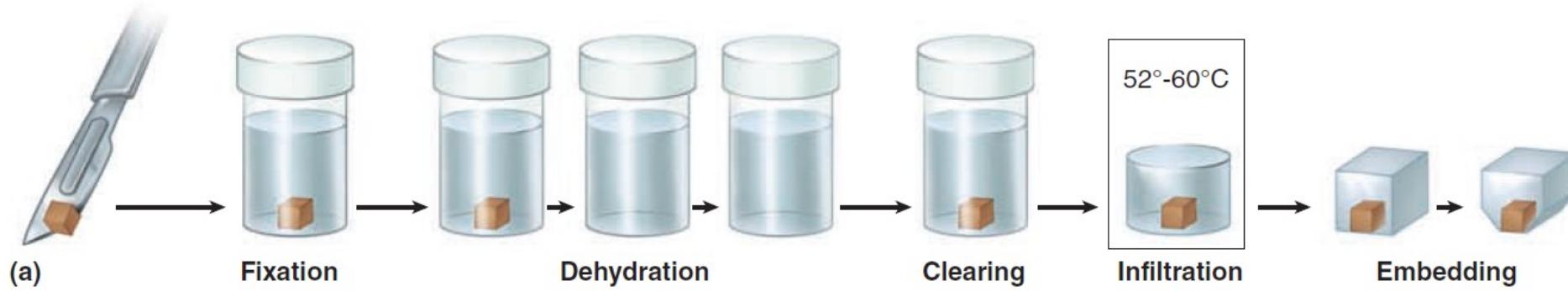
Evaluation of a specimen obtained during surgery by frozen-section technique.

a. This photomicrograph shows a specimen obtained from the large intestine that was prepared by frozen-section technique and stained with methylene blue.

b. Part of the specimen was fixed in formalin and processed as a routine H&E preparation.

Examination of the frozen section revealed it to be normal. This diagnosis was later confirmed by examining the routinely prepared H&E specimen.

Most tissues studied histologically are prepared as shown, with this sequence of steps:



- 1. Fixation:** Small pieces of tissue are placed in solutions of chemicals that preserve by cross-linking proteins and inactivating degradative enzymes.
- 2. Dehydration:** The tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water.
- 3. Clearing:** Alcohol is removed in toluene or other agents in which both alcohol and paraffin are miscible.
- 4. Infiltration:** The tissue is then placed in melted paraffin until it becomes completely infiltrated with this substance.
- 5. Embedding:** The paraffin-infiltrated tissue is placed in a small mold with melted paraffin and allowed to harden
6. The resulting paraffin block is trimmed to expose the tissue for sectioning (slicing) on a microtome.

A microtome is used for sectioning paraffin-embedded tissues for light microscopy

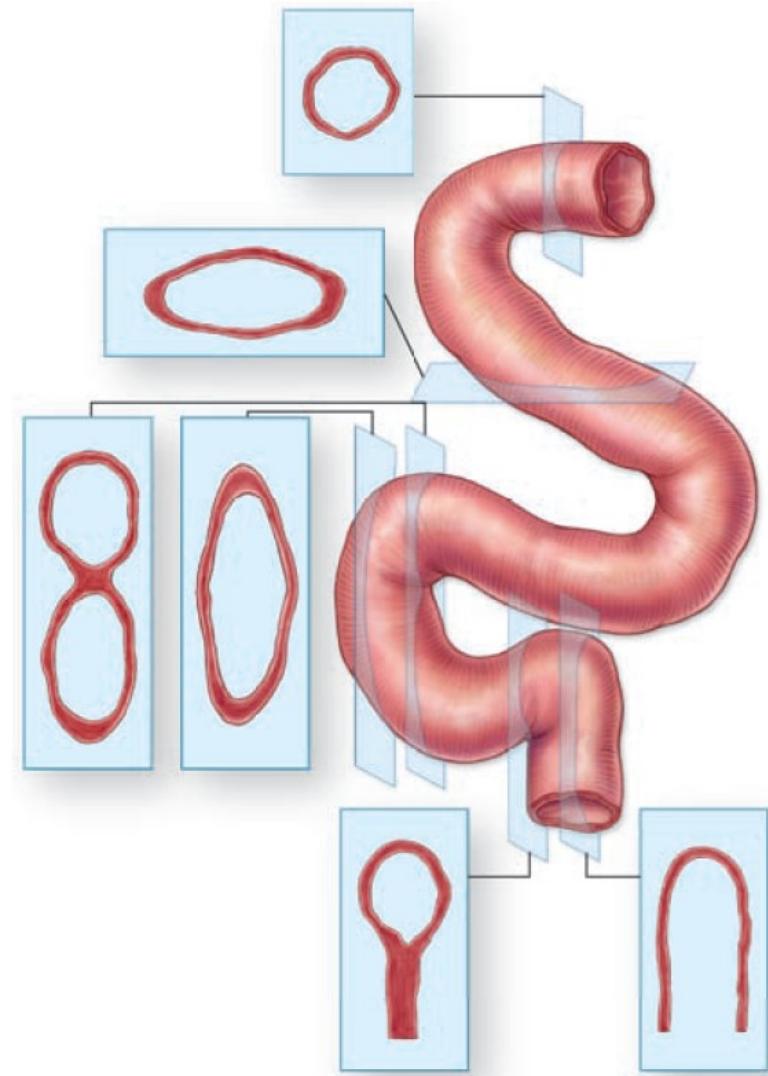


1. The trimmed tissue specimen is mounted in the paraffin block holder, and each turn of the drive wheel by the histologist advances the holder a controlled distance, generally between 1 and 10 μm .
2. After each forward move, the tissue block passes over the steel knife edge and a section is cut at a thickness equal to the distance the lock advanced.
3. The paraffin sections are placed on glass slides and allowed to adhere, deparaffinized, and stained for light microscope study.

Sectional Planes

FIGURE 1–14 Interpretation of 3D structures in 2D sections.

- When a structure's three-dimensional volume is cut into very thin sections, the sections appear microscopically to have only two dimensions: length and width.
- When examining a section under the microscope, the viewer must always keep in mind that components are missing in front of and behind what is being seen because many tissue structures are thicker than the section.



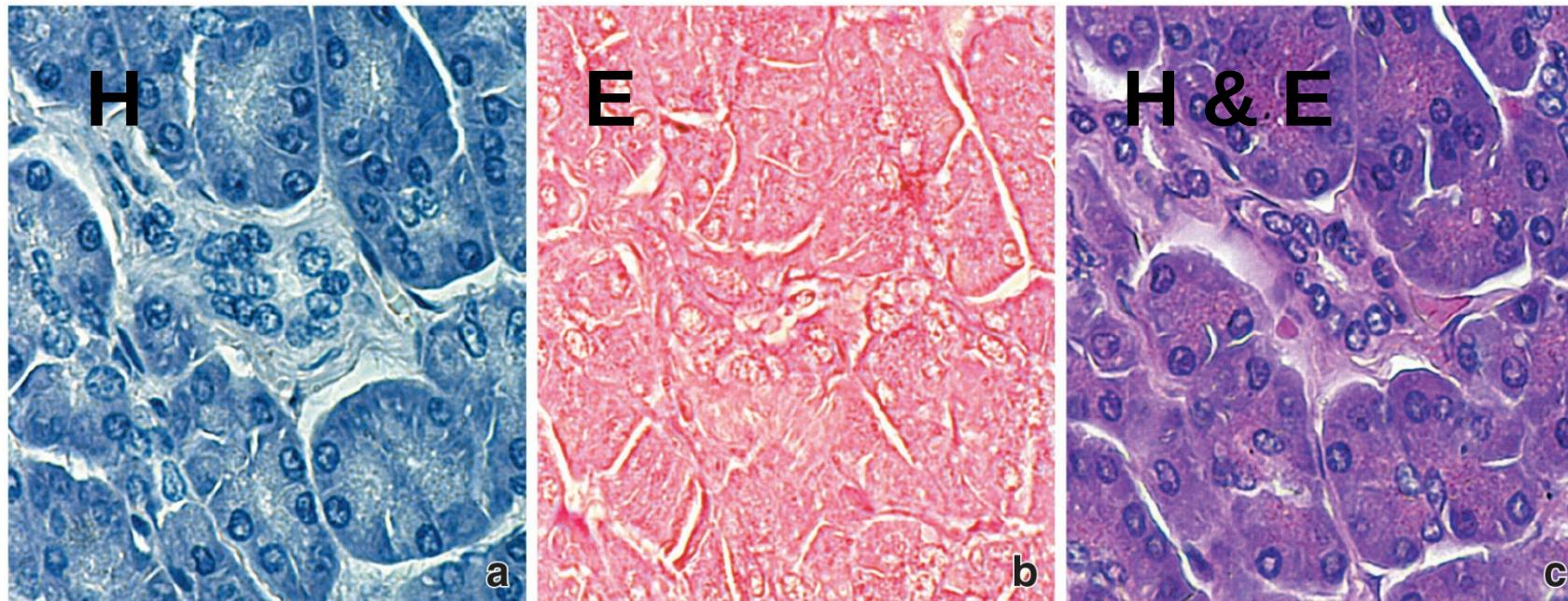
Staining

- Most cells and extracellular material are **completely colorless**, and to be studied microscopically sections must typically be **stained (dyed)**.
- Methods of staining have been devised that not only make the various tissue components conspicuous but also permit distinctions to be made between them
- Dyes stain tissue components more or less selectively, with many behaving like acidic or basic compounds and forming electrostatic (salt) linkages with ionizable radicals of molecules in tissues

TABLE	1.2	Some Basic and Acidic Dyes
Dye		Color
<i>Basic dyes</i>		
Methyl green		Green
Methylene blue		Blue
Pyronin G		Red
Toluidine blue		Blue
<i>Acidic dyes</i>		
Acid fuchsin		Red
Aniline blue		Blue
Eosin		Red
Orange G		Orange

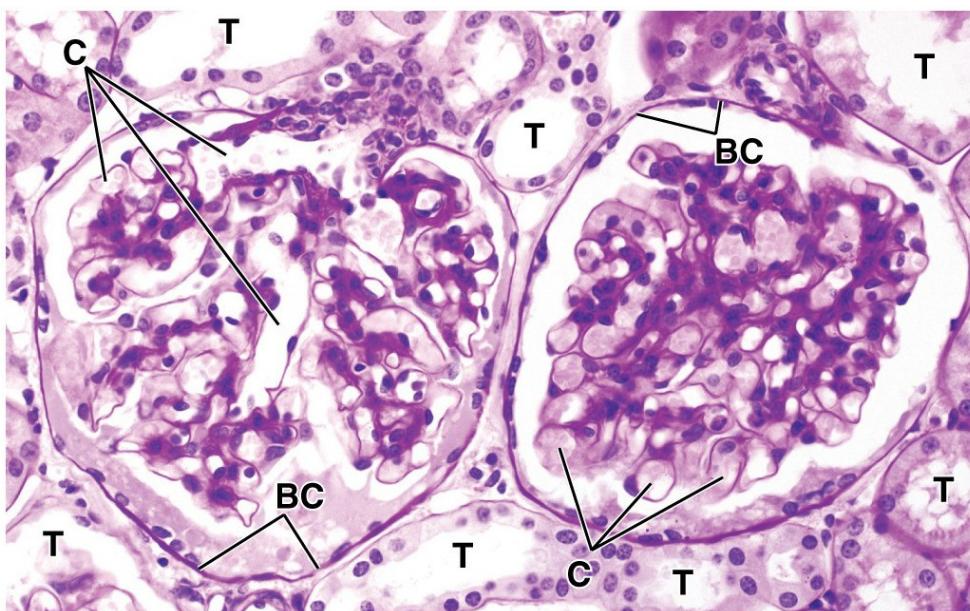
The Hematoxylin and Eosin (H&E) stain is used most commonly

- **Hematoxylin** (almost basic) produces a dark blue or purple color, staining **DNA** in the cell nucleus and other acidic structures (such as **RNA-rich** portions of the cytoplasm and the **matrix of cartilage**).
- **Eosin** stains other **cytoplasmic components and collagen** pink



Periodic Acid-Schiff (PAS) reagent.

- The PAS reaction is based on the transformation of 1,2-glycol groups present in sugars into aldehyde residues, which then react with Schiff reagent to produce a purple or magenta color



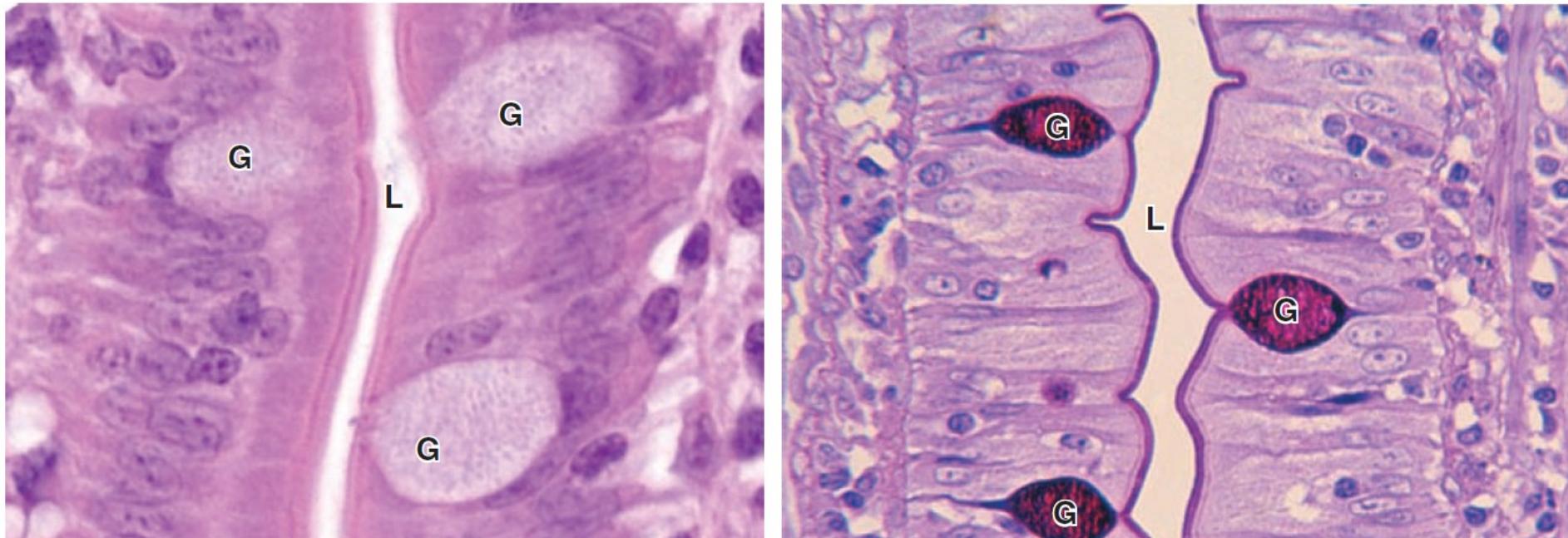
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This histochemical method demonstrates and localizes carbohydrates and carbohydrate-rich macromolecules.

The basement membranes in this kidney tissue are PAS positive as evidenced by the magenta staining of these sites. The kidney tubules (T) are sharply delineated by the stained basement membrane surrounding the tubules. The glomerular capillaries (C) and the epithelium of Bowman's capsule (BC) also show PAS-positive basement membranes.

H & E vs PAS

FIGURE 1–2 Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining.



Micrograph of epithelium lining the small intestine, (a) stained with H&E, and (b) stained with the PAS reaction for glycoproteins. With H&E, basophilic cell nuclei are stained purple while cytoplasm stains pink. Cell regions with abundant oligosaccharides on glycoproteins, such as the ends of the cells at the lumen (L) or the scattered mucus-secreting goblet cells (G), are poorly stained. With PAS, however, cell staining

is most intense at the lumen, where projecting microvilli have a prominent layer of glycoproteins at the lumen (L) and in the mucin-rich secretory granules of goblet cells. Cell surface glycoproteins and mucin are PAS-positive because of their high content of oligosaccharides and polysaccharides respectively. The PAS-stained tissue was counterstained with hematoxylin to show the cell nuclei. Both X300.

Enzyme histochemistry (cytochemistry): localizes cellular structures using a specific enzymatic activity present in those structures

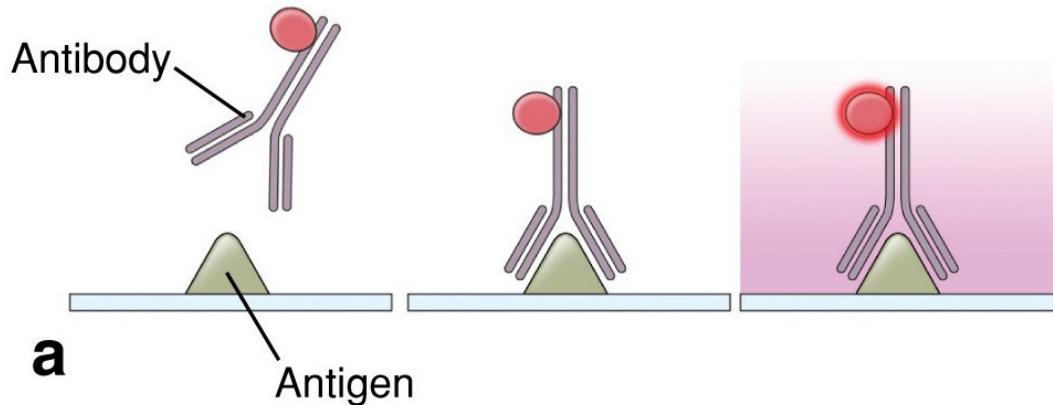


Micrograph of cross sections of kidney tubules treated histochemically to demonstrate alkaline phosphatases shows strong activity of this enzyme at the apical surfaces of the cells at the lumens (L) of the tubules.

- (1) Tissue sections are immersed in a solution containing the substrate of the enzyme to be localized
- (2) The enzyme is allowed to act on its substrate
- (3) The section is put in contact with a marker compound that reacts with a product of the enzymatic action on the substrate
- (4) The final product has color or electron density, and precipitates over the site of the enzymes causing contrast between enzymatically active vs inactive areas

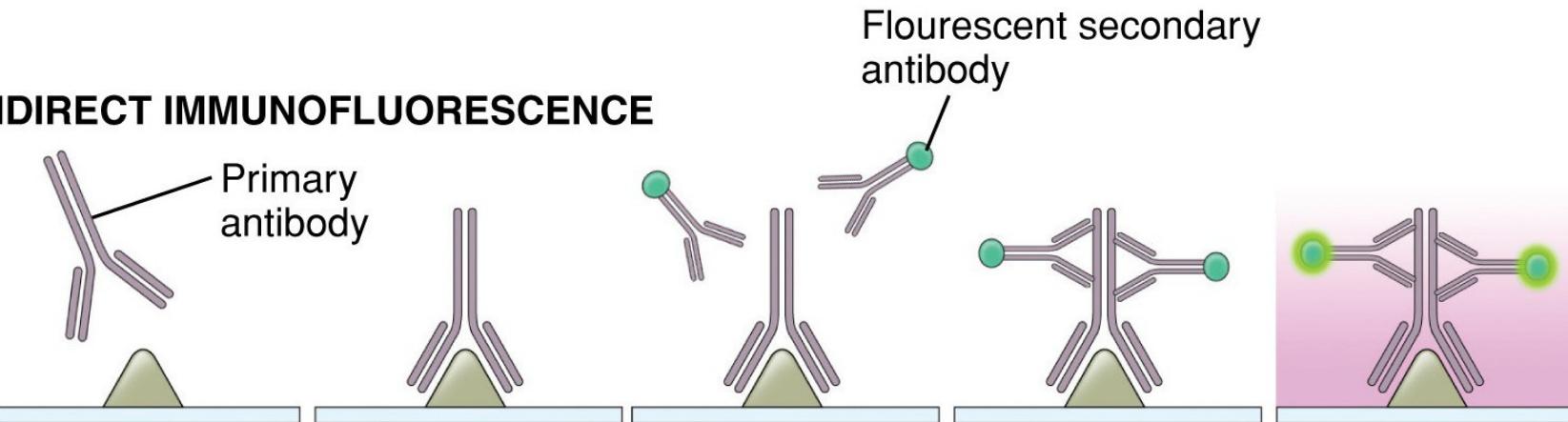
Immunocytochemistry: uses reaction between an antigen and an antibody to visualize proteins

DIRECT IMMUNOFLUORESCENCE



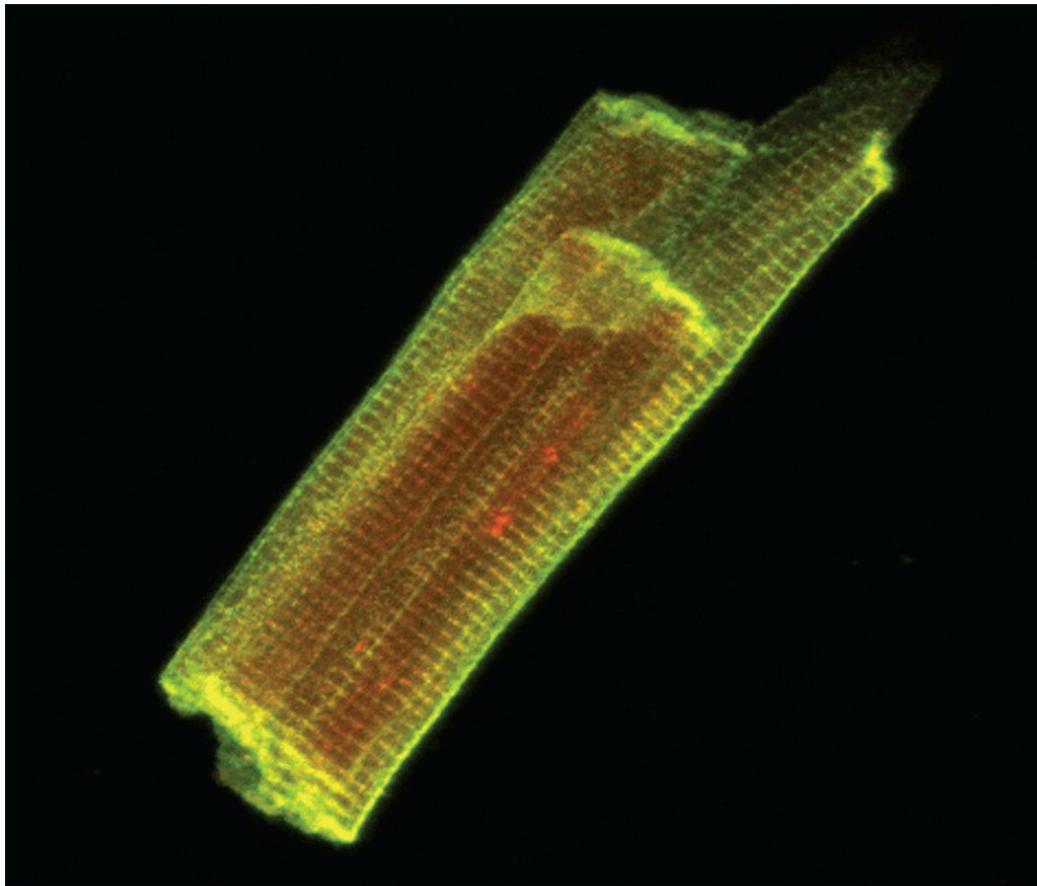
a

INDIRECT IMMUNOFLUORESCENCE



b

Immunocytochemistry: uses reaction between an antigen and an antibody to visualize proteins



Rat Cardiac Muscle Cell:

RED: lactate transporter (MCT1) antibody is detected with a secondary antibody conjugated with rhodamine (red).

GREEN: transmembrane protein CD147 antibody is detected by a secondary antibody labeled with fluorescein (green).

YELLOW: secondary antibodies exactly co-localize within the cardiac muscle cell.

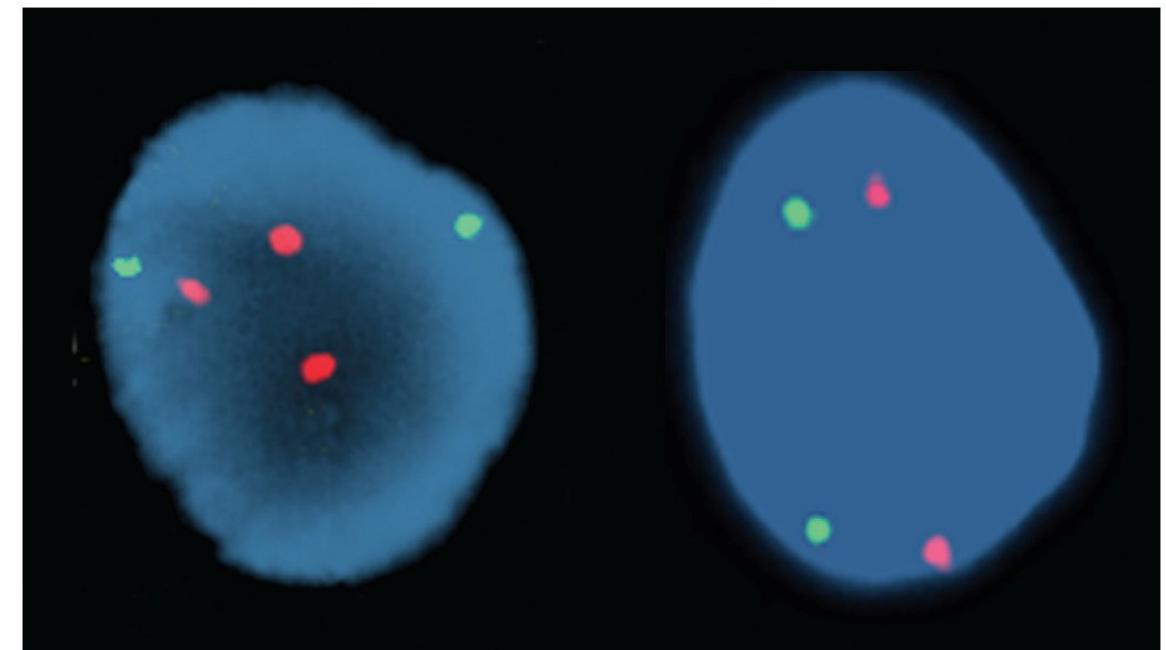
Hybridization: visualizing gene expression by hybridizing an added nucleotide probe to mRNA in the cell

Prenatal screening test. Interphase nuclei of cells obtained from amniotic fluid specimens were hybridized with two specific DNA probes.

ORANGE (LSI 21) chromosome 21 probe
GREEN (LSI 13) chromosome 13 probe

The right nucleus is from a normal Amniotic fluid specimen

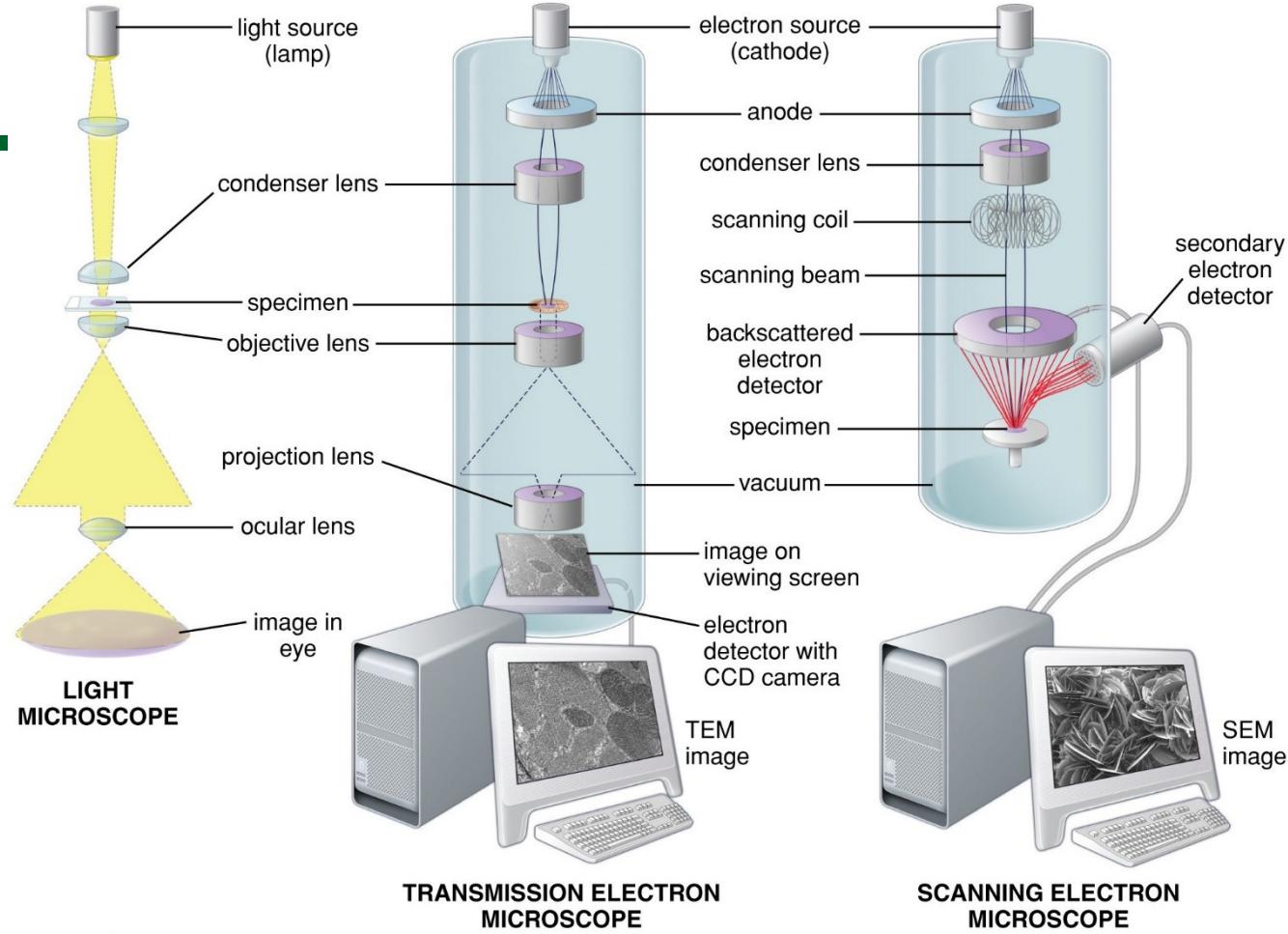
The left nucleus has three orange signals, which indicate trisomy 21 (Down syndrome).



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Microscopy

1. Light Microscopy
 - a. Bright-field
 - b. Phase Contrast
 - c. Fluorescence
 - d. Confocal
2. Electron Microscopy (EM)
3. Atomic Forces Microscopy
4. Virtual Microscopy



How to focus a microscope:
<https://www.youtube.com/watch?v=scEhgAiazzU>

Resolving Power

- The distance by which two objects must be separated to be seen as two objects

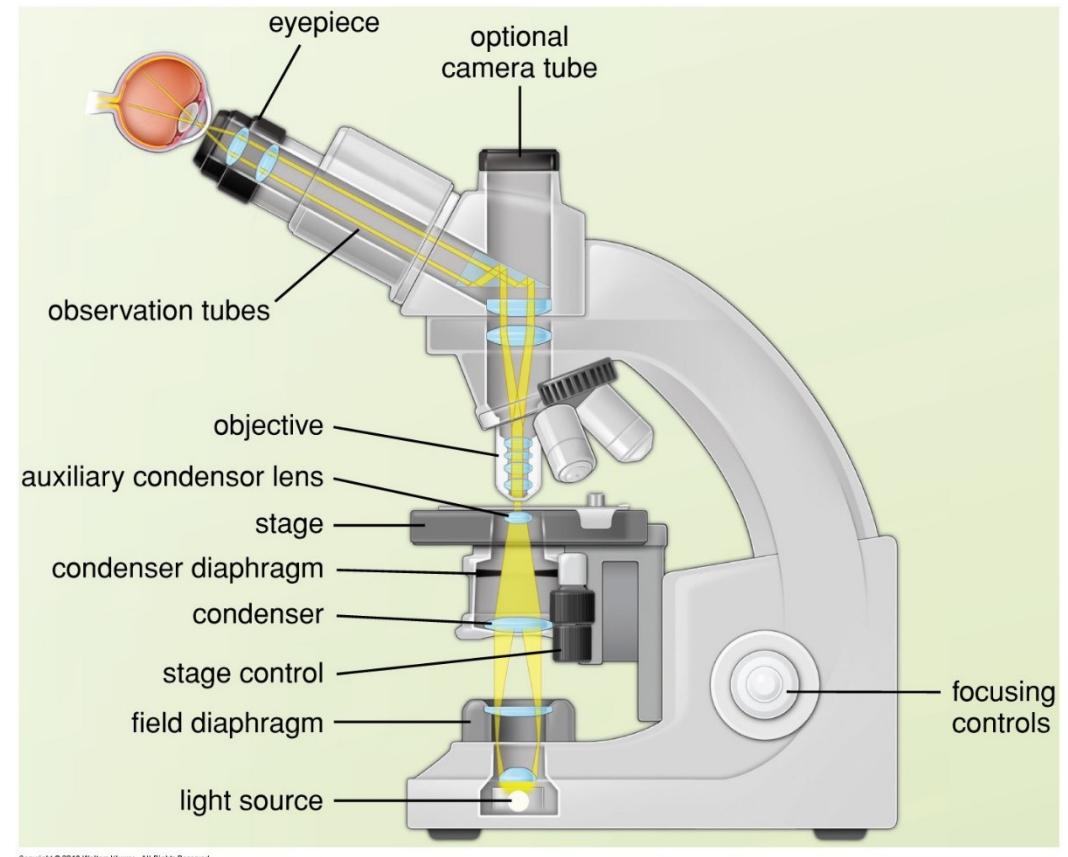
TABLE	1.3	Eye Versus Instrument Resolution
Distance Between Resolvable Points		
Human eye		0.2 mm
Bright-field microscope		0.2 μ m
SEM		2.5 nm
TEM		
Theoretical		0.05 nm
Tissue section		1.0 nm

Light Microscopy: interaction of light with tissue components and are used to reveal and study tissue features in different ways

Bright-field microscopy (which we will use this term): stained preparations are examined as ordinary light passes through the specimen.

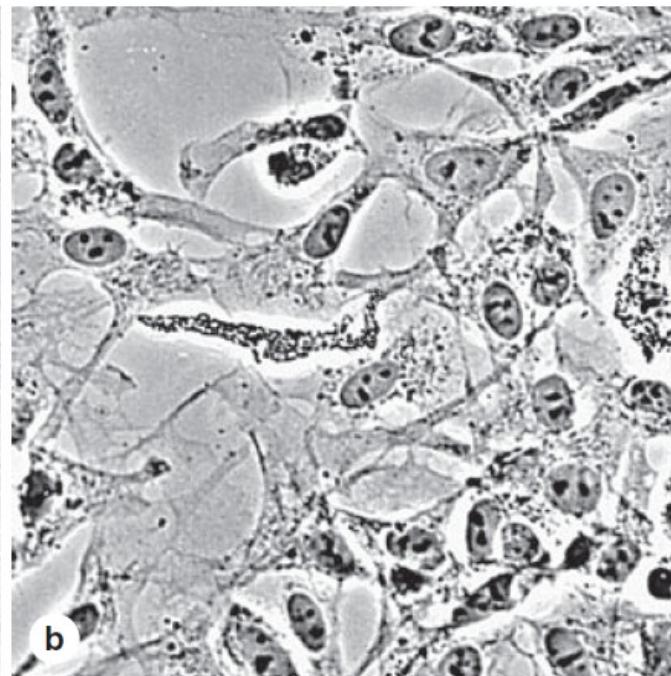
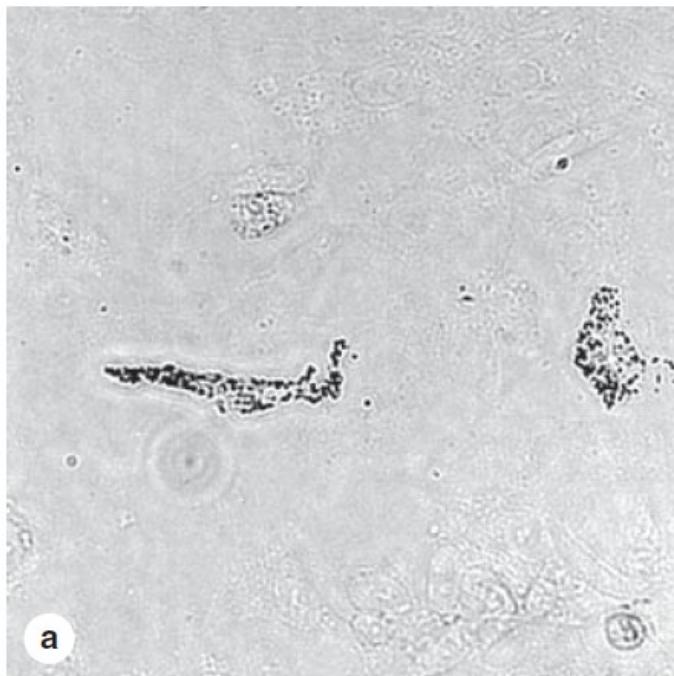
Optical Components:

- **condenser:** collects and focuses light
- **objective lens:** enlarges and projects the image of the object toward eyepiece.
- **ocular lens (eyepiece):** further magnifies image low light levels with a monitor and camera.
- **total magnification:** product of magnifying power of the objective and ocular lenses.



Other types of light microscopy

- **Phase-contrast microscopy** uses the differences in refractive index of various natural cell and tissue components to produce an image without staining, allowing observation of living cells.

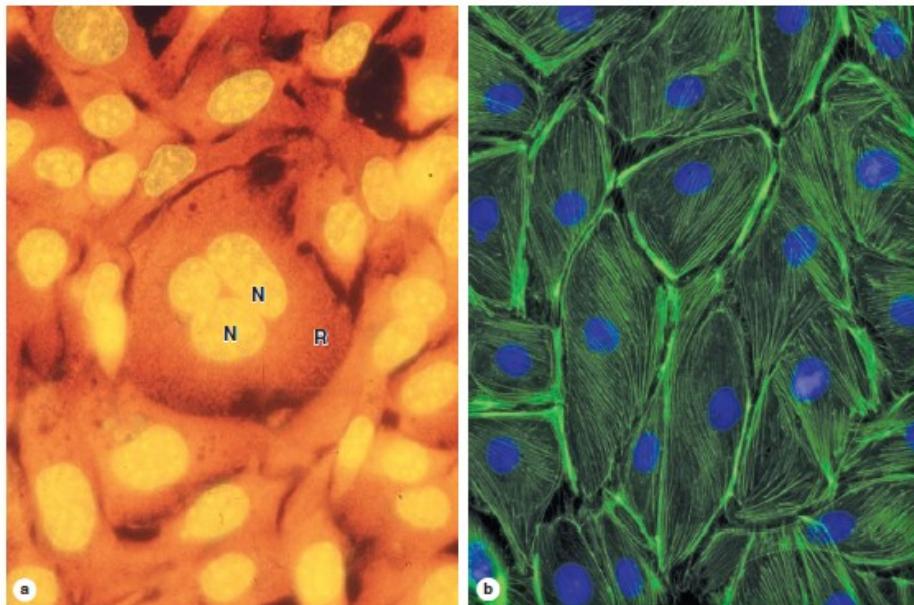


(a) Bright-field microscopy: Without fixation and staining, only the two pigment cells can be seen.

(b) Phase-contrast microscopy: Cell boundaries, nuclei, and cytoplasmic structures with different refractive indices affect in-phase light differently and produce an image of these features in *all* the cells.

Other types of light microscopy

- **Fluorescence microscopy** uses ultraviolet light, under which only fluorescent molecules are visible, allowing localization of fluorescent probes which can be much more specific than routine stains.



Components of cells are often stained with compounds visible by fluorescence microscopy.

(a) Acridine orange binds nucleic acids and causes DNA in cell nuclei (**N**) to emit yellow light and the RNA-rich cytoplasm (**R**) to appear orange in these cells of a kidney tubule.

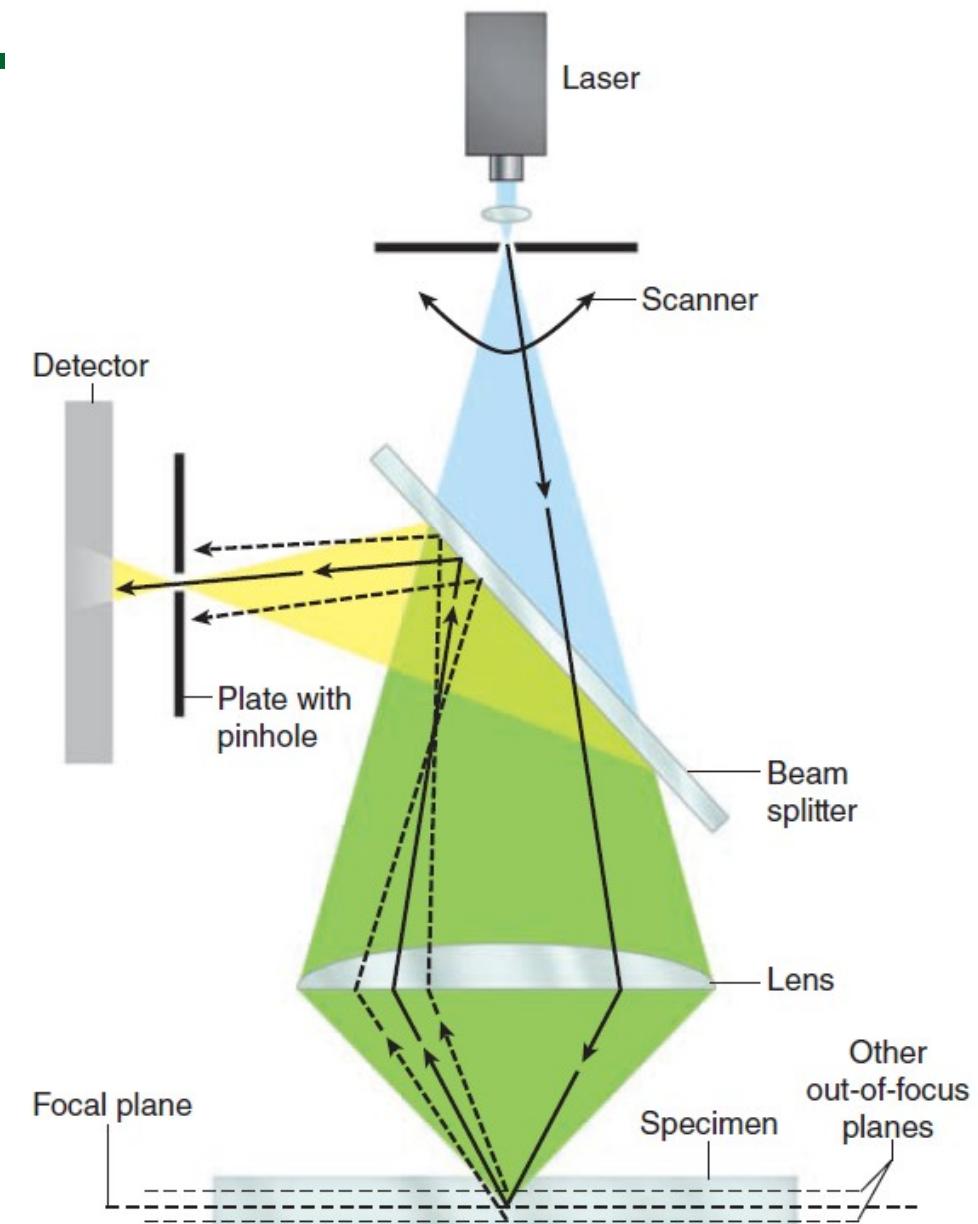
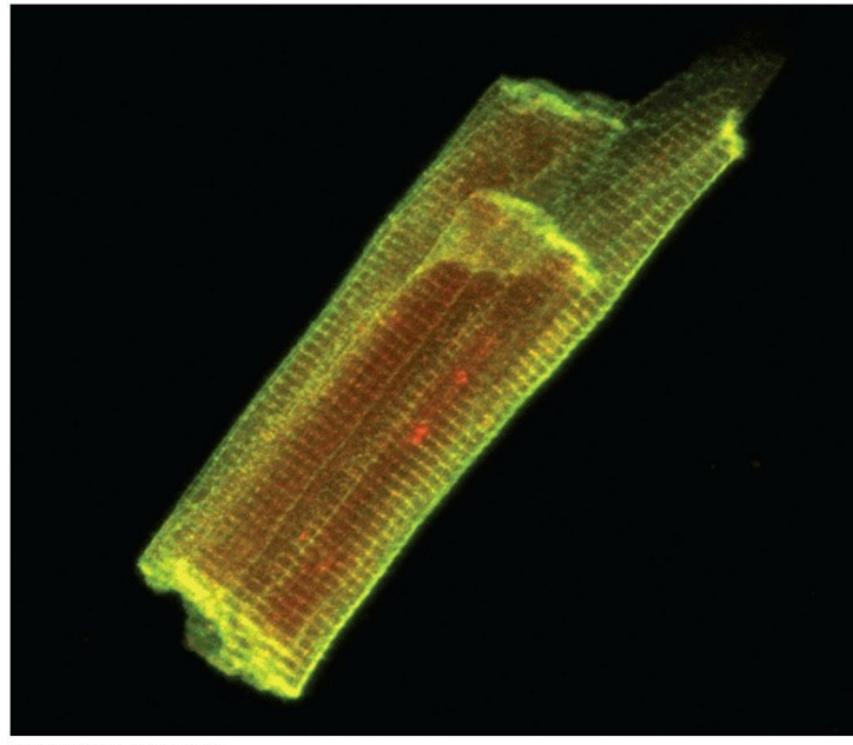
(b) Cultured cells stained with DAPI (4',6-diamino-2-phenylindole) that binds DNA and with fluorescein-phalloidin that binds actin filaments show nuclei with blue fluorescence and actin filaments stained green. Important information such as the greater density of microfilaments at the cell periphery is readily apparent. Both X500.

(Figure 1–4b, contributed with permission, from Drs Claire E. Walczak and Rania Risk, Indiana University School of Medicine, Bloomington.)

FIGURE 1–6 Principle of confocal microscopy.

Other types of light microscopy

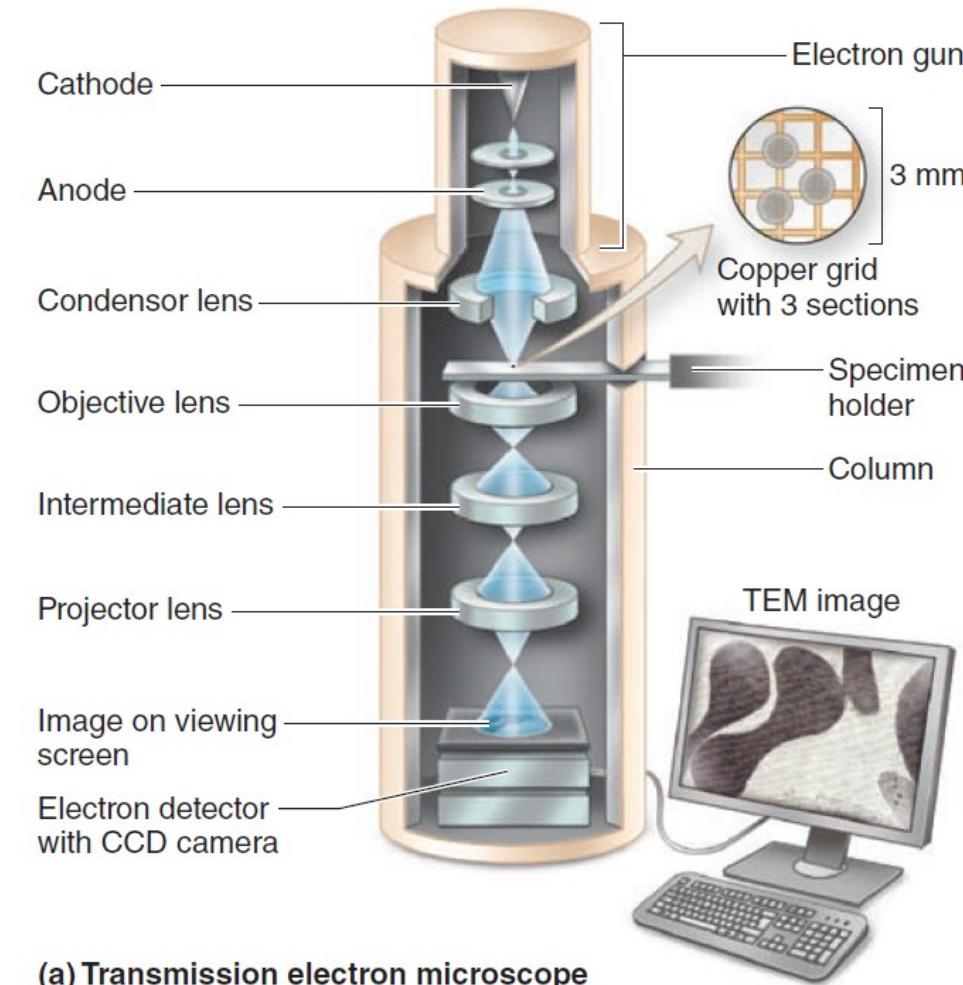
- **Confocal microscopy** involves scanning the specimen at successive focal planes with a focused light beam, often from a laser, and produces a 3D reconstruction from the images.

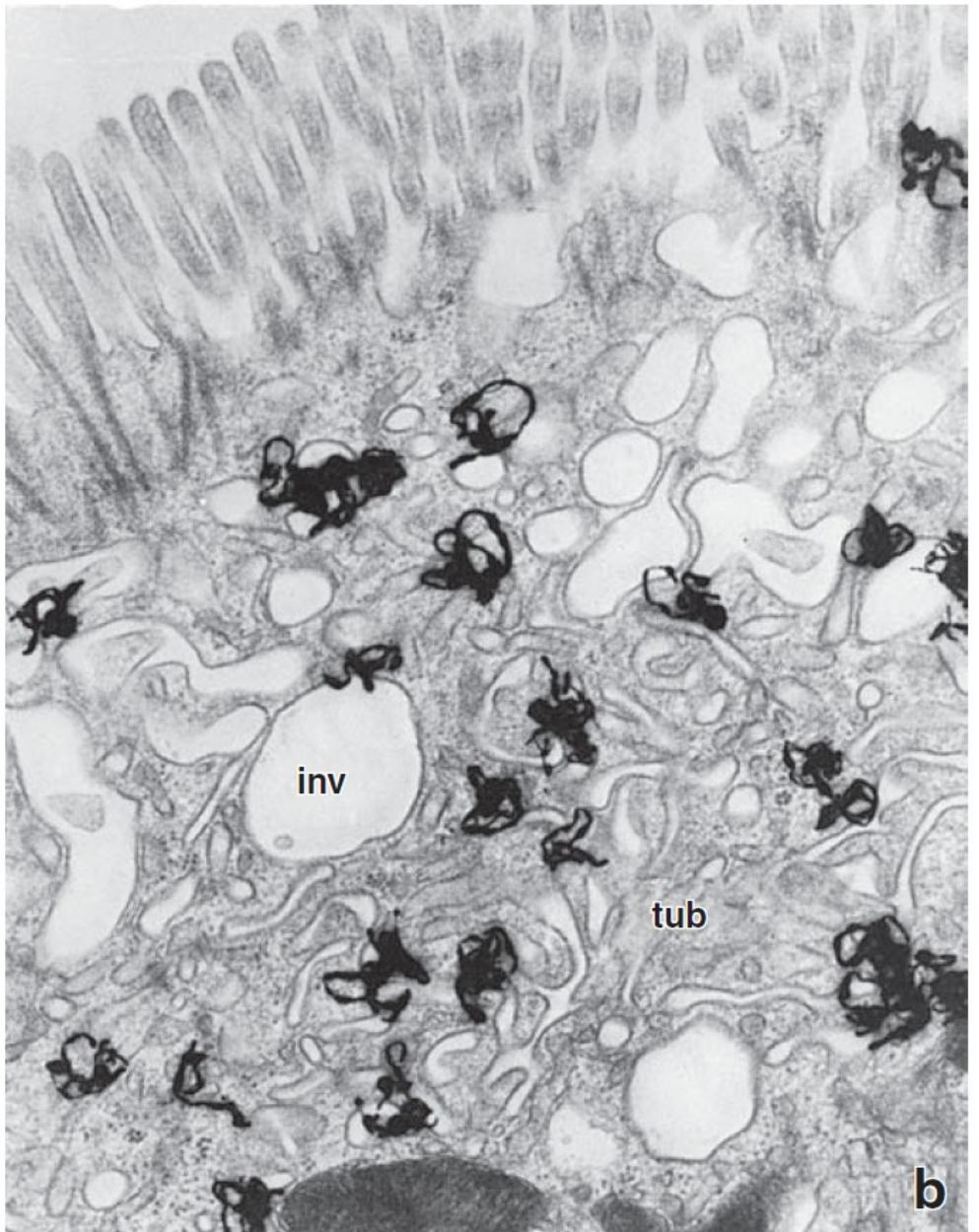


Electron Microscopy

- Transmission and scanning electron use beams of electrons rather than light. The wavelength in the electron beam is much shorter than that of light, allowing a 1000-fold increase in resolution.
- **Transmission EM** sends an electromagnetically focused beam of electrons at very high voltage through ultrathin sections of tissue.
- Tissue preparation for TEM involves adding **heavy metal ions** that associate at different electron densities with cell and tissue components, improving contrast in the resulting image

FIGURE 1–8 Electron microscopes.



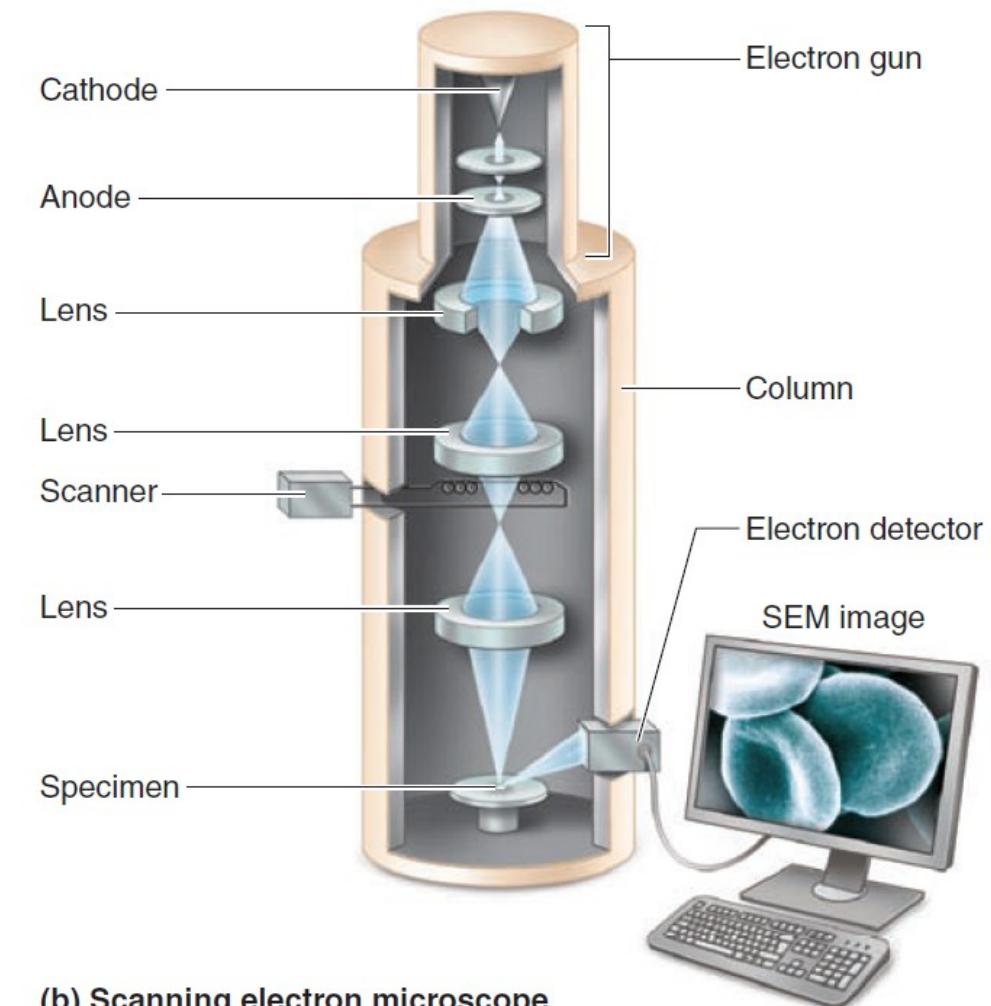


Electron microscopic autoradiograph of the apical region of an intestinal absorptive cell. In this specimen, ^{125}I bound to nerve growth factor (NGF) was injected into the animal, and the tissue was removed 1 hour later.

The specimen was prepared in a manner similar to that for light microscopy. The relatively small size of the silver grains aids precise localization of the ^{125}I -NGF complexes. Note that the silver grains are concentrated over apical invaginations (*inv*) and early endosomal tubular profiles (*tub*). 32,000.

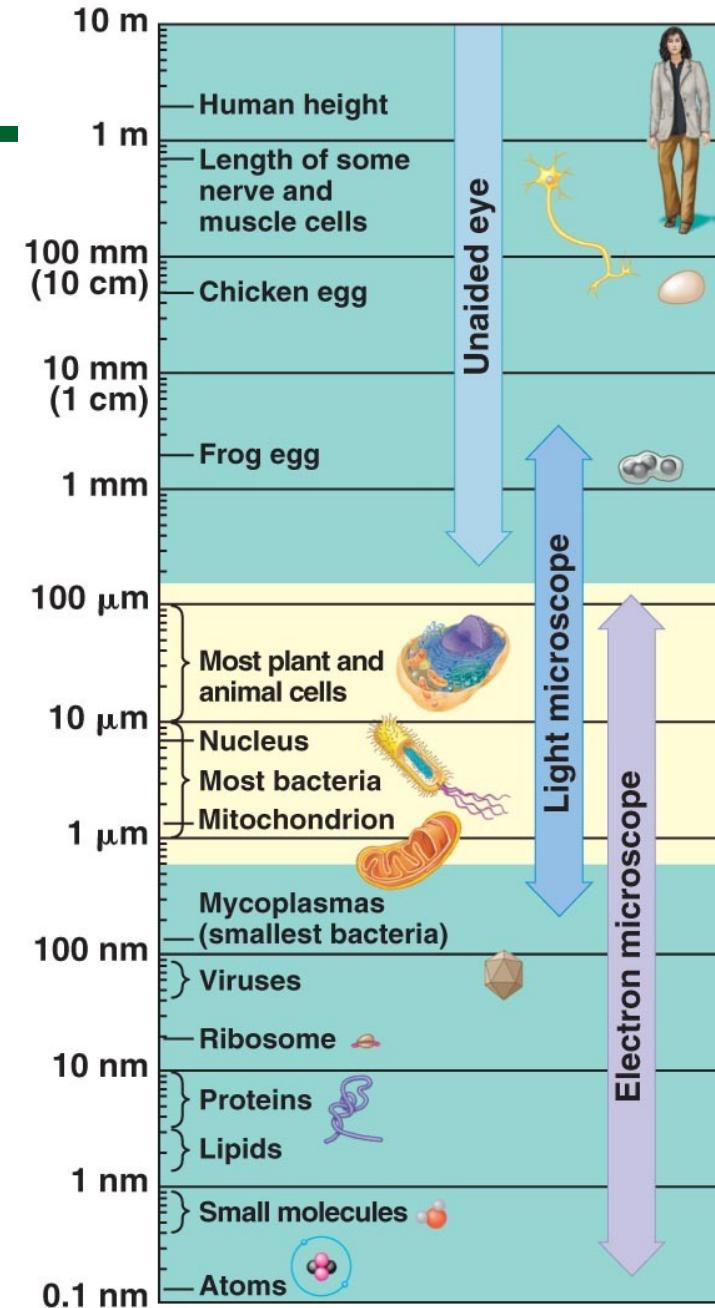
Electron Microscopy

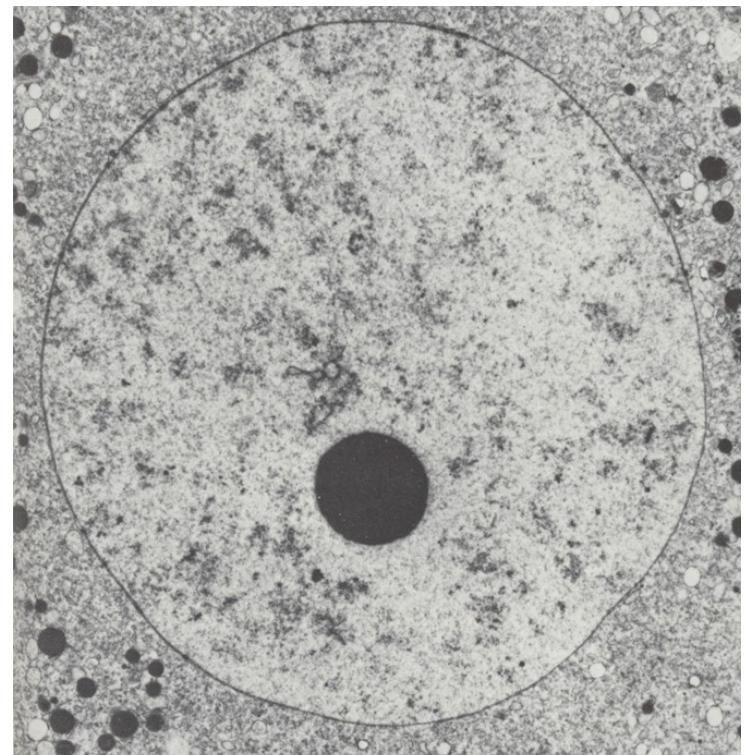
- **Scanning EM** scans an electron beam across a specimen coated with a thin layer of heavy metal; reflected and secondary electrons from the specimen are processed into a 3D ultrastructural image



Most cells are microscopic

- Most cells cannot be seen without a microscope
 - Bacteria are the smallest of all cells and require magnifications up to 1,000X
 - Plant and animal cells are 10 times larger than most bacteria





Membranous/Non-Membranous Organelles, Inclusions, Cytoplasmic Matrix

CHAPTER 2: CELL CYTOPLASM

An Introduction to Cells

The four major life processes in eukaryotic cells & their organelles

1. Manufacturing: nucleus, ribosomes, endoplasmic reticulum, and Golgi apparatus
2. Breakdown of molecules: lysosomes, vacuoles, and peroxisomes
3. Energy processing: mitochondria in animal cells and chloroplasts in plant cells
4. Structural support, movement, and communication: cytoskeleton, plasma membrane, and cell wall

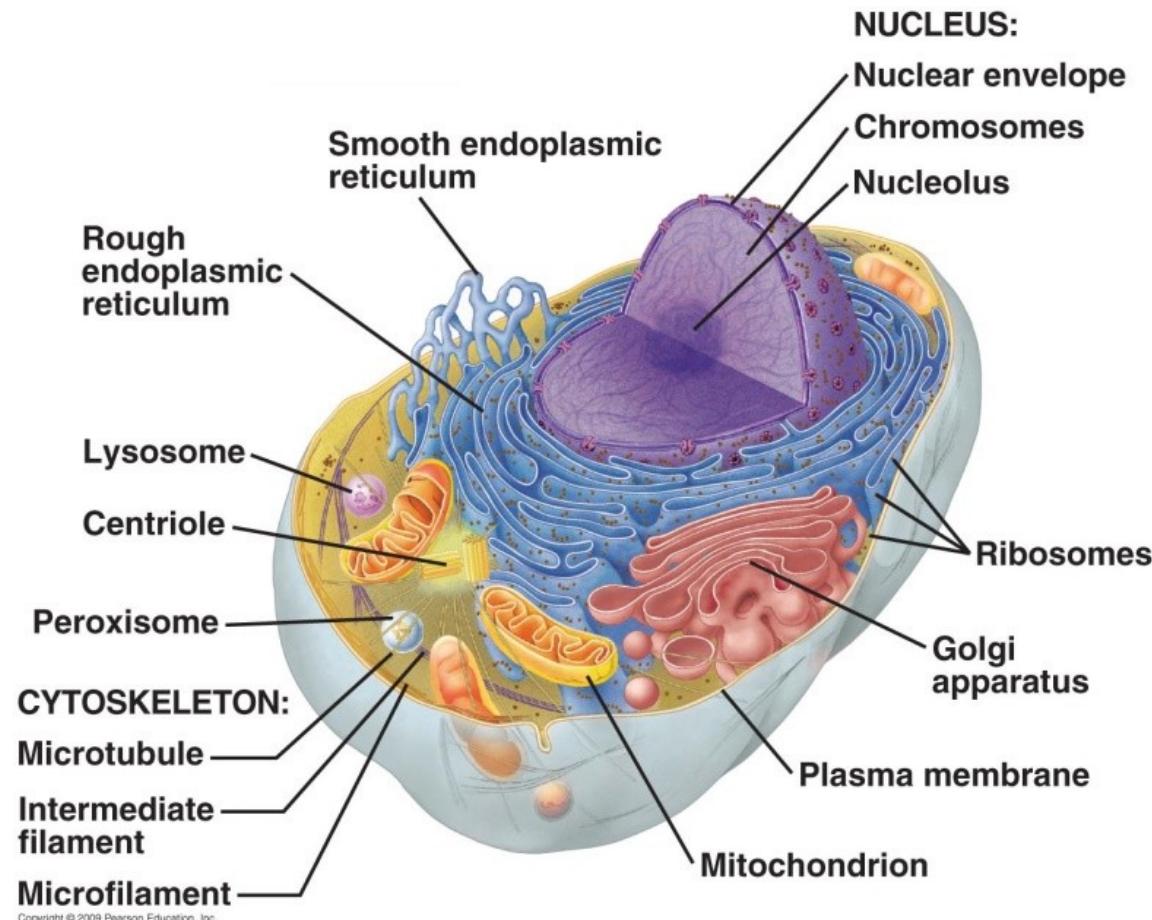
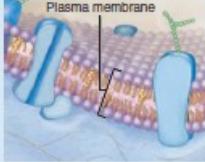
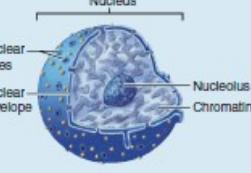
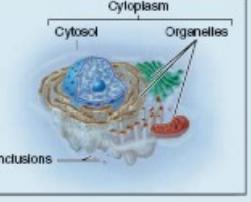


TABLE 2-6 Summary of cellular structural components.

Component	Structure	Major Function	Appearance
Plasma membrane	Phospholipid bilayer containing cholesterol and proteins (integral and peripheral) and some carbohydrates (externally); forms a selectively permeable boundary of the cell	Acts as a physical barrier to enclose cell contents; regulates material movement into and out of the cell; establishes and maintains an electrical charge difference across the plasma membrane; functions in cell communication	
Cilia	Short, numerous membrane extensions supported by microtubules, which occur on exposed membrane surfaces of some cells	Move substances (eg, mucus, and dissolved materials) over the cell surface	
Flagellum	Long, singular membrane extension supported by microtubules; present on sperm cells	Propels sperm	
Microvilli	Numerous thin membrane folds projecting from the free cell surface; supported by microfilaments	Increase membrane surface area for greater absorption	
Nucleus	Large structure enclosed within a double membrane; contains chromatin, nucleolus, and nucleoplasm	Houses the DNA that serves as the genetic material for directing protein synthesis	
Nuclear envelope	Double membrane boundary between cytoplasm and nuclear contents; continuous with rough endoplasmic reticulum	Separates nucleus from cytoplasm	
Nuclear pores	Openings through the nuclear envelope	Allow passage of materials between the cytoplasm and nucleoplasm, including ribonucleic acid (RNA), protein, ions, and small water-soluble molecules	
Nucleolus	Large, prominent structure within the nucleus	Functions in synthesis of ribosomes	
Cytoplasm	Contents of cells between the plasma membrane and nuclear envelope	Responsible for many cellular processes	
Cytosol	Viscous fluid medium with dissolved solutes (eg, ions, proteins, carbohydrates, lipids)	Provides support for organelles; serves as the viscous fluid medium through which diffusion occurs	
Organelles	Membrane-bound and non-membrane-bound structures	Carry out specific metabolic activities of the cell	
Rough endoplasmic reticulum (rough ER)	Extensive interconnected membrane network that varies in shape (eg, cisternae, tubules); ribosomes attached on cytoplasmic surface	Modifies, transports, and stores proteins produced by attached ribosomes; these proteins are secreted, become components of the plasma membrane, or serve as enzymes of lysosomes	
Smooth endoplasmic reticulum (smooth ER)	Extensive interconnected membrane network lacking ribosomes	Synthesizes, transports, and stores lipids (eg, steroids); metabolizes carbohydrates; detoxifies drugs, alcohol, and poisons; forms vesicles and peroxisomes	

(Continued)

An excellent summary of chapter 2

Crash Course Cells

<https://www.khanacademy.org/partner-content/crash-course1/crash-course-biology/v/crash-course-biology-104>

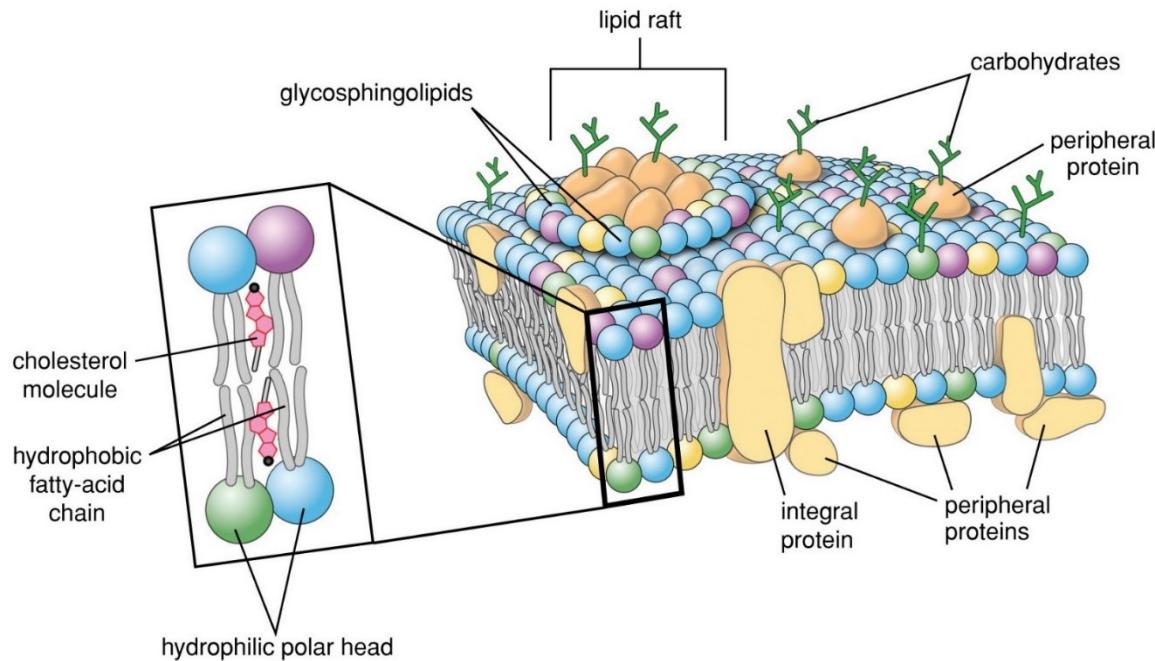
The membranous organelles: with plasma membranes that separate the internal environment of the organelle from the cytoplasm

- **plasma (cell) membrane**, a lipid bilayer that forms the cell boundary as well as the boundaries of many organelles within the cell;
- **rough-surfaced endoplasmic reticulum (rER)**, a region of endoplasmic reticulum associated with ribosomes and the site of protein synthesis and modification of newly synthesized proteins;
- **smooth-surfaced endoplasmic reticulum (sER)**, a region of endoplasmic reticulum involved in lipid and steroid synthesis but not associated with ribosomes;
- **Golgi apparatus**, a membranous organelle composed of multiple flattened cisternae responsible for modifying, sorting, and packaging proteins and lipids for intracellular or extracellular transport;

The membranous organelles: with plasma membranes that separate the internal environment of the organelle from the cytoplasm

- **Endosomes:** membrane-bounded compartments interposed within endocytotic pathways that have the major function of sorting proteins delivered to them via endocytotic vesicles and redirecting them to different cellular compartments for their final destination;
- **Lysosomes,** small organelles containing digestive enzymes that are formed from endosomes by targeted delivery of unique lysosomal membrane proteins and lysosomal enzymes
- **transport vesicles (pinocytotic, endocytotic, and coated):** involved in both endocytosis and exocytosis and vary in shape and the material that they transport
- **mitochondria,** organelles that provide most of the energy to the cell by producing adenosine triphosphate (ATP) in the process of oxidative phosphorylation; and
- **peroxisomes,** small organelles involved in the production and degradation of H₂O₂ and degradation of fatty acids.

The Plasma Membrane

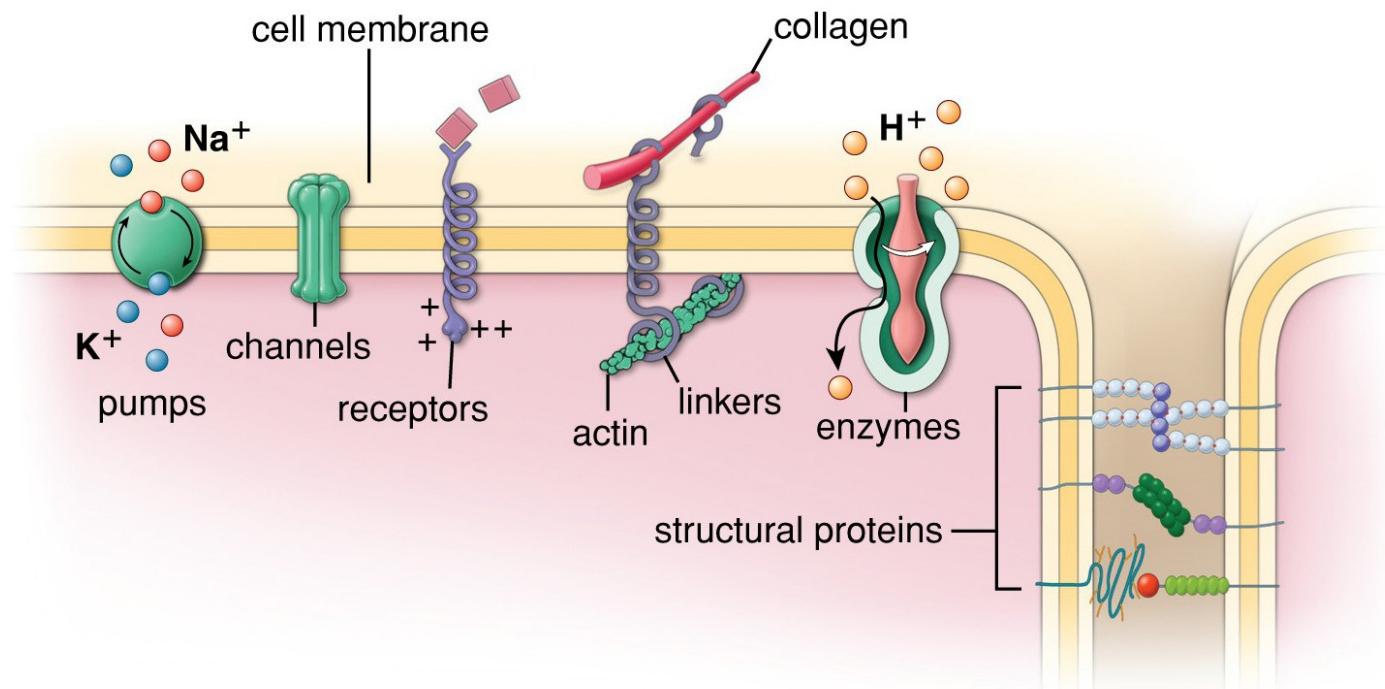


The plasma membrane is a **lipid bilayer** consisting primarily of phospholipid molecules, cholesterol, and protein molecules.

The **hydrophobic fatty-acid chains** of phospholipids face each other to form the inner portion of the membrane, whereas the **hydrophilic polar heads** of the phospholipids form the extracellular and intracellular surfaces of the membrane.

Cholesterol molecules are incorporated within the gaps between phospholipids equally on both sides of the membrane.

Integral Membrane Proteins



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Different functions of integral membrane proteins. The six major categories of integral membrane proteins are shown in this diagram:

1. pumps
2. channels
3. receptors
4. linkers
5. enzymes
6. structural proteins.

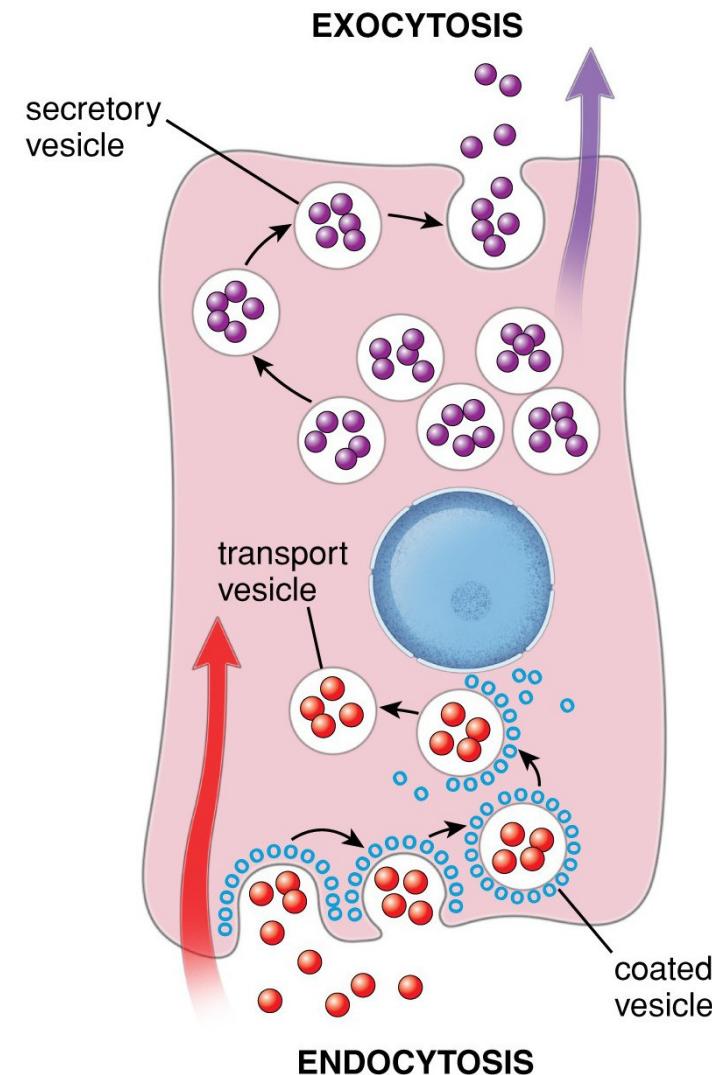
These categories are not mutually exclusive.

A structural membrane protein involved in cell-to-cell junctions might simultaneously serve as a receptor, enzyme, linker, or a combination of these functions.

Exocytosis and endocytosis transport large molecules across membranes

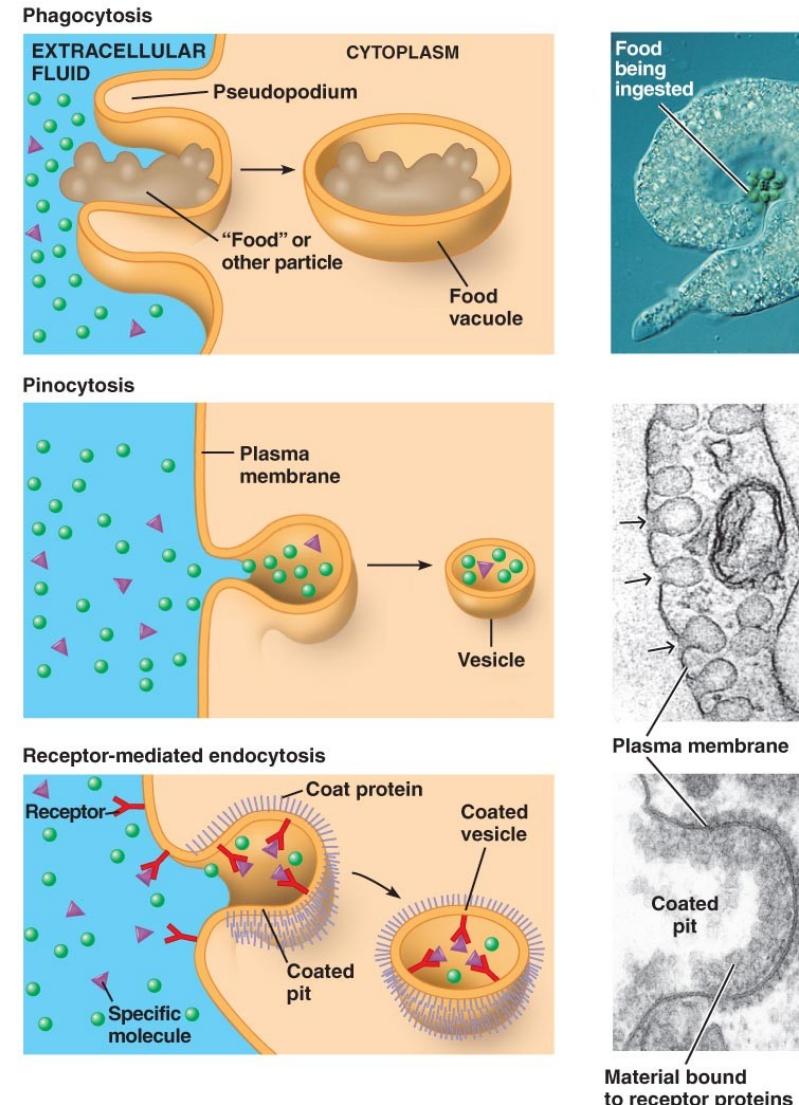
- A cell uses two mechanisms for moving large molecules across membranes
 - **Exocytosis** is used to export bulky molecules, such as proteins or polysaccharides
 - **Endocytosis** is used to import substances useful to the livelihood of the cell
- In both cases, material to be transported is packaged within a vesicle that fuses with the membrane

http://www.mhhe.com/biosci/ap/ap_prep/bioC8.html

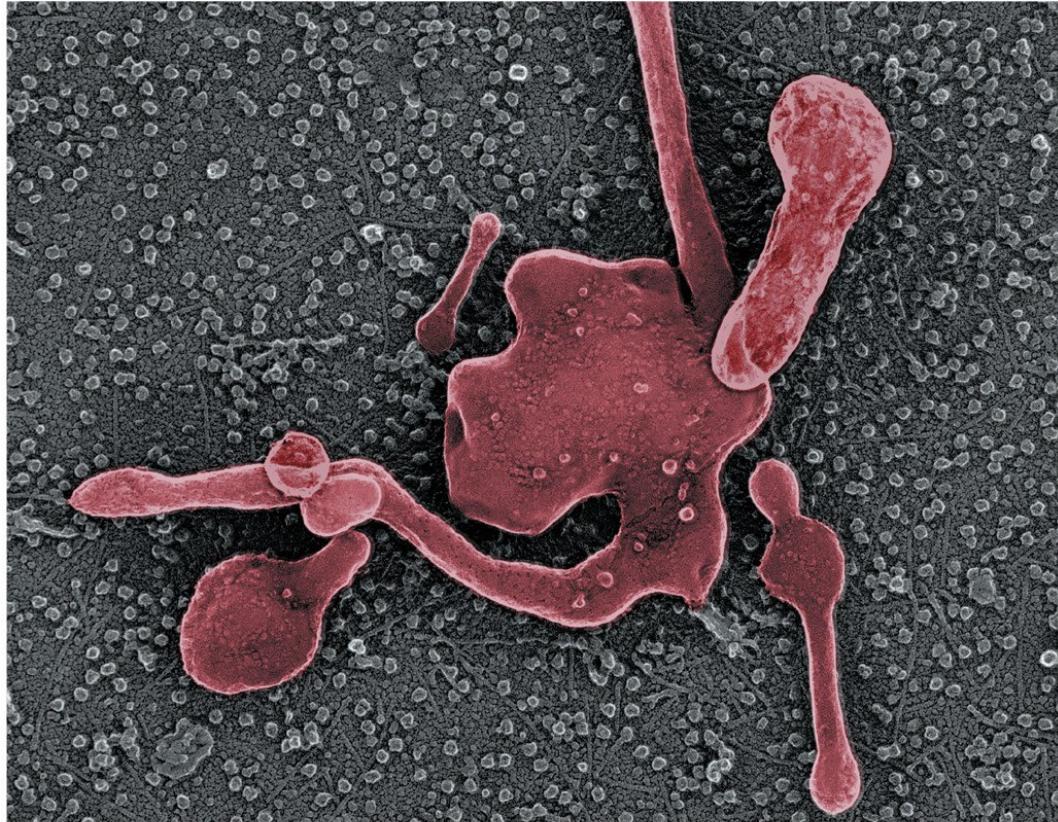


Exocytosis and endocytosis transport large molecules across membranes

- Three kinds of endocytosis
 - **Phagocytosis** is engulfment of a particle by wrapping cell membrane around it, forming a vacuole
 - **Pinocytosis** is the same thing except that fluids are taken into small vesicles
 - **Receptor-mediated endocytosis** is where receptors in a receptor-coated pit interact with a specific protein, initiating formation of a vesicle



Endosomes can be viewed either as stable cytoplasmic organelles or as transient structures formed as the result of endocytosis.

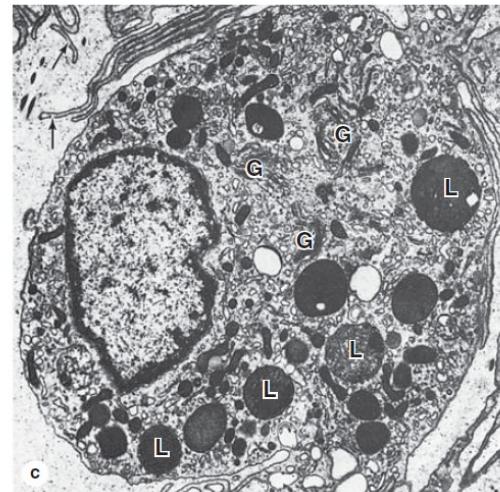
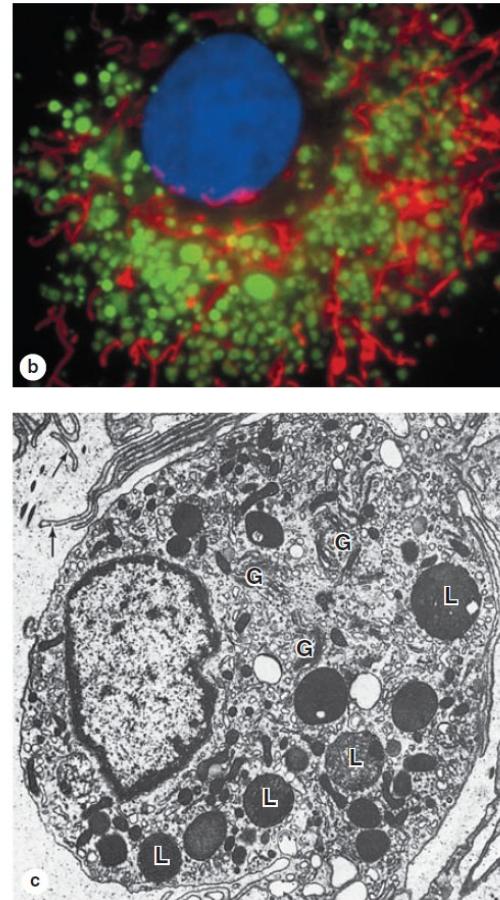
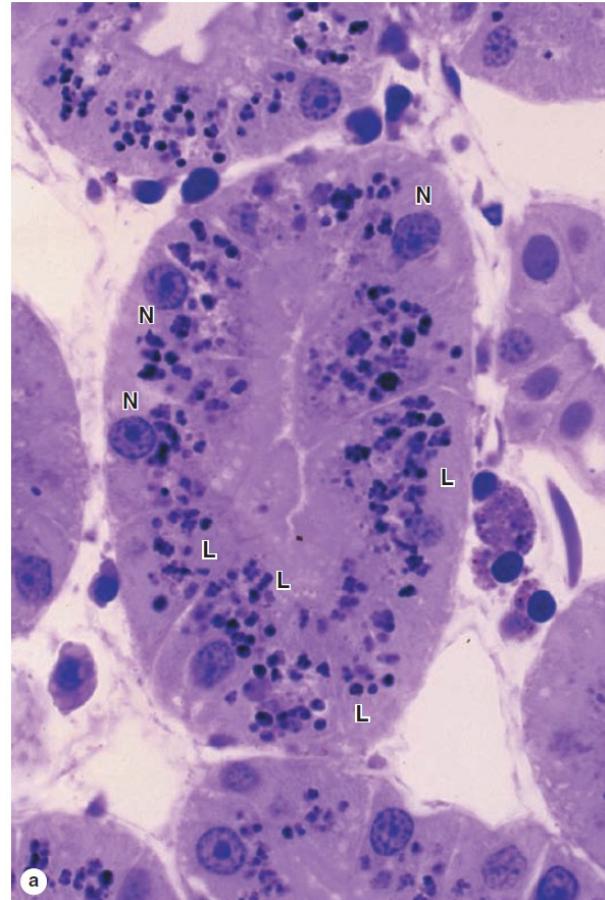


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The TEM reveals the presence in the cytoplasm of membrane enclosed compartments associated with all the endocytotic pathways described above (Fig. 2.15). These compartments, called **early endosomes**, are restricted to a portion of the cytoplasm near the cell membrane where vesicles originating from the cell membrane fuse. From here, many vesicles return to the plasma membrane.

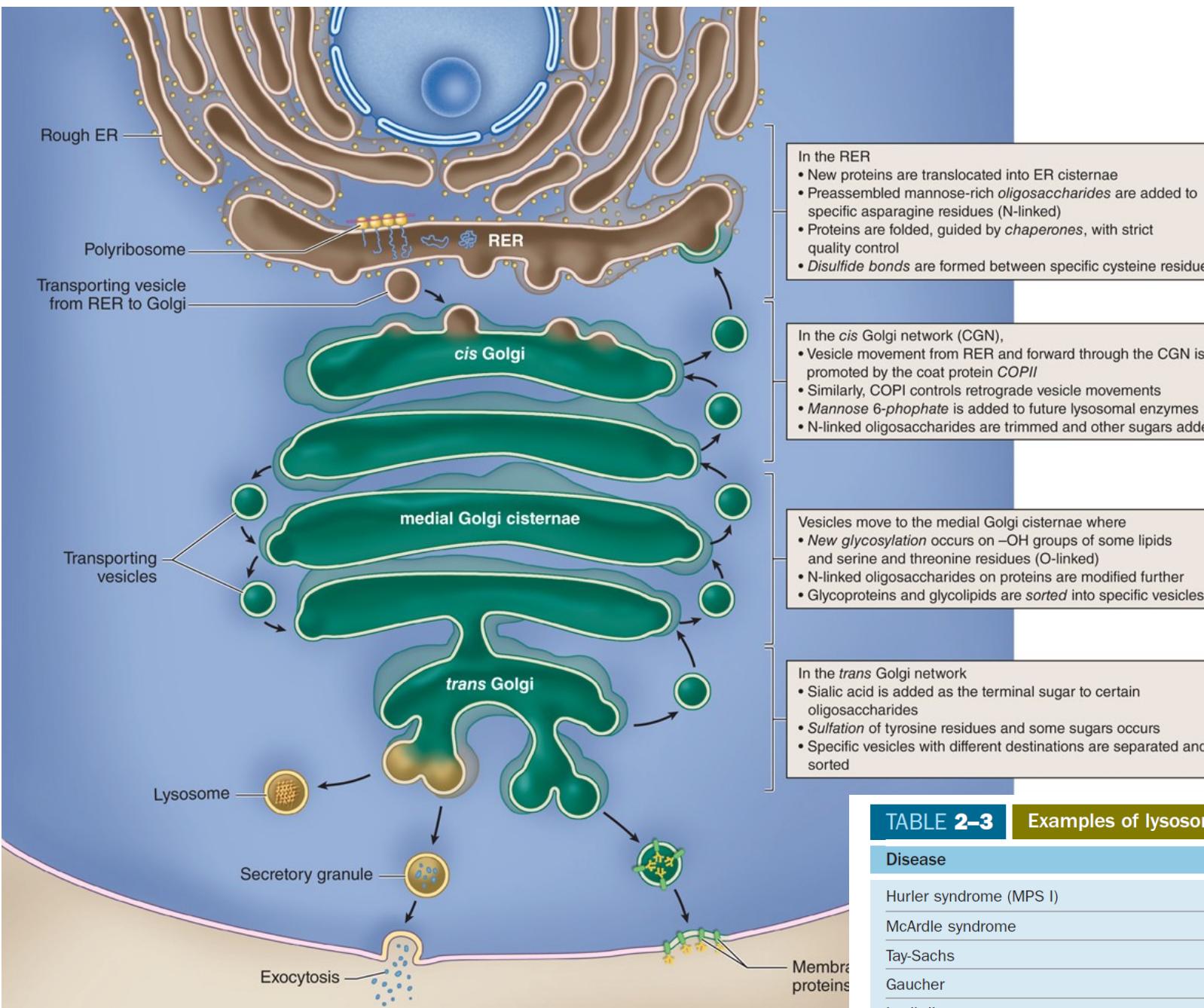
However, large numbers of vesicles originating in early endosomes travel to deeper structures in the cytoplasm called **late endosomes**. The latter typically mature into **lysosomes**.

Lysosomes are spherical membrane-enclosed vesicles that function as sites of intracellular digestion and are particularly numerous in cells active after the various types of endocytosis.



Lysosomes are not well shown on H&E-stained cells but can be visualized by light microscopy after staining with toluidine blue.

- (a) Cells in a kidney tubule show numerous purple lysosomes
- (b) Lysosomes in cultured vascular endothelial cells can be specifically stained using fluorescent dyes: lysosomes (green), Mitochondria (red) are scattered among the lysosomes.
- (c) In the TEM lysosomes (L) have a characteristic very electron-dense appearance and are shown here near groups of Golgi cisternae (G). The less electron-dense lysosomes represent heterolysosomes in which digestion of the contents is under way.

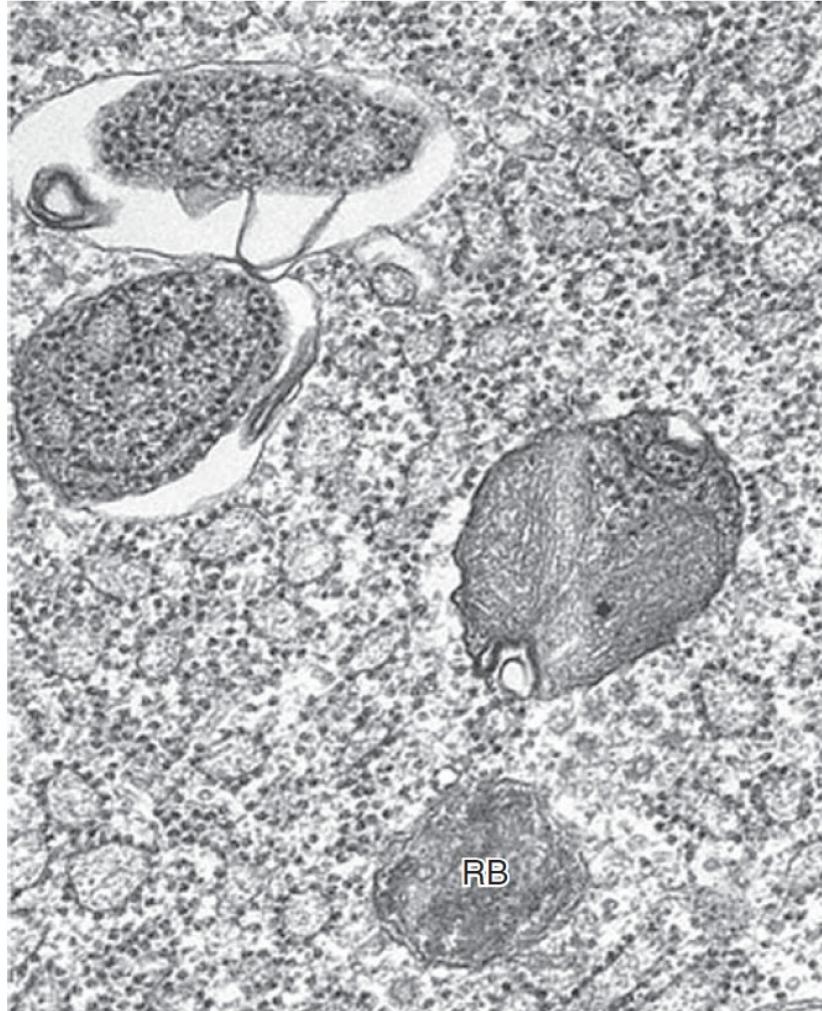


Lysosomal Storage Diseases, Lysosome Development:
<https://www.youtube.com/watch?v=-q8voqiXmF8>

TABLE 2-3 Examples of lysosomal storage diseases caused by defective lysosomal enzymes.

Disease	Faulty Enzyme	Main Organs Affected
Hurler syndrome (MPS I)	α -L-Iduronidase	Skeleton and nervous system
McArdle syndrome	Muscle phosphorylase	Skeletal muscles
Tay-Sachs	GM ₂ -gangliosidase	Nervous system
Gaucher	Glucocerebrosidase	Liver and spleen
I-cell disease	Phosphotransferase for M6P formation	Skeleton and nervous system

Autophagy: proteins, organelles, and other cellular structures are degraded in the lysosomal compartment

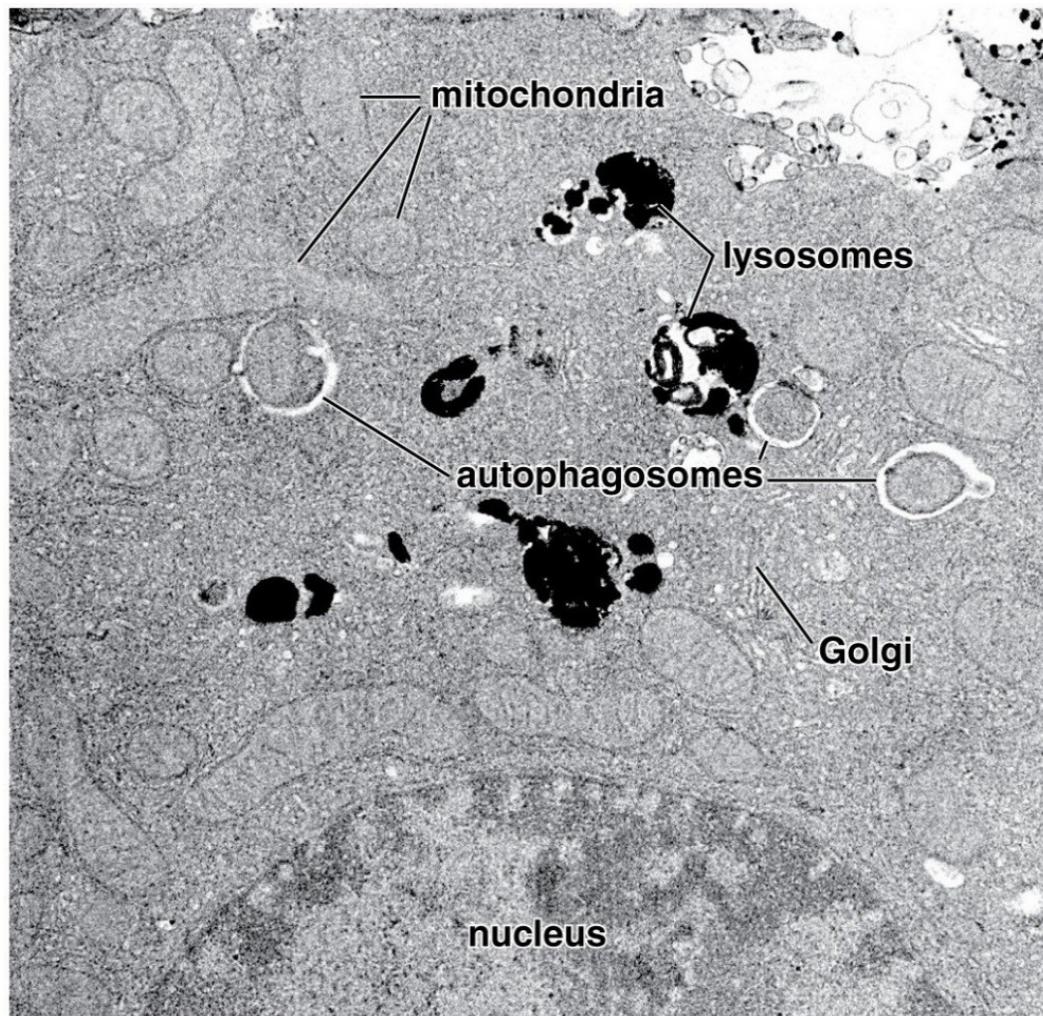


Autophagy is a process in which the cell uses lysosomes to dispose of excess or nonfunctioning organelles or membranes.

Membrane that appears to emerge from the SER encloses the organelles to be destroyed, forming an autophagosome that then fuses with a lysosome for digestion of the contents. In this TEM the two autophagosomes at the upper left contain portions of RER more electron dense than the neighboring normal RER and one near the center contains what may be mitochondrial membranes plus RER.

Also shown is a vesicle with features of a residual body (RB).

Proteasomes are protein complexes that destroy proteins without involvement of lysosomes.

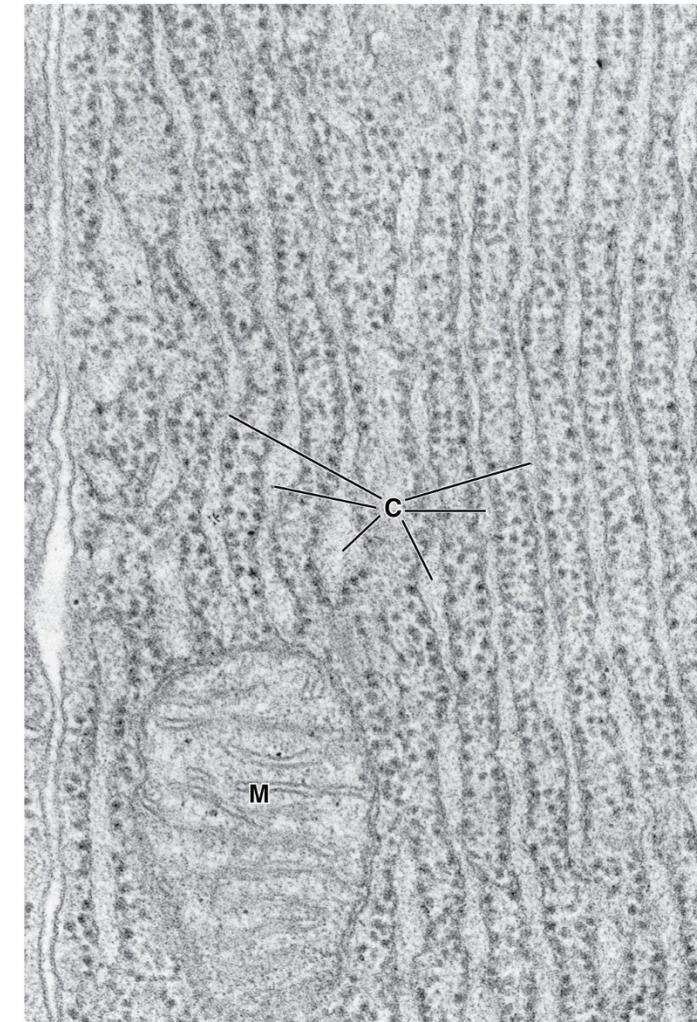


Electron micrograph of autophagosomes in a hepatocyte
shows several autophagosomes containing degenerating mitochondria.
Note the surrounding lysosomes that have been stained with acid phosphatase

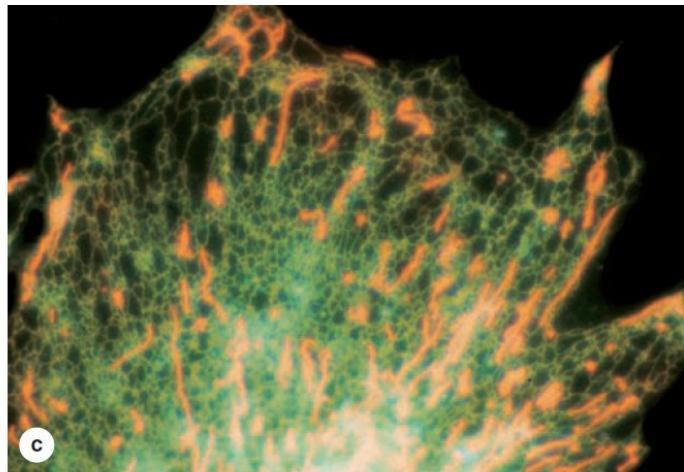
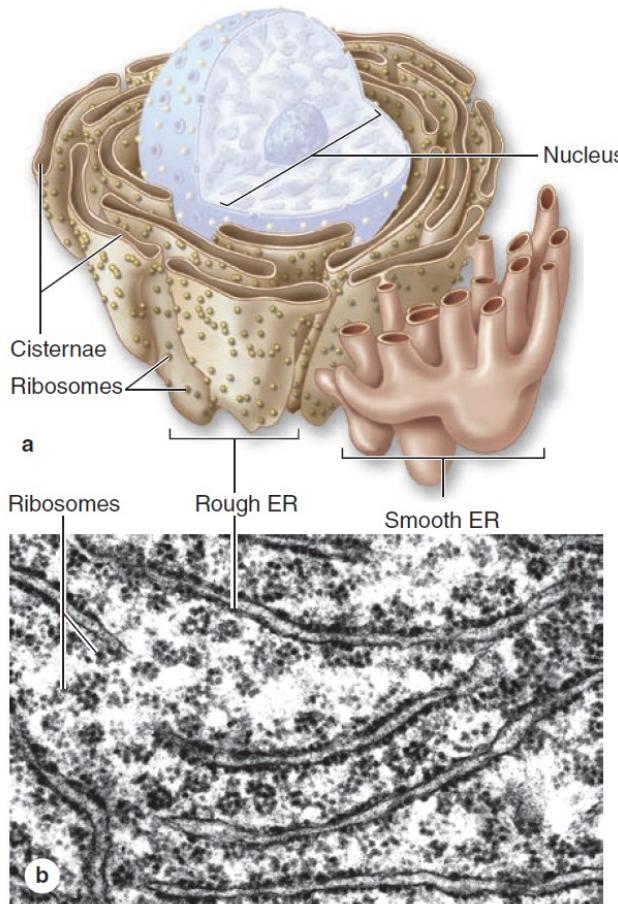
Rough ER manufactures proteins targeted for secretion or other organelles and membranes

Electron micrograph of the rER. This image of the rER in a chief cell of the stomach shows the membranous cisternae (C) closely packed in parallel arrays.

Polyribosomes are present on the cytoplasmic surface of the membrane surrounding the cisternae. The image of a ribosome-studded membrane is the origin of the term *rough endoplasmic reticulum*. A few ribosomes are free in the cytoplasm. M, mitochondrion.



Smooth ER lacks ribosomes and synthesizes lipids



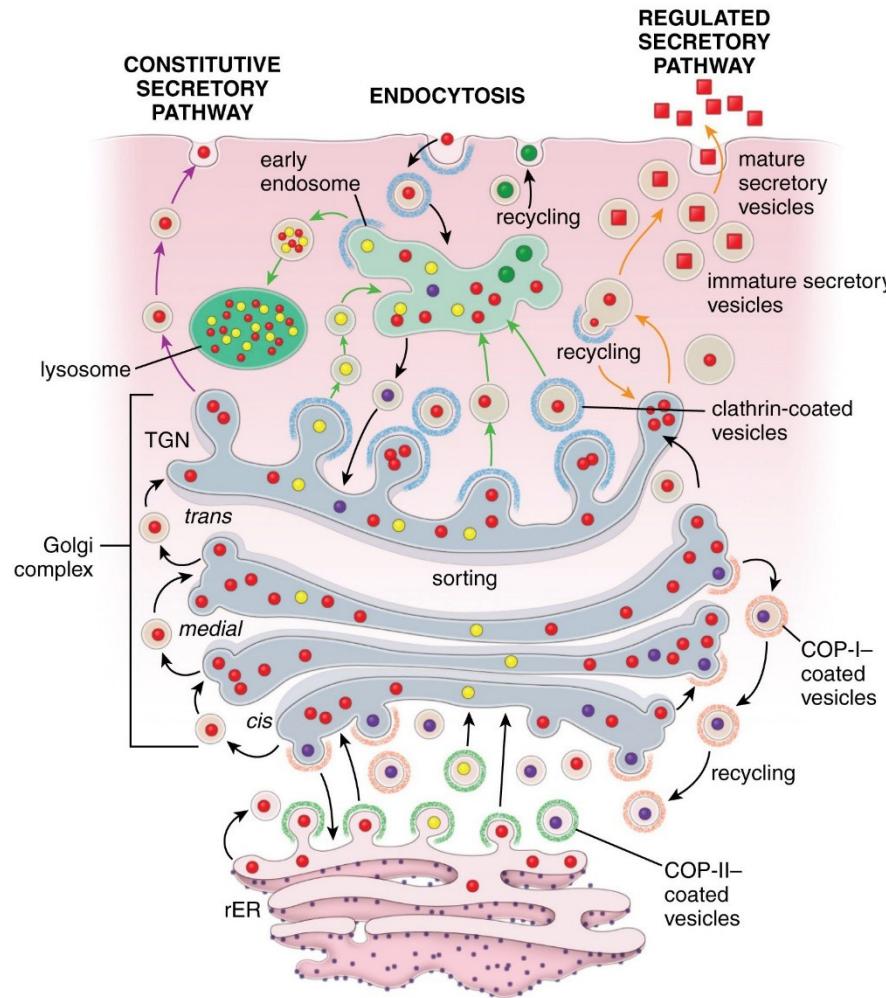
Functions of Endoplasmic Reticulum

1. **Synthesis:** Provides a place for chemical reactions
 - a. Smooth ER is the site of lipid synthesis and carbohydrate metabolism
 - b. Rough ER synthesizes proteins for secretion, incorporation into the plasma, membrane, and as enzymes within lysosomes
2. **Transport:** Moves molecules through cisternal space from one part of the cell to another, sequestered away from the cytoplasm
3. **Storage:** Stores newly synthesized molecules
4. **Detoxification:** Smooth ER detoxifies both drugs and alcohol

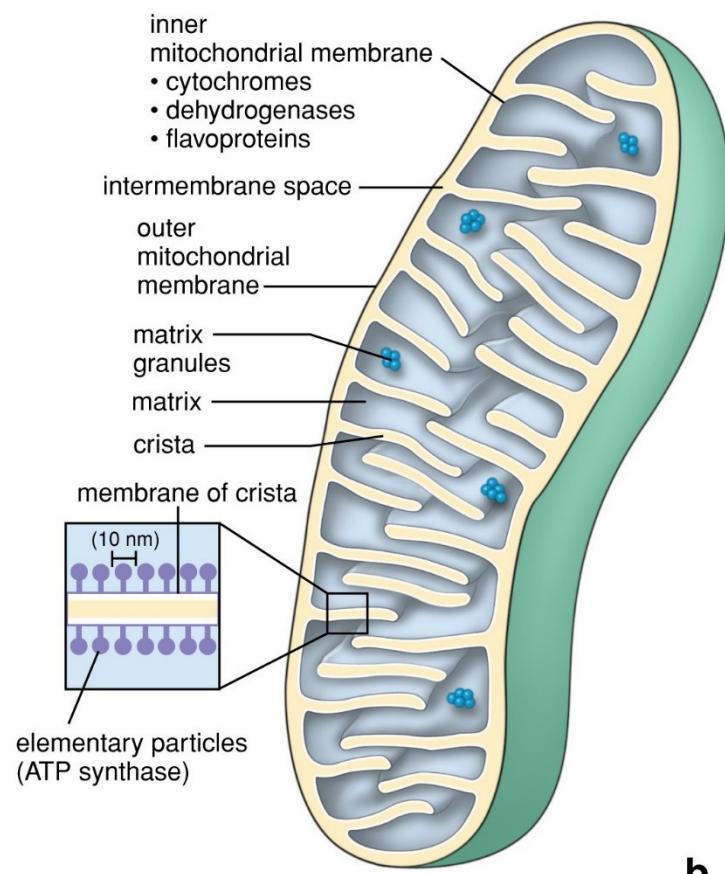
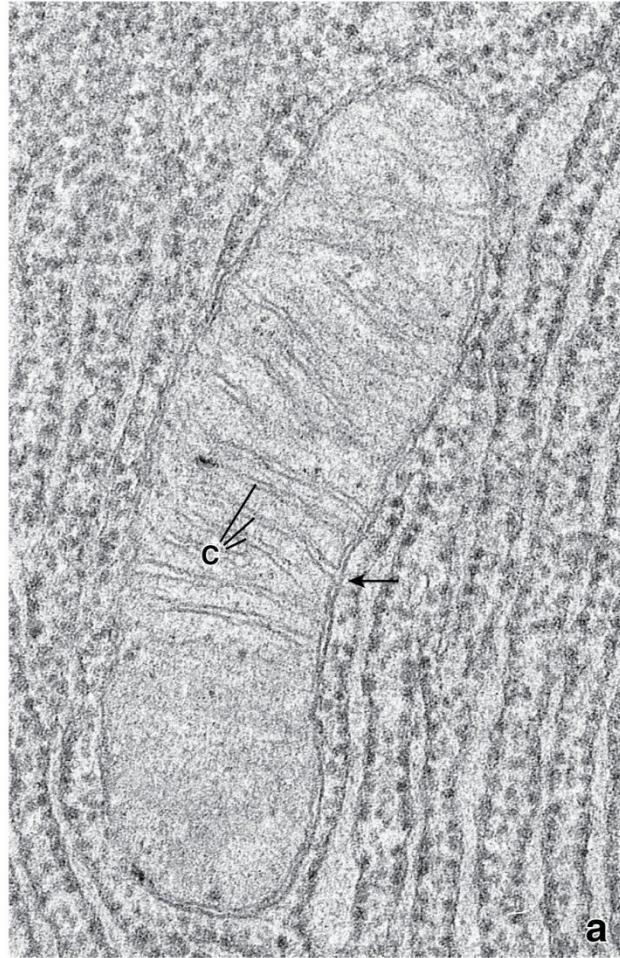
» MEDICAL APPLICATION

Jaundice denotes a yellowish discoloration of the skin and is caused by accumulation in extracellular fluid of bilirubin and other pigmented compounds, which are normally metabolized by SER enzymes in cells of the liver and excreted as bile. A frequent cause of jaundice in newborn infants is an underdeveloped state of SER in liver cells, with failure of bilirubin to be converted to a form that can be readily excreted.

The Golgi Apparatus and vesicular trafficking



Mitochondria are abundant in cells that generate and expend large amounts of energy



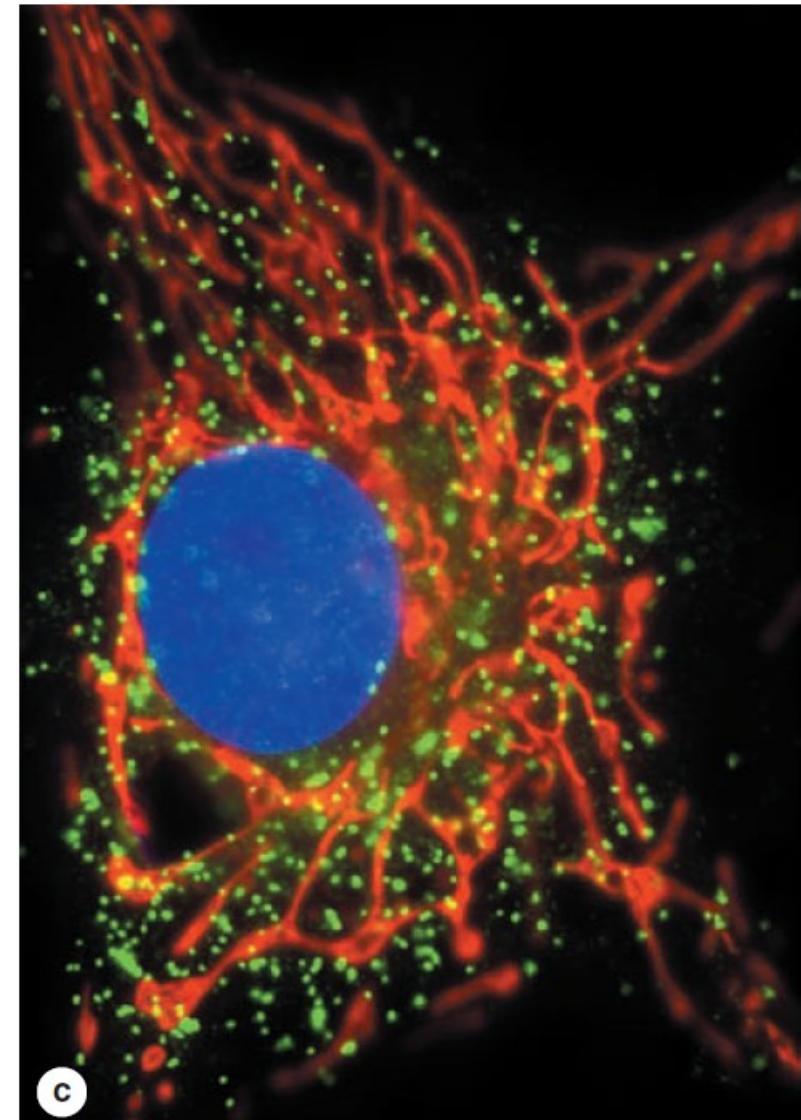
a. This electron micrograph shows a mitochondrion in a pancreatic acinar cell. Note that the inner mitochondrial membrane forms the cristae (C) through a series of infoldings, as is evident in the region of the arrow. The outer mitochondrial membrane is a smooth continuous envelope that is separate and distinct from the inner membrane.

b. Schematic diagram showing the components of a mitochondrion. Note the location of the elementary particles (inset), the shape of which reflects the three-dimensional structure of ATP synthase.

Peroxisomes are spherical organelles enclosed by a single membrane

Peroxisomes produce and degrade hydrogen peroxide inactivate various potentially toxic molecules, including some prescription drugs, particularly in the large and abundant peroxisomes of liver and kidney cells

A cultured endothelial cell processed by immunocytochemistry shows many peroxisomes (green) distributed throughout the cytoplasm among the vitally stained elongate mitochondria (red) around the DAPI-stained nucleus (blue). Peroxisomes shown here were specifically stained using an antibody against the membrane protein PMP70.



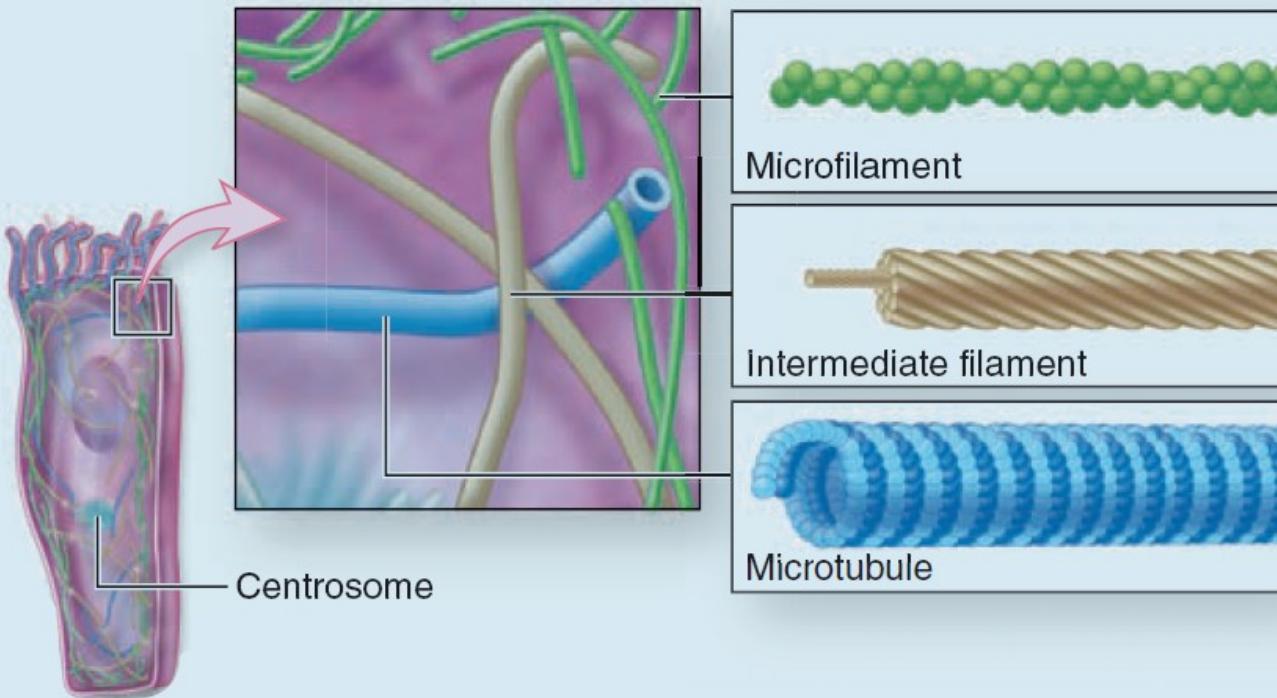
Non-membranous organelles

- **Microtubules:** form elements of the **cytoskeleton** and continuously elongate (by adding tubulin dimers) and shorten (by removing tubulin dimers), a property referred to as **dynamic instability**;
- **Filaments**, which are also part of the cytoskeleton
 - **actin filaments:** flexible chains of actin molecules,
 - **intermediate filaments:** which are ropelike fibers formed from a variety of proteins—both groups providing tensile strength to withstand tension and confer resistance to shearing forces;
- **Centrioles**, or short, paired cylindrical structures found in the center of the **microtubule-organizing center (MTOC)** or **centrosome** and whose derivatives give rise to basal bodies of cilia
- **Ribosomes**, structures essential for protein synthesis and composed of ribosomal RNA (rRNA) and ribosomal proteins (including proteins attached to membranes of the rER and proteins free in the cytoplasm).

Microtubules & Filaments

TABLE 2-4

Properties of cytoskeletal components (microtubules, microfilaments, and intermediate filaments).



General Function of Cytoskeleton

- Structural:** Provides structural support to cell; stabilizes junctions between cells
- Movement:** Assists with cytosol streaming and cell motility; helps move organelles and materials throughout cell; helps move chromosomes during cell division

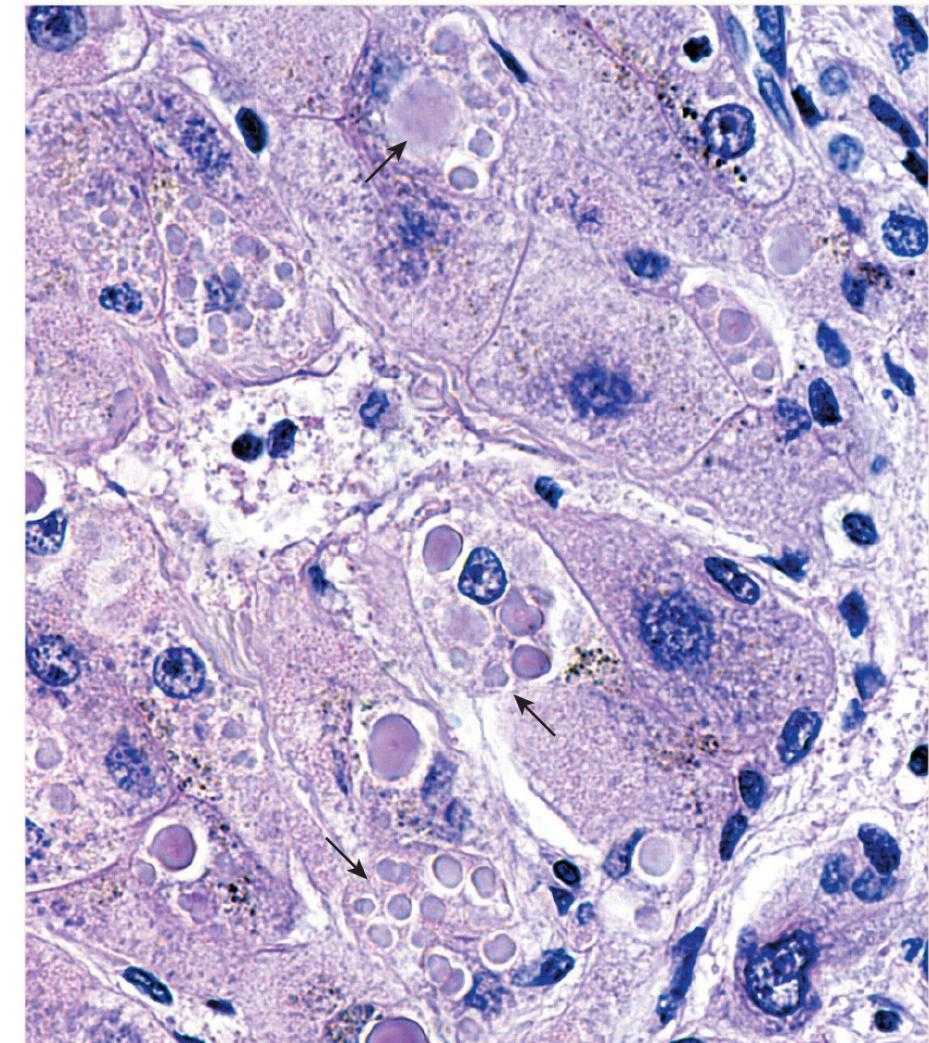
Abnormalities in microtubules and filaments

Photomicrograph of Mallory bodies.

Accumulation of keratin intermediate filaments forming intercellular inclusions is frequently associated with specific cell injuries.

In **alcoholic liver cirrhosis**, hepatocytes exhibit such inclusions (arrows), which are known as Mallory bodies.

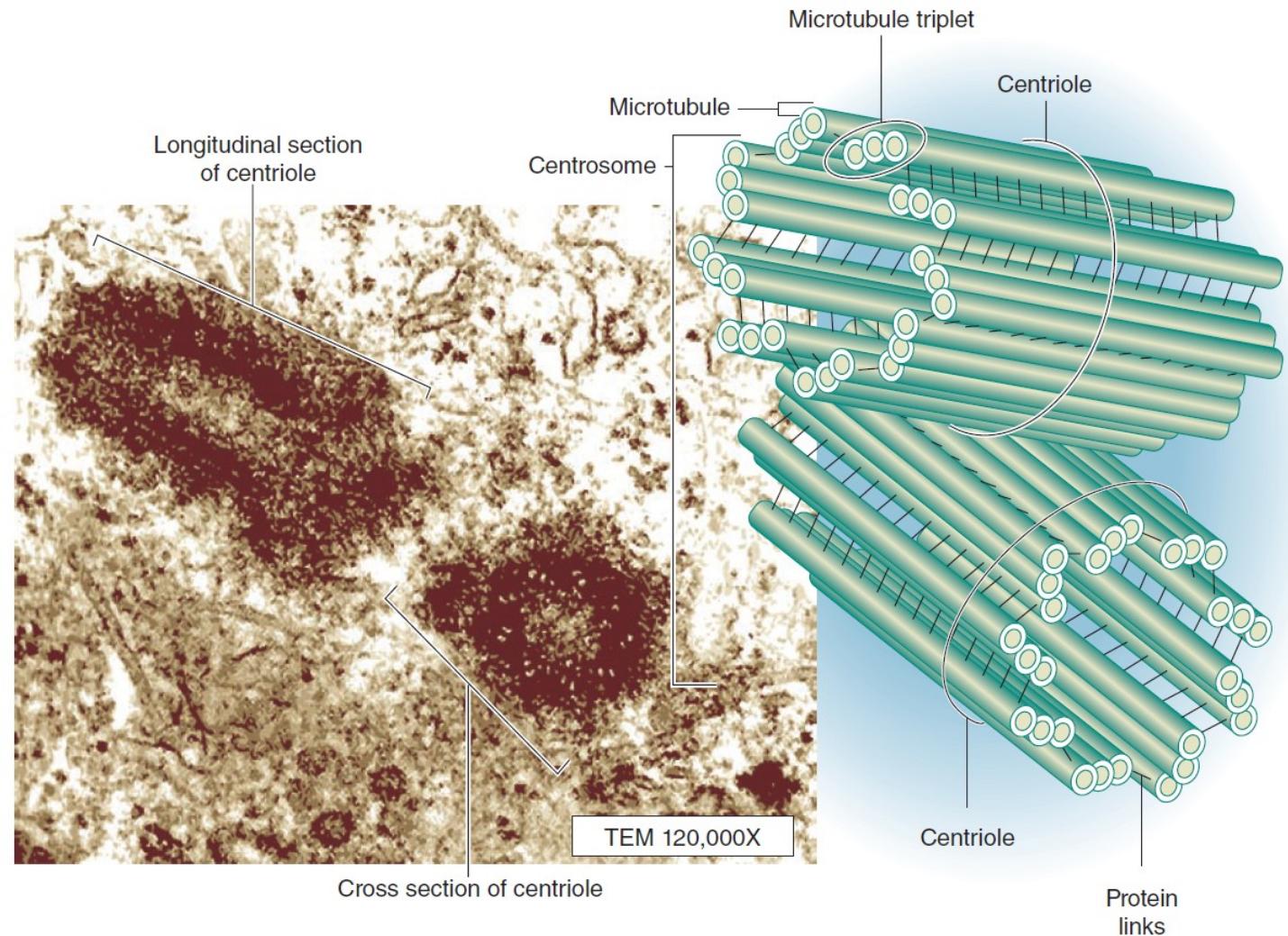
Lymphocytes and macrophages responsible for an intense inflammatory reaction surround cells containing Mallory bodies



Centrioles

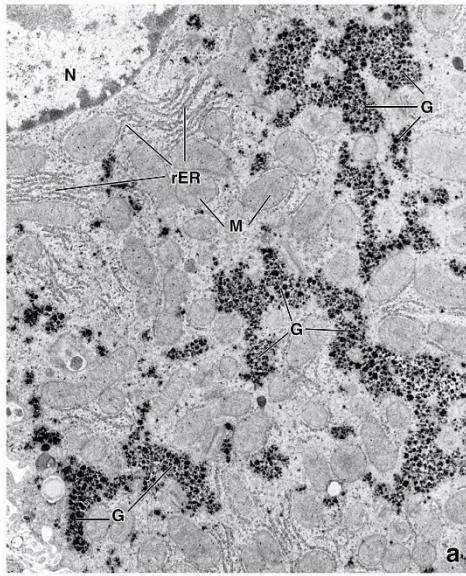
The centrosome is the microtubule-organizing center for the mitotic spindle and consists of paired centrioles. The TEM reveals that the two centrioles in a centrosome exist at right angles to each other in a dense matrix of free tubulin subunits and other proteins

FIGURE 2-24 Centrosome.



Inclusions contain accumulated metabolites or other substances not enclosed by membrane

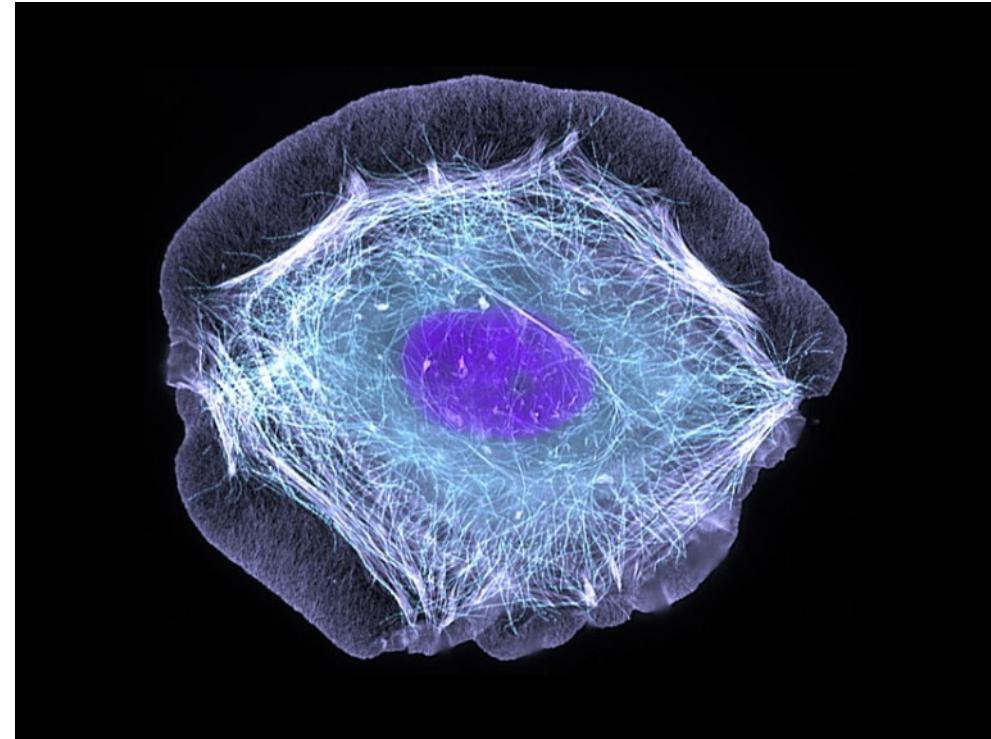
- **Fat droplets:** accumulations of lipid
- **Glycogen granules:** aggregates of glycogen mainly liver cells
- **Lipofuscin:** yellowish-brown pigment found in stable nondividing cells (eg, neurons, cardiac muscle).
- **Hemosiderin:** dense brown aggregate of denatured proteins bound to iron. Found in liver and spleen



Electron micrographs of a hepatocyte (liver cell) with glycogen inclusions.

- Nucleus (*N, upper left*).
- Glycogen (*G*) appears as irregular electron-dense masses.
- Profiles of rough endoplasmic reticulum (*rER*) and mitochondria (*M*) are also evident.

A Human Skin Cell. The purple in the center is the cell's nucleus. Surrounding it are wispy blue and white microtubules and filaments that make up the cell's cytoskeleton



Nuclear Components, Cell Renewal, Cell Cycle, Cell Death

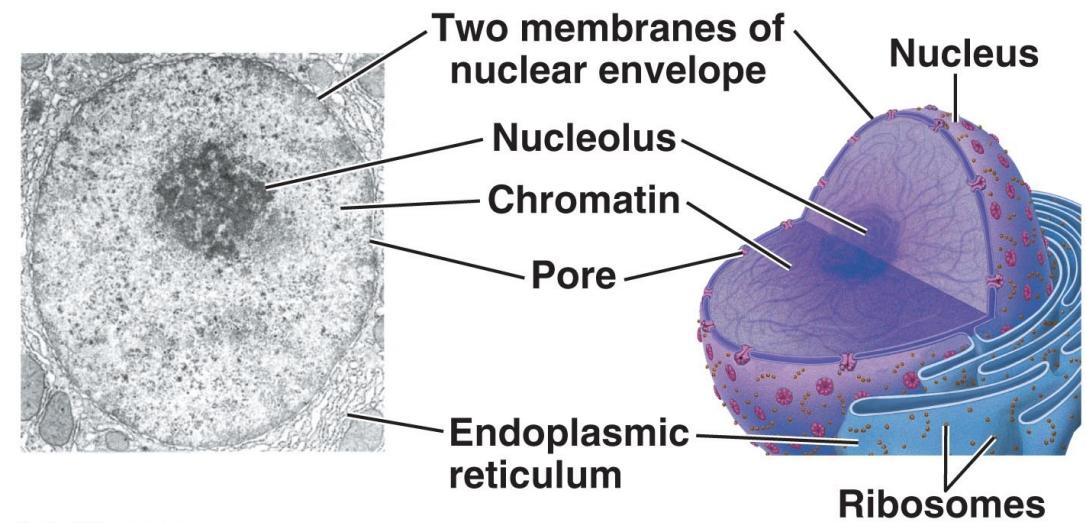
CHAPTER 3: THE NUCLEUS

The nucleus is the cell's genetic control center

- The **nucleus** controls the cell's activities and is responsible for inheritance
 - Inside is a complex of proteins and DNA called **chromatin**, which makes up the cell's chromosomes
- The **nuclear envelope** is a double membrane with pores that allow material to flow in and out of the nucleus

Nucleolus: contains DNA in the form of transcriptionally active ribosomal RNA (rRNA) genes, RNA, and proteins.

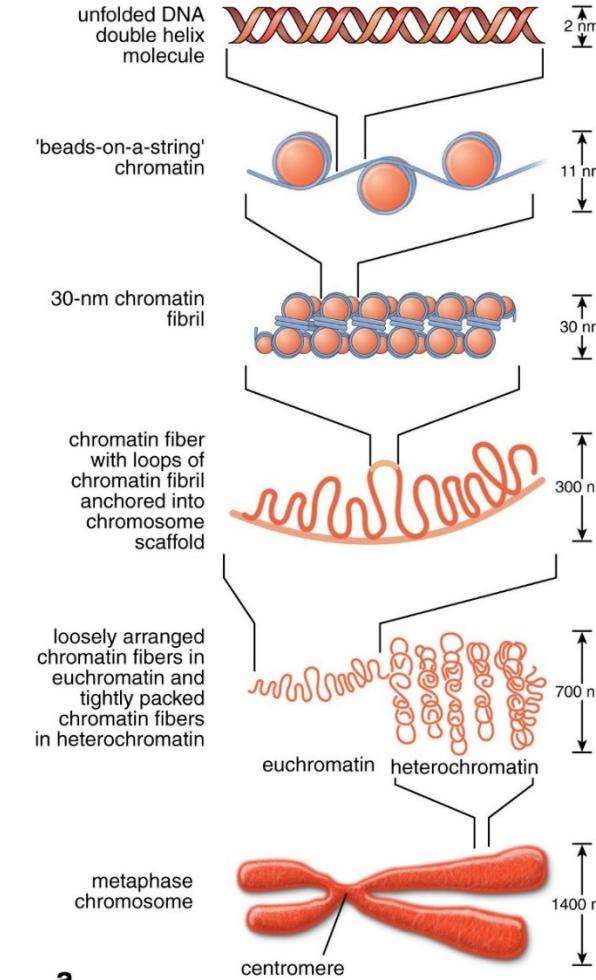
Nucleoplasm: nuclear content other than chromatin and nucleolus



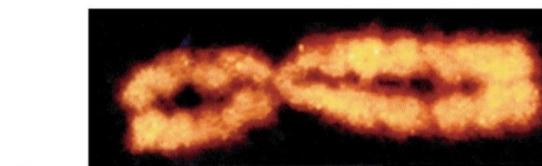
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Packaging of chromatin into the chromosomal structure

a. Sequential steps in the packaging of nuclear chromatin are shown in this diagram, beginning with the DNA double helix and ending with the highly condensed form found in chromosomes.



b. Structure of human metaphase chromosome 2
(Courtesy of Dr. Tatsuo Ushiki.)

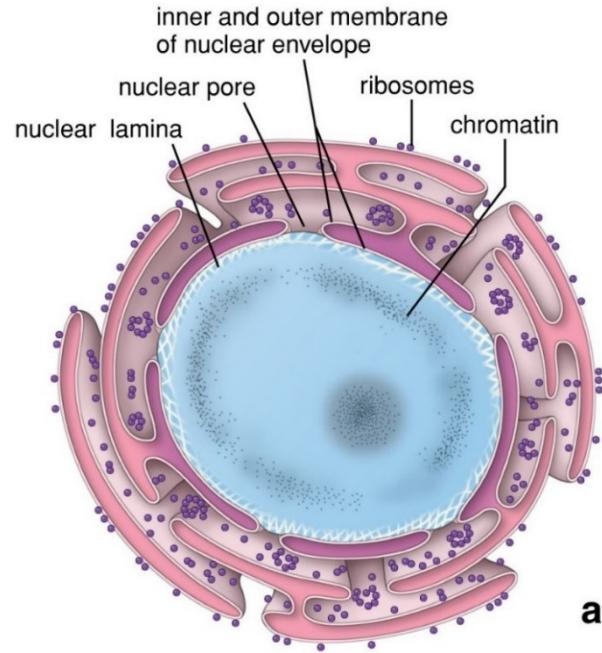


b

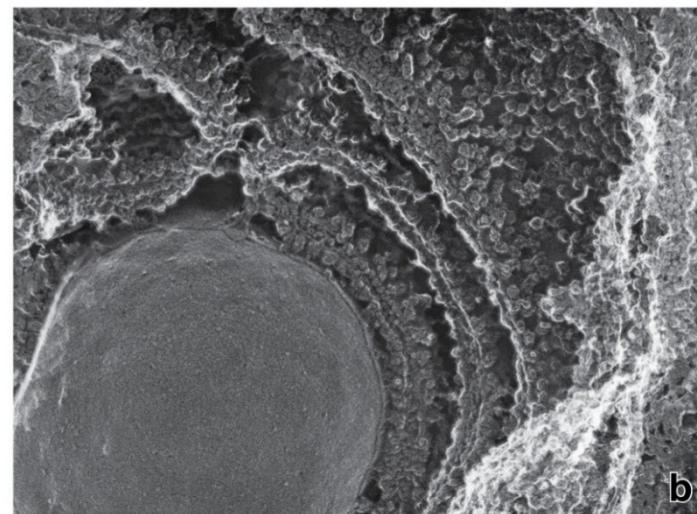
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Structure of the nuclear envelope and its relationship to the rER

- a. Double membrane envelope surrounds nucleus: outer membrane is continuous with the membranes of the rER; Perinuclear space communicates with the rER lumen. The inner membrane is adjacent to nuclear intermediate filaments that form the nuclear lamina.
- b. Electron micrograph of nucleus surrounded by the nuclear envelope. Note that the outer membrane possesses ribosomes and is continuous with the rER.

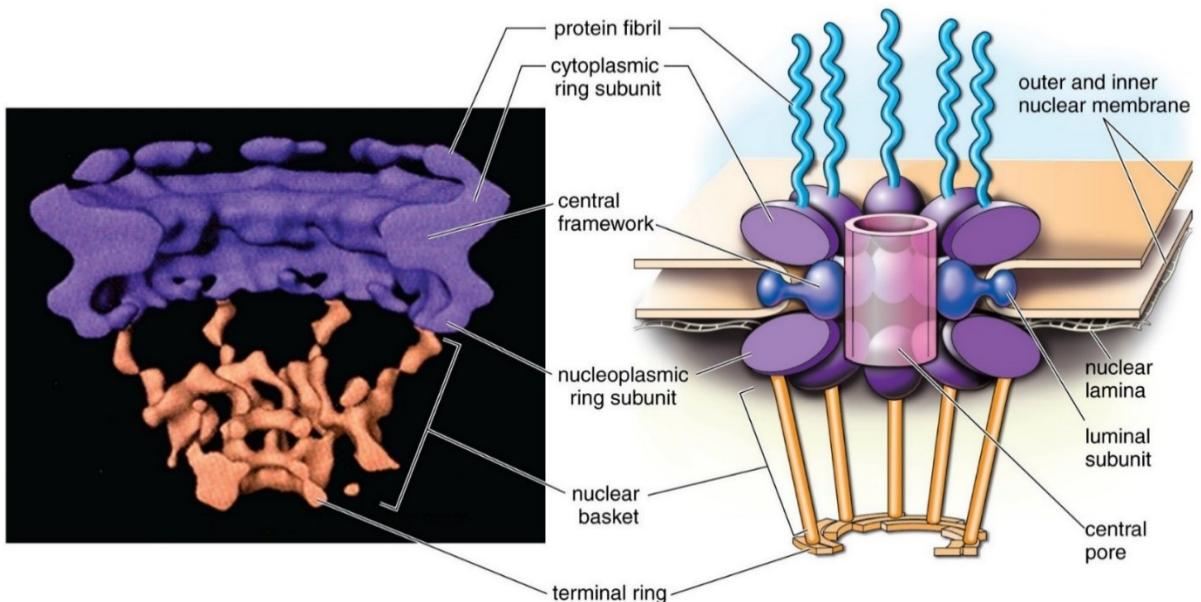


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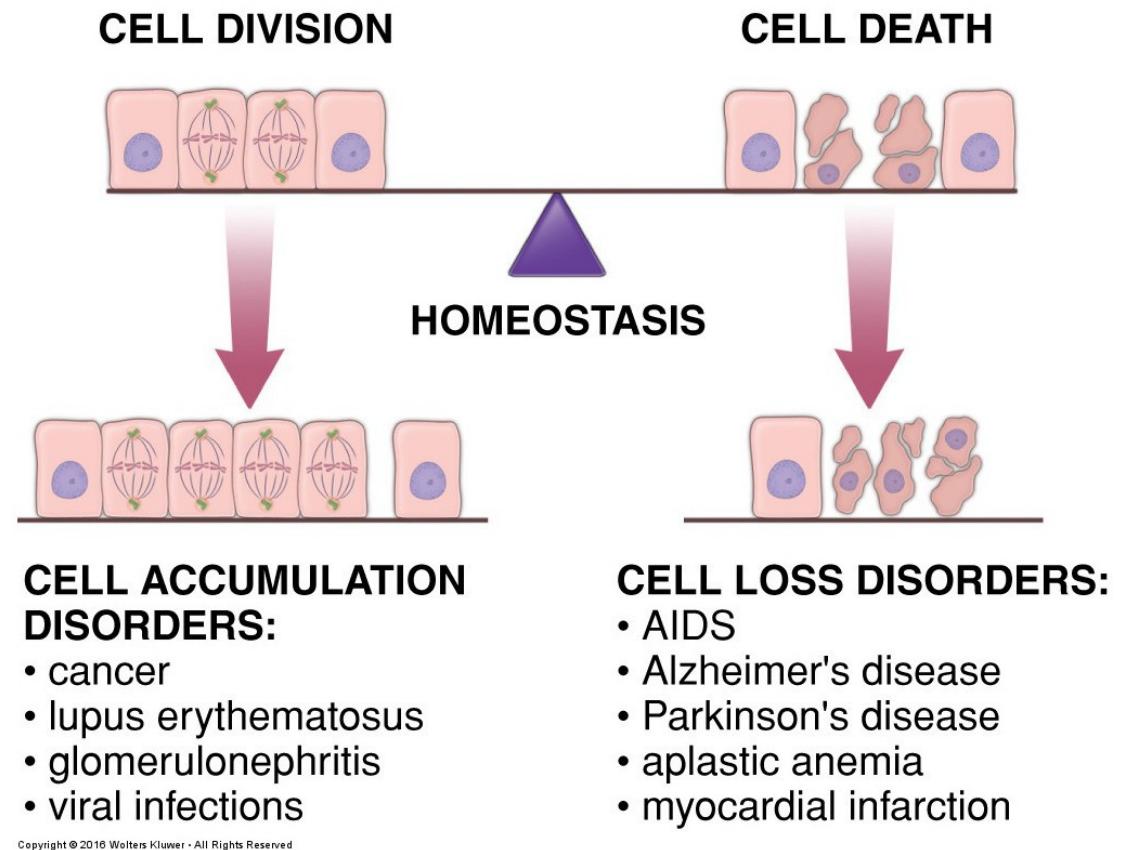
Nuclear Pore Complex



- Each pore contains eight protein subunits arranged in an octagonal central framework at the periphery of the pore.
- These subunits form a nuclear pore complex that is inserted between two cytoplasmic and nucleoplasmic rings
- The cylindrical central framework encircles the central pore, which acts as a close-fitting diaphragm.

Cell Division and Cell Death

- Under normal physiologic conditions (homeostasis), the rates of cell division and cell death are similar.
- If the rate of cell death is higher than that of cell division, then a net loss of cell number will occur. Such conditions are categorized as **cell loss disorders**.
- When the situation is reversed and the rate of cell division is higher than the rate of cell death, then the net gain in cell number will be prominent, leading to a variety of disorders of **cell accumulation**.



Cell Renewal and the Cell Cycle

Somatic cells in the adult organism may be classified according to their mitotic activity

Interphase:

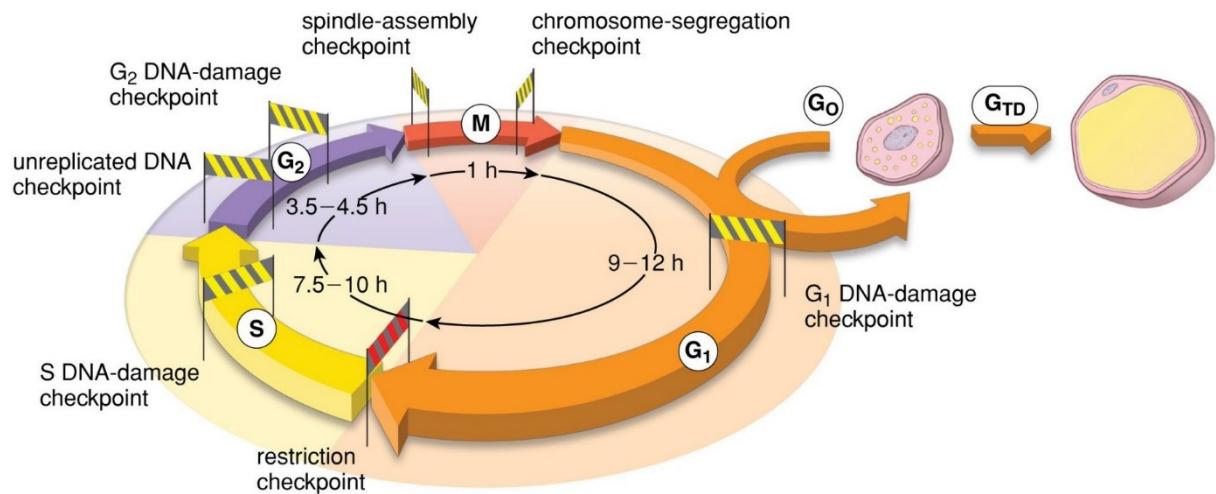
1. **G₁** (growth phase 1) acquisition of nutrients, growth
2. **S** (DNA synthesis): chromosome replication
3. **G₂** (growth phase 2) completion of cell growth, preparation for cell division

Cell division:

4. **Mitosis:** division of the nucleus
5. **Cytokinesis:** cytoplasmic division

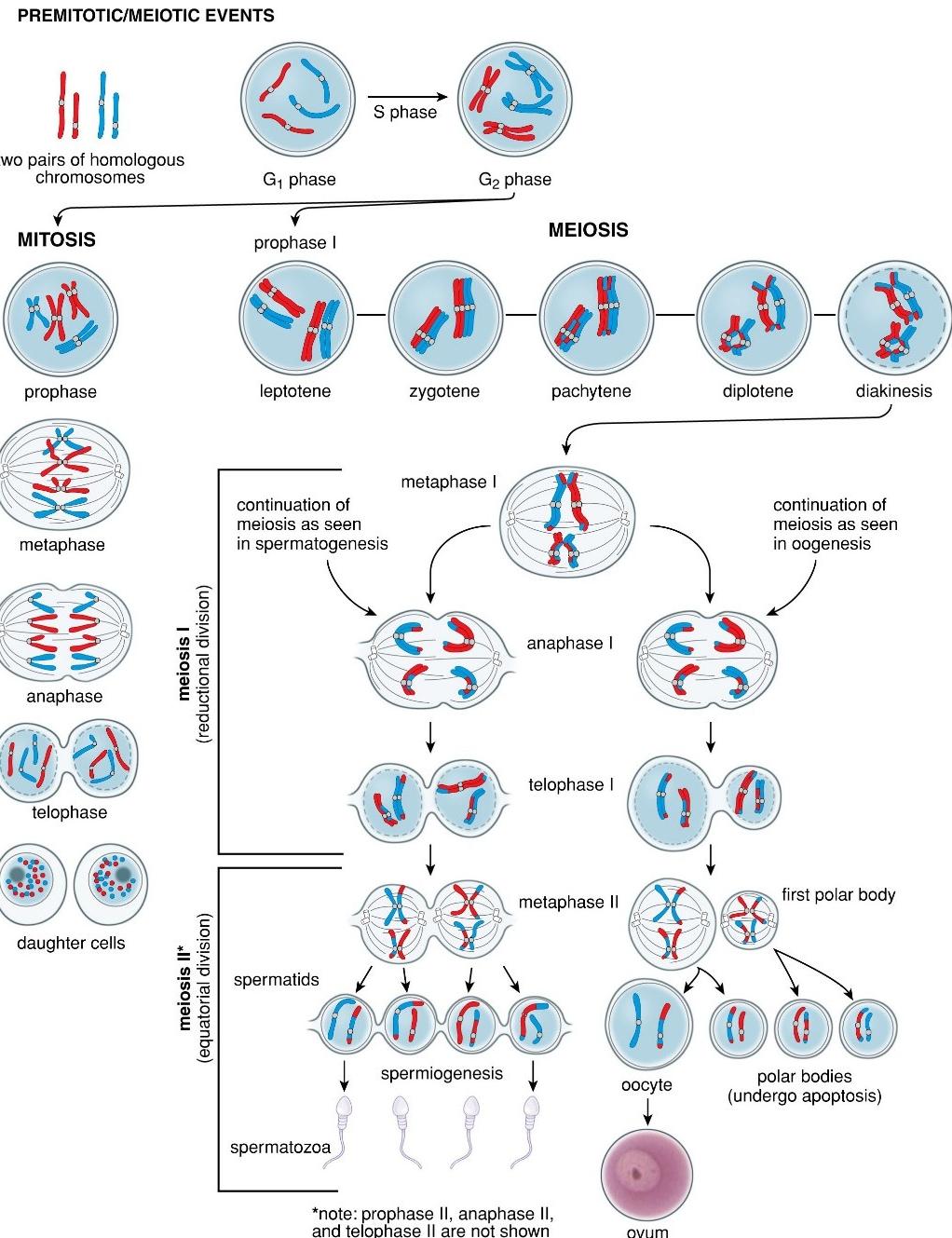
» MEDICAL APPLICATION

Tissues with either stable or rapidly renewing cell populations can include cells that become transformed to grow at a higher rate and in an uncoordinated manner. Such **neoplastic proliferation** typically follows damage to the DNA of proto-oncogenes and failure of the cells to be eliminated. Neoplastic growth can be either benign (with slow growth and no invasiveness to neighboring organs) or malignant (with rapid growth and great capacity to invade other organs). **Cancer** is the common term for all malignant tumors.



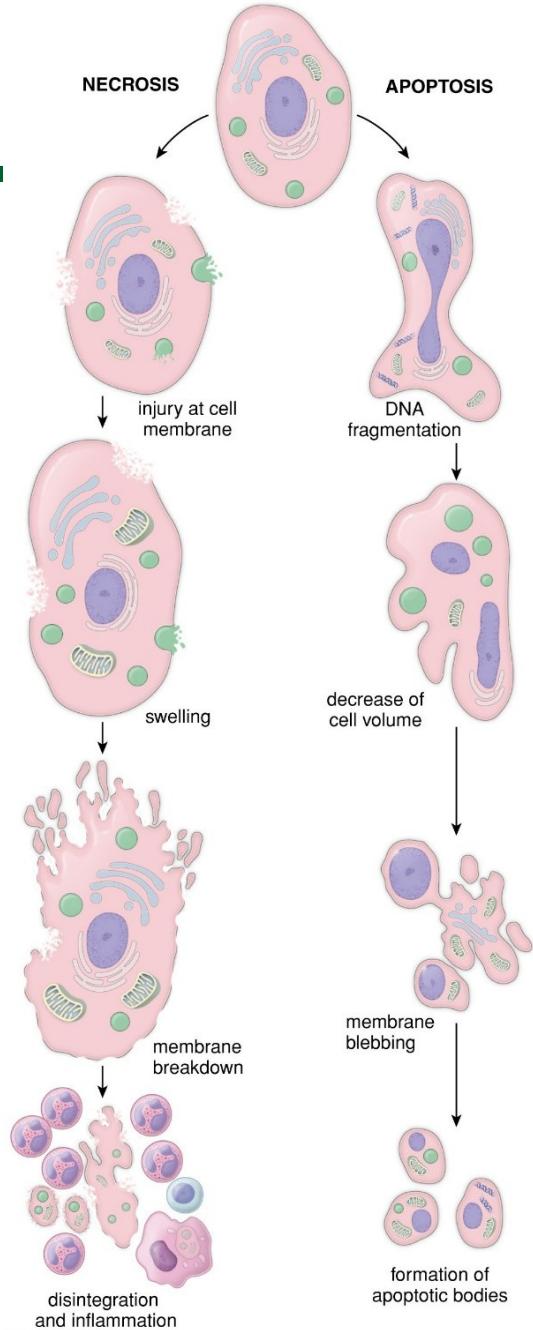
The Stages of Mitosis

1. Prophase
2. Metaphase
3. Anaphase
4. Telophase

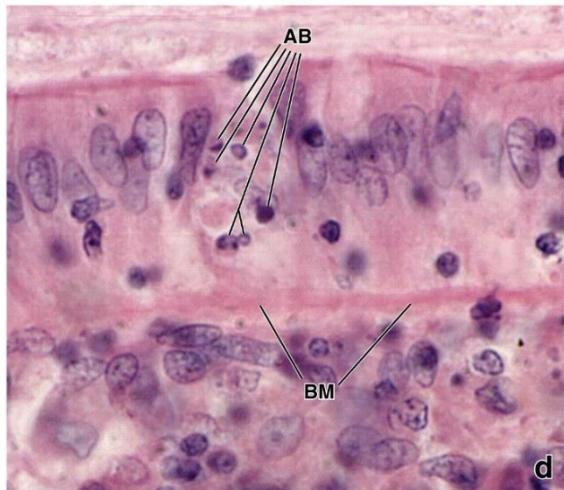
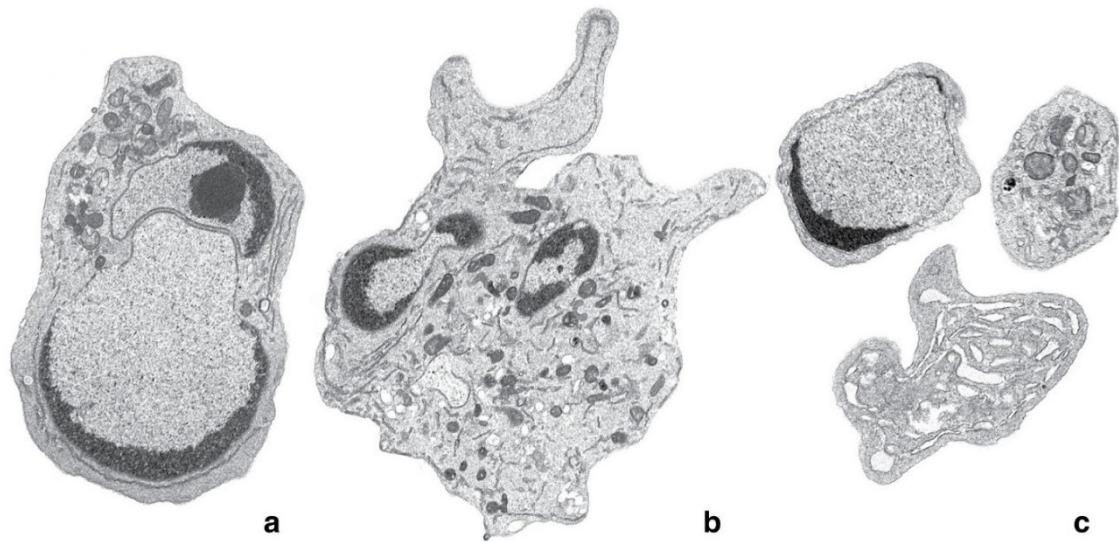


Apoptosis vs Necrosis

- In **necrosis** (*left side*), breakdown of the cell membrane results in an influx of water and extracellular ions, causing the organelles to undergo irreversible changes. Lysosomal enzymes are released into the extracellular space, causing damage to neighboring tissue and an intense inflammatory response.
- In **apoptosis** (*right side*), the cell shows characteristic morphologic and biochemical features such as DNA fragmentation, decrease in cell volume, membrane blebbing without loss of membrane integrity, and formation of apoptotic bodies, causing cell breakage. Apoptotic bodies are later removed by phagocytotic cells without inflammatory reactions.



Electron micrographs of apoptotic cells



- a. The nucleus is already fragmented, and the irreversible process of DNA fragmentation is turned on. Note the regions containing condensed heterochromatin adjacent to the nuclear envelope.
- b. Further fragmentation of DNA.
- c. Apoptotic bodies
- d. This photomicrograph taken with light microscopy of intestinal epithelium from the human colon shows apoptotic bodies (AB) within a single layer of absorptive cells. BM, basement membrane