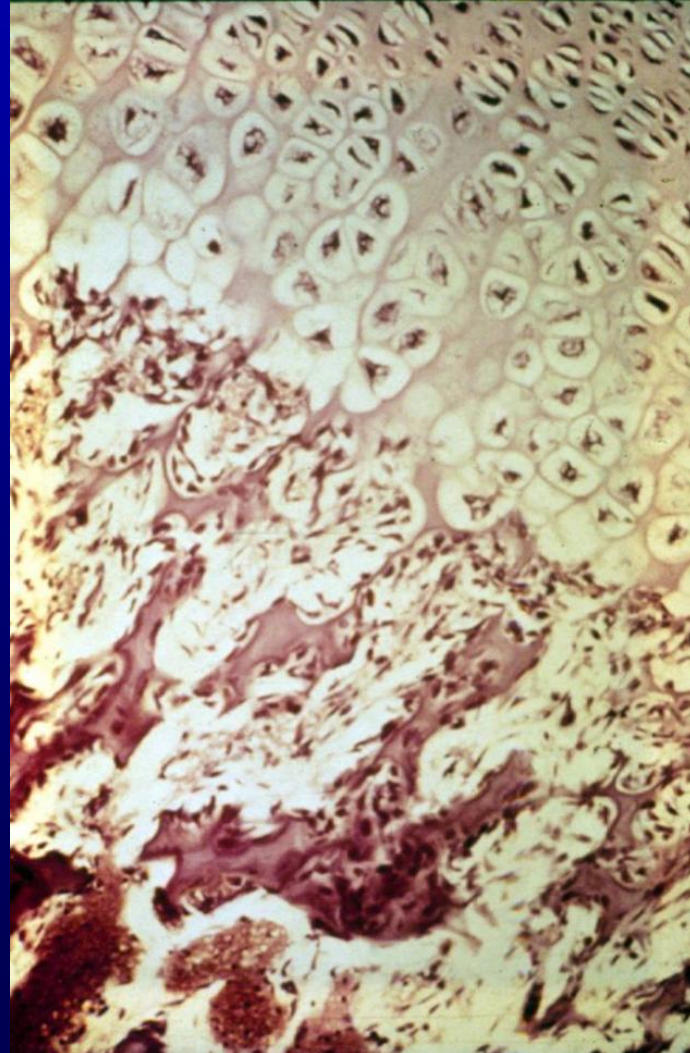


# 1. Introduction to Cells, Tissues, and Microscopy

VIBS 443 and VIBS 602



Undergraduate – Graduate  
Histology Lecture Series

Larry Johnson, Professor  
Veterinary Integrative Biosciences  
Texas A&M University  
College Station, TX 77843

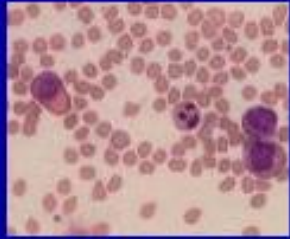


Figure 1-5. Photograph of the Zeiss model EM 10 electron microscope. (Courtesy of Carl Zeiss Co.)

# ***OBJECTIVES***

- 1. Preview cellular ultrastructure***
- 2. Preview cells, tissues, and organs***
- 3. Overview of light and electron microscopy***
- 4. Preparation of specimens – types of visions***
- 5. Section orientation***

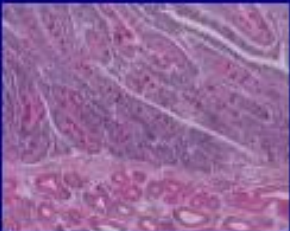
# Introduction to HISTOLOGY



**CELL**



**TISSUE**



**ORGAN**



**SYSTEM**

## PROTOPLASM – Living Substance

CELL – Smallest unit of protoplasm

Simplest animals consist of a single cell.

TISSUE – Groups of cells with same general function and texture (texture = tissue)

e.g., muscle, nerve, connective tissue, epithelium

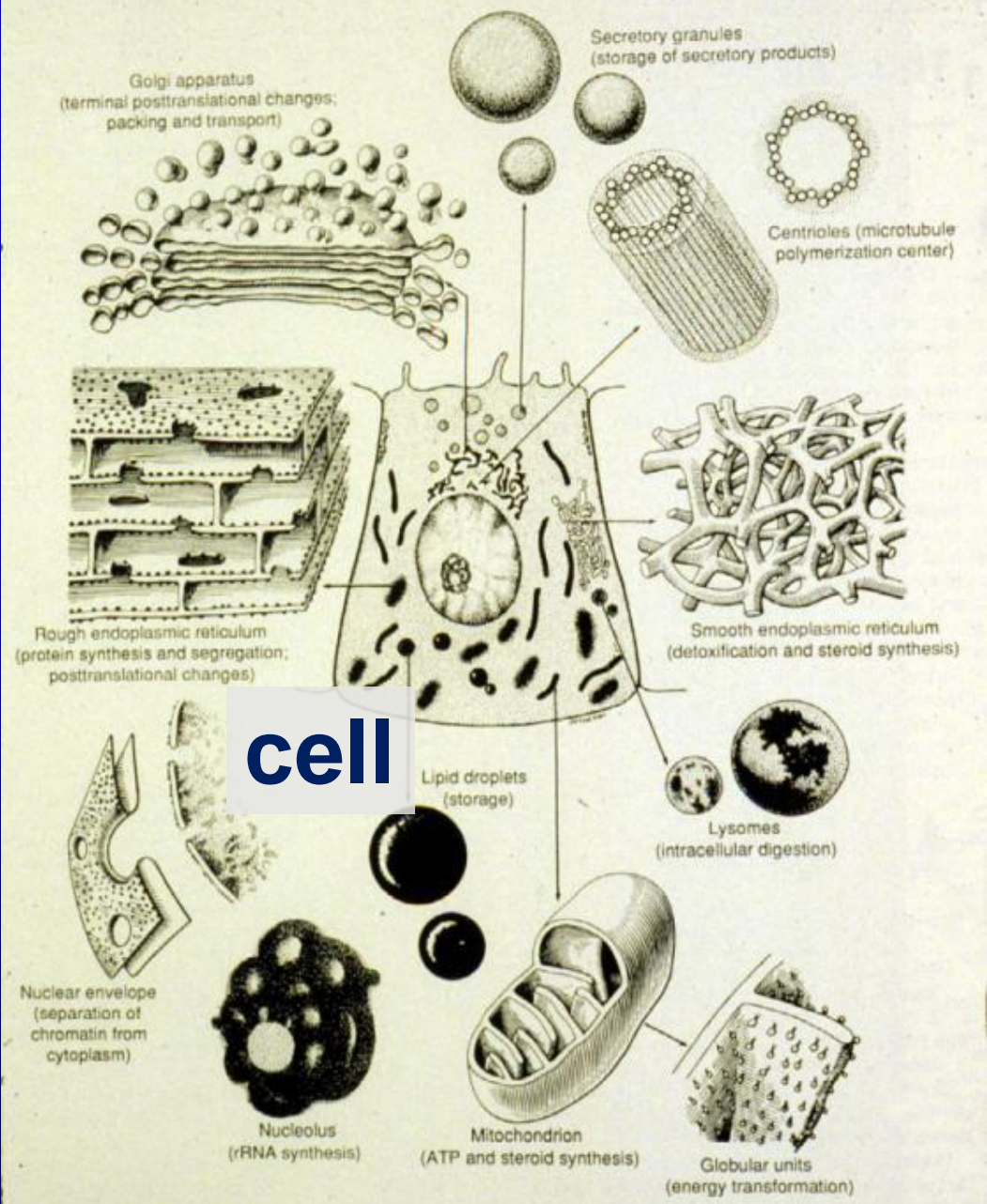
ORGAN – Two or more types of tissues; larger functional unit

e.g., skin, kidney, intestine, blood vessels

ORGAN SYSTEM - Several organs

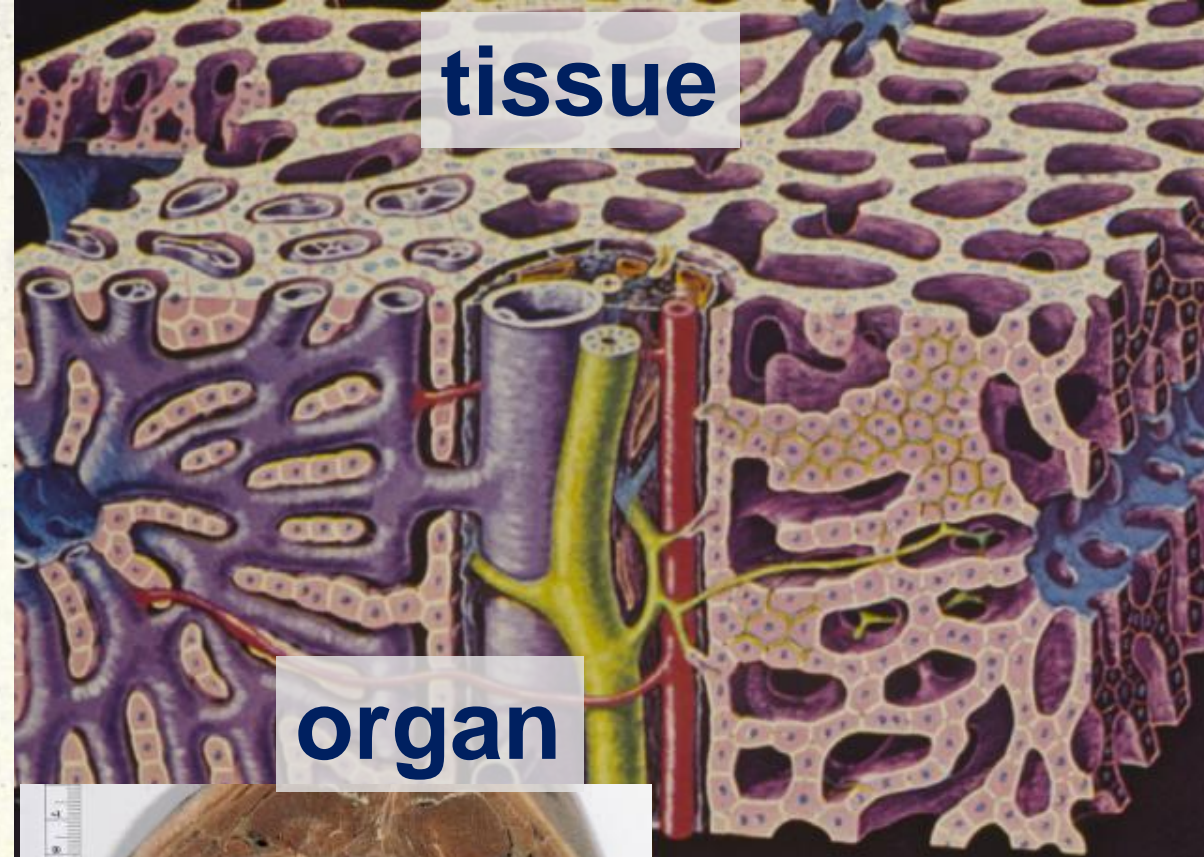
e.g., respiratory, digestive, reproductive systems





**cell**

**Figure 3-1.** Diagram showing a hypothetical eukaryotic cell, in the center, as seen with the light microscope. It is surrounded by its various structures as seen with the electron microscope. (Redrawn and reproduced, with permission, from Bloom W, Fawcett DW. *A Textbook of Histology*, 9th ed. Saunders, 1968.)



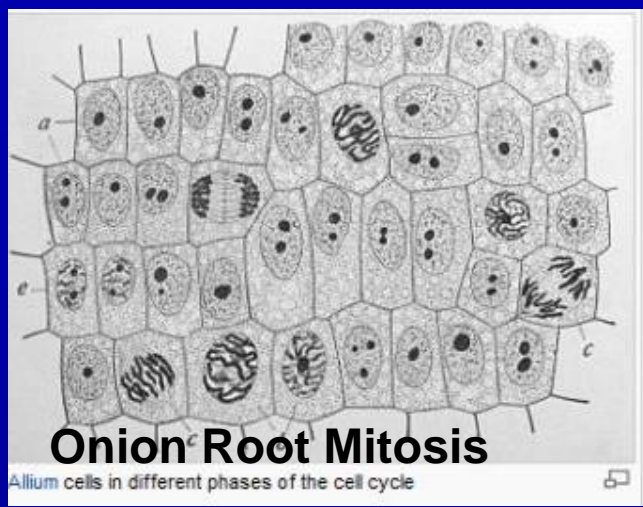
**tissue**

**organ**



**organ system**



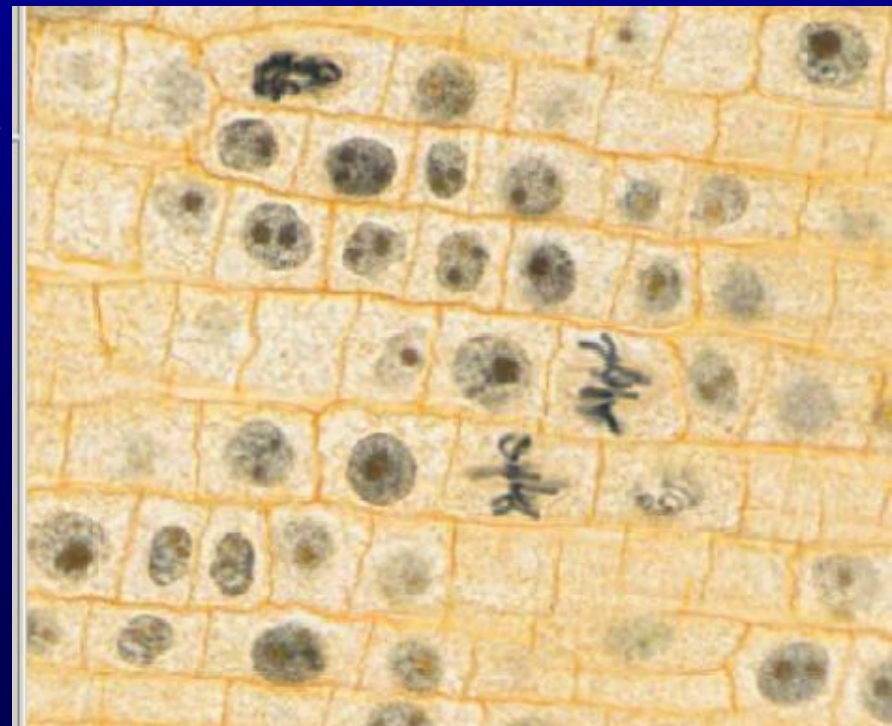


In the 1660s, Robert Hooke looked through a primitive microscope at a thinly cut piece of cork. He saw a series of walled boxes that reminded him of the tiny rooms, or *cellula*, occupied by monks, and he coined the word "**cell**."



Cells  
in a →  
plant

Although animal cells do not have cell walls like plants, cells are the “building blocks of life” of both.





# Cell Theory

1. All organisms are made of 1 or more cells.

2. Cells are the basic building blocks of life.

3. All cells come from existing cells.

Rudolf Virchow

Theodore Shwann



Theodore Shwann

Rudolf Virchow





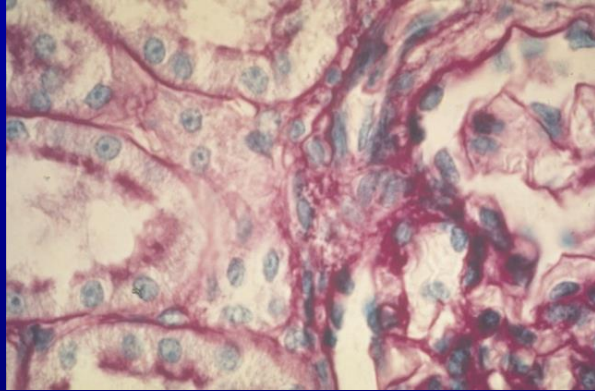
# The Cell Theory

1. All living things are made up of one or more cells
2. Cells are the basic unit of structure and function
3. All cells come from pre-existing cells
4. Cells contain the hereditary information which is passed from cell to “daughter” cell during cell division
5. All cells are basically the same in chemical composition

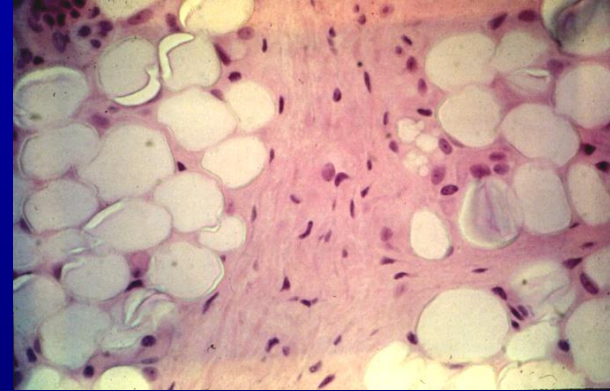
# FOUR BASIC TYPES OF TISSUES IN THE BODY

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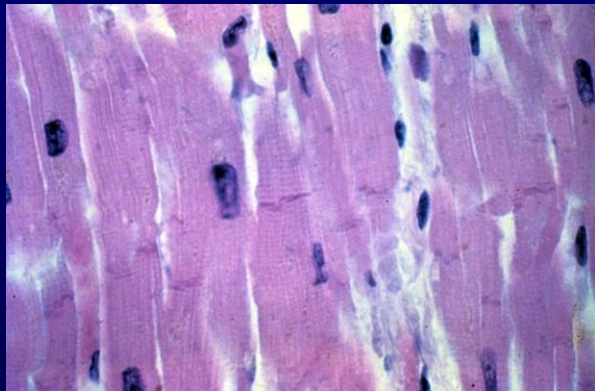
**Epithelium**



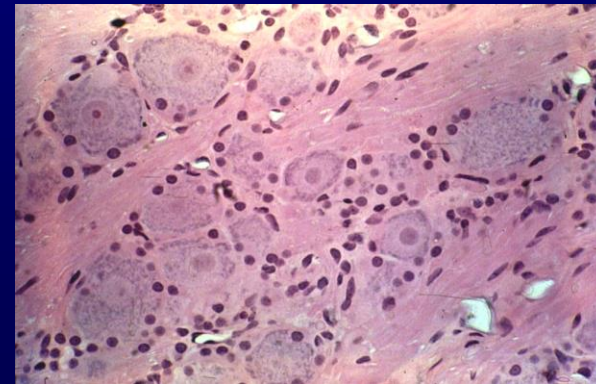
**Connective tissue**



**Muscular tissue**



**Nervous tissue**

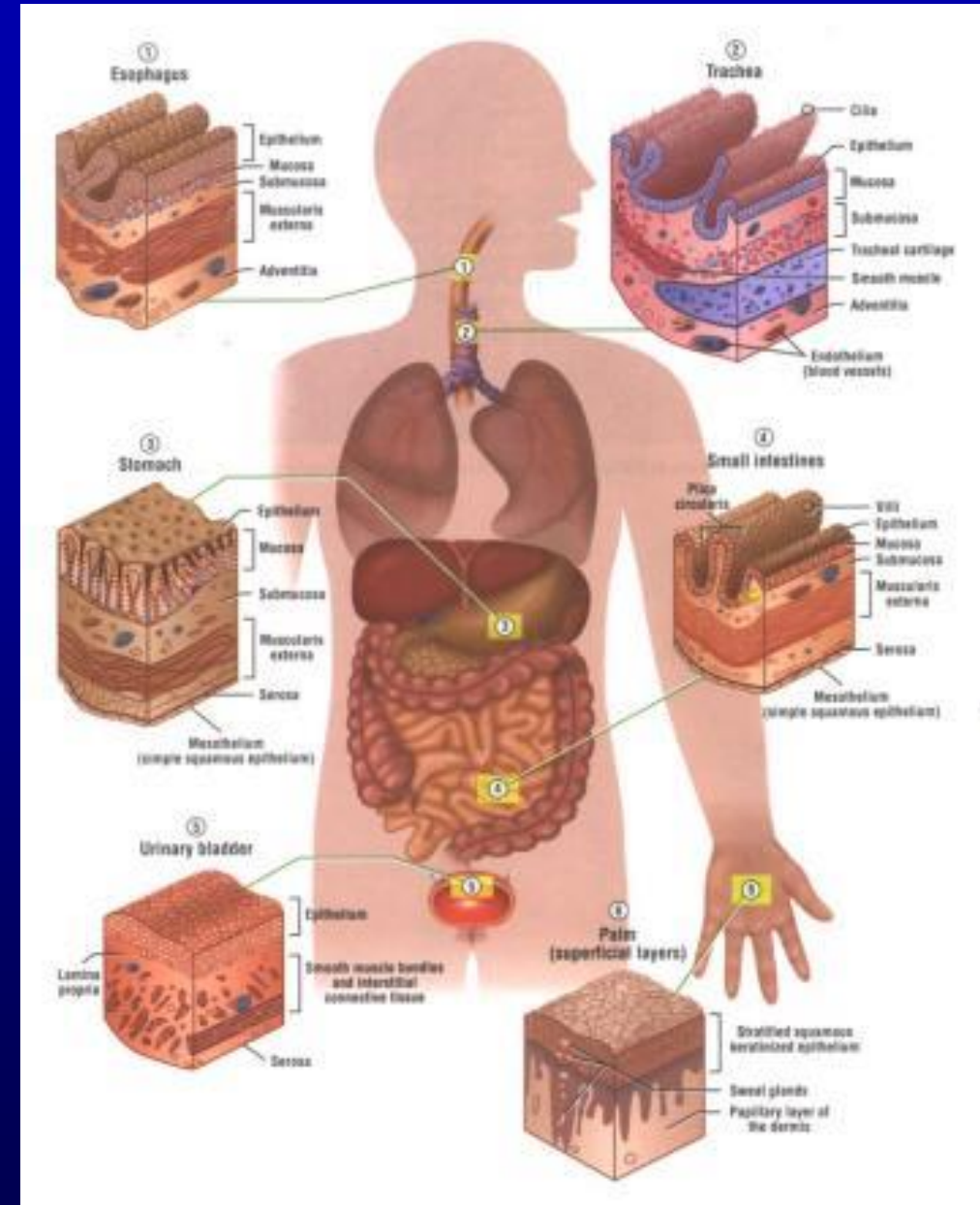
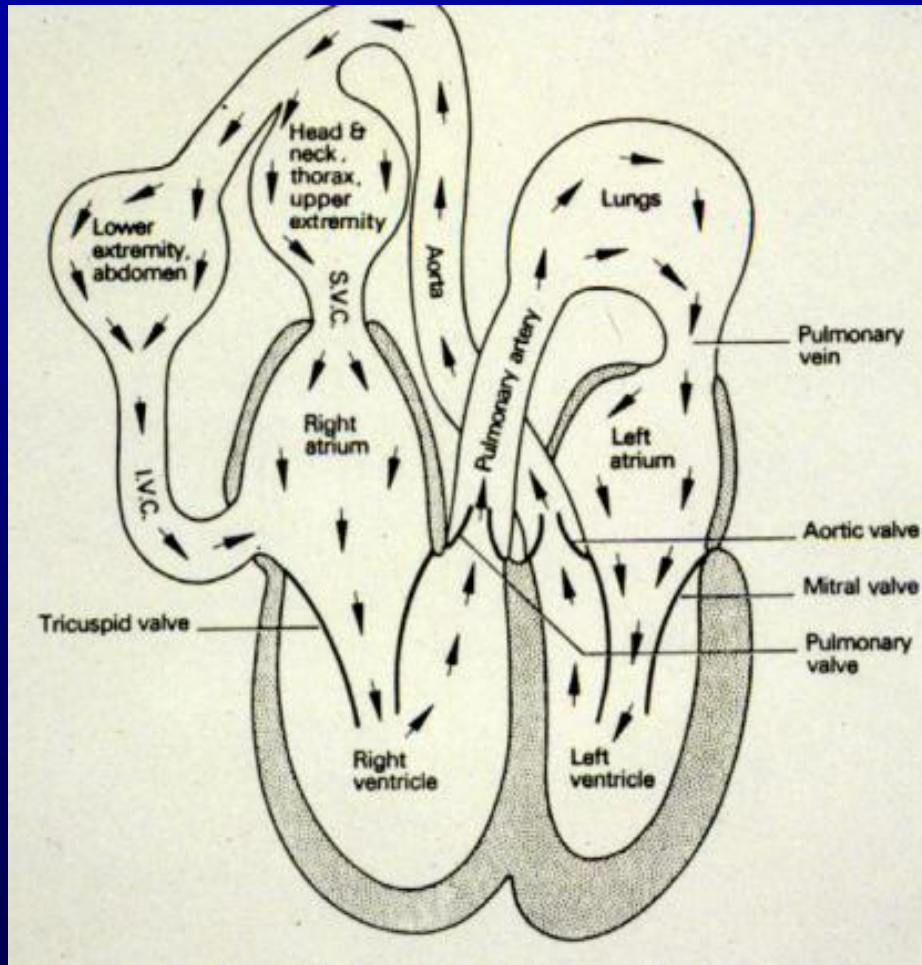




# Epithelium

## Functions:

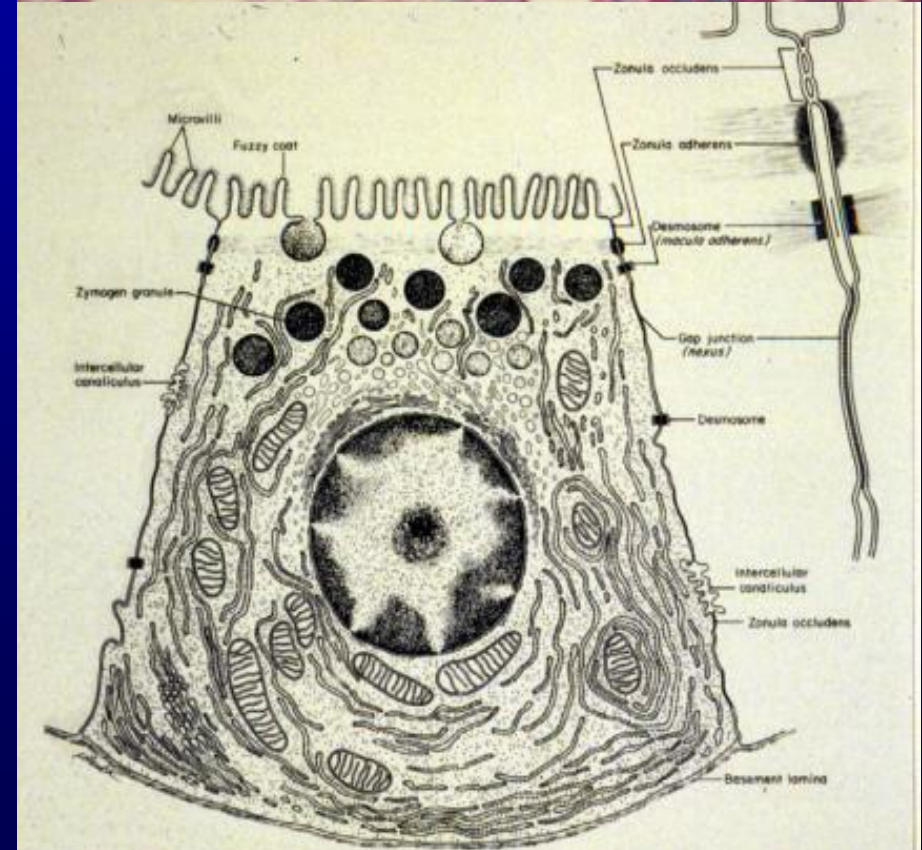
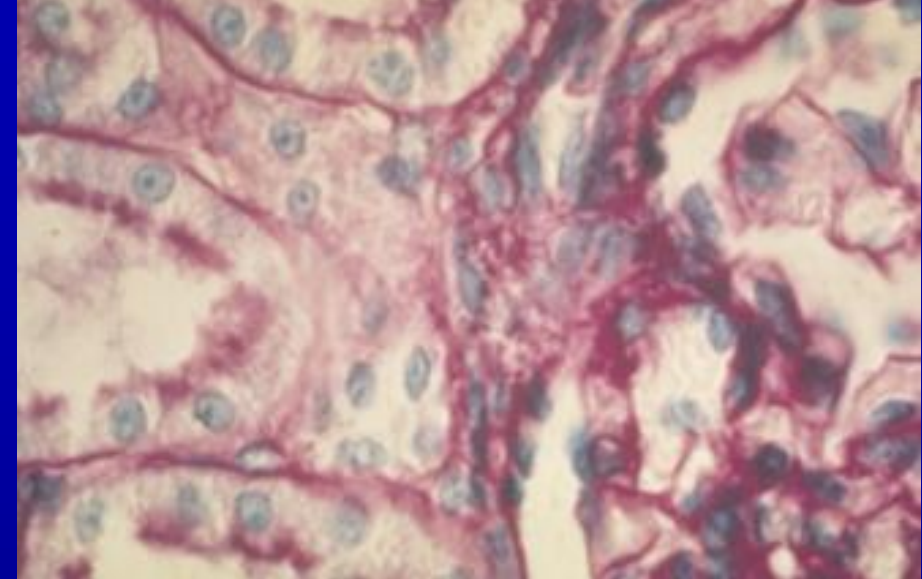
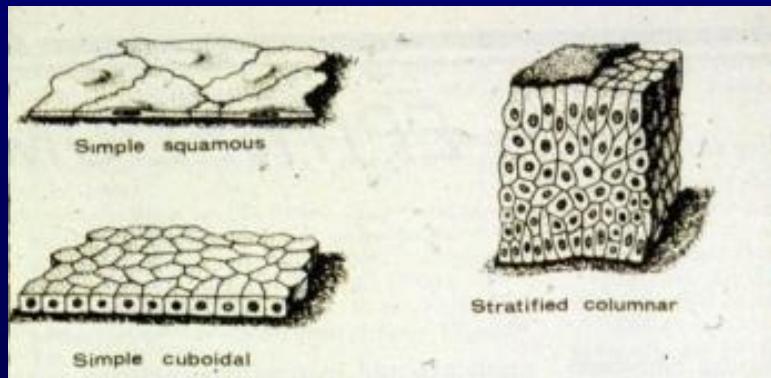
Cover organs, line viscera and blood vessels, secretory cells of glands



# Epithelium

## Distinguishing features and distribution:

- Always sit on a **basement membrane**, but come in a variety of configurations: classified on the basis of their shape and of the surface cells and whether one (**simple**) or more (**stratified**) layers of cells are stacked upon each other.
- These cells are always **attached** to their neighbors by desmosomes, tight junctions, and gap **junctions**.





# Epithelium

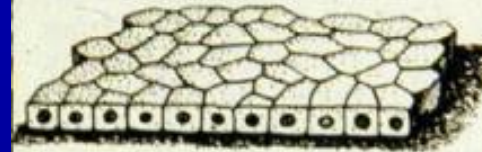
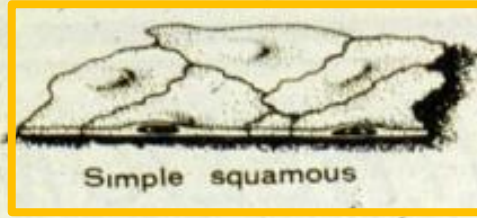
## Histological Identification

### Simple Squamous –

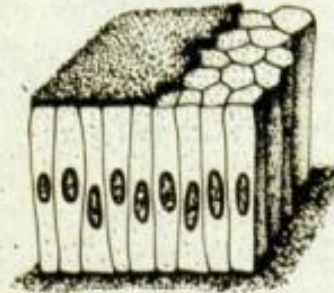
single layer of flat cells  
(blood vessels, covering of organs)

### Stratified Squamous –

Multiple layers of cells with  
flat ones at the surface  
(skin, gums)



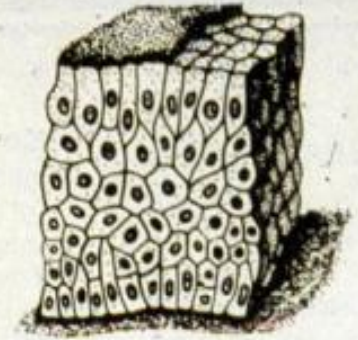
Simple cuboidal



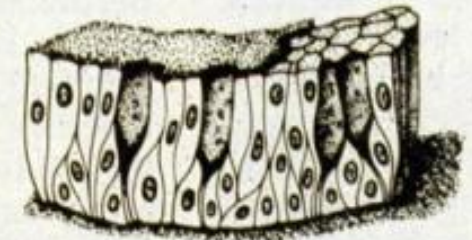
Simple columnar



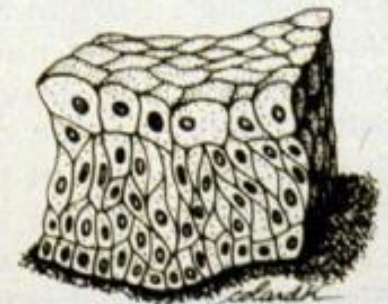
Stratified squamous



Stratified columnar



Pseudostratified columnar



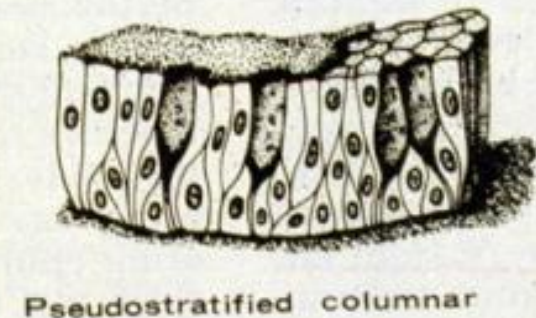
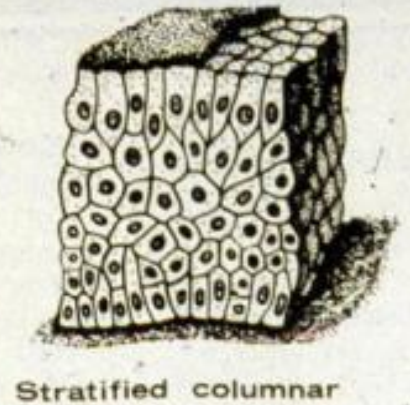
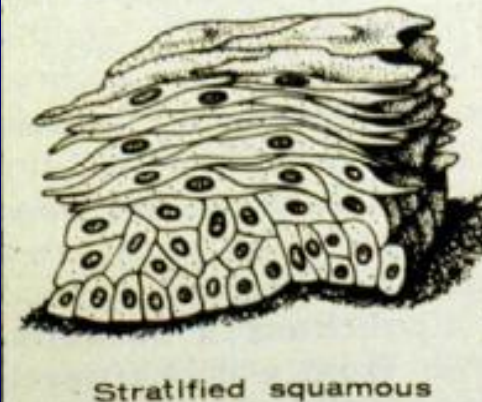
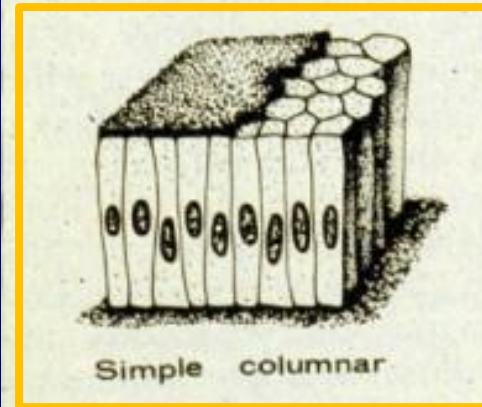
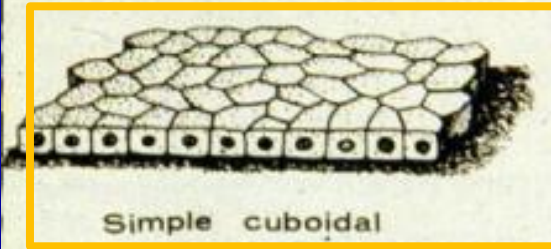
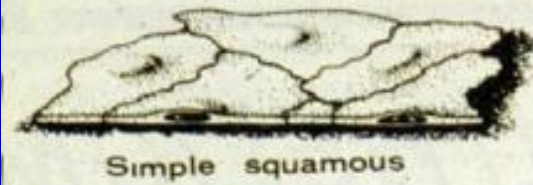
Transitional

# Epithelium

## Histological Identification

**Simple cuboidal** – Single layer of square cells (kidney tubules, liver cells, many others)

**Simple columnar** – Single layer of tall, thin cells (intestinal epithelium)



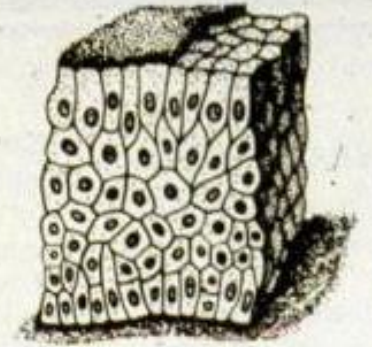


# Epithelium

## Histological Identification

**Pseudostratified columnar** – single layer of tall, thin cells packed together in such a jumble that they seem to be in layers, although all of the cells reach the basement membrane (respiratory passage)

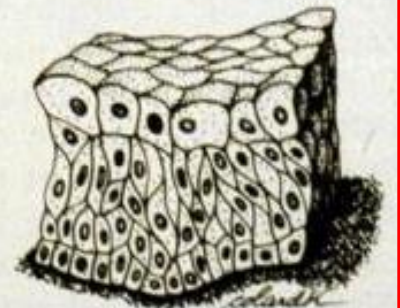
**Transitional** – stratified cuboidal epithelium of urinary passages



Stratified columnar



Pseudostratified columnar

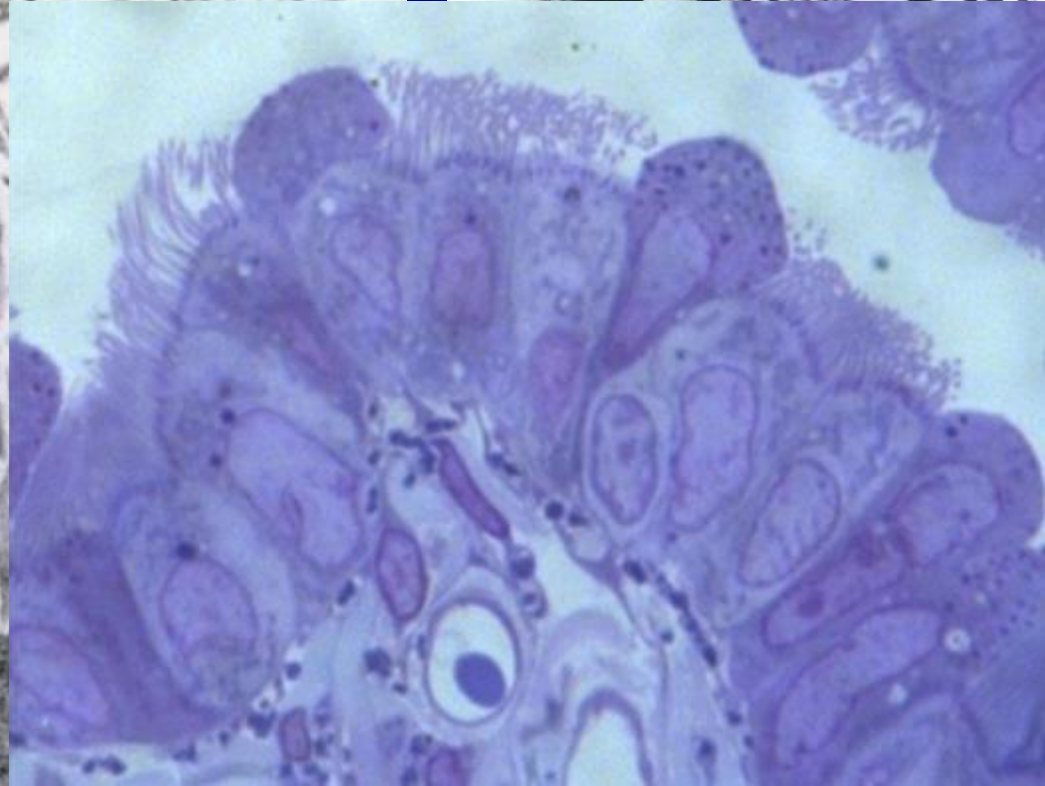
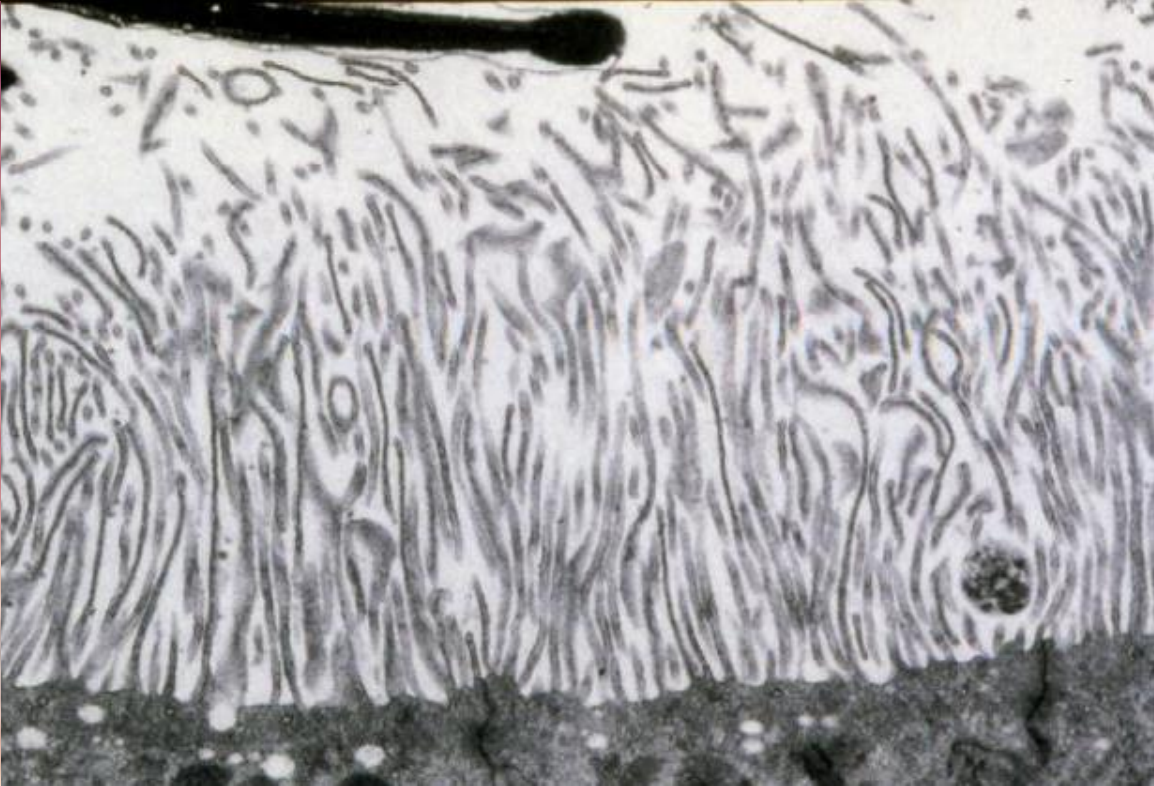


Transitional

# Epithelium

## Histological Identification

Some epithelia have **surface specializations** such as numerous microvilli or cilia.



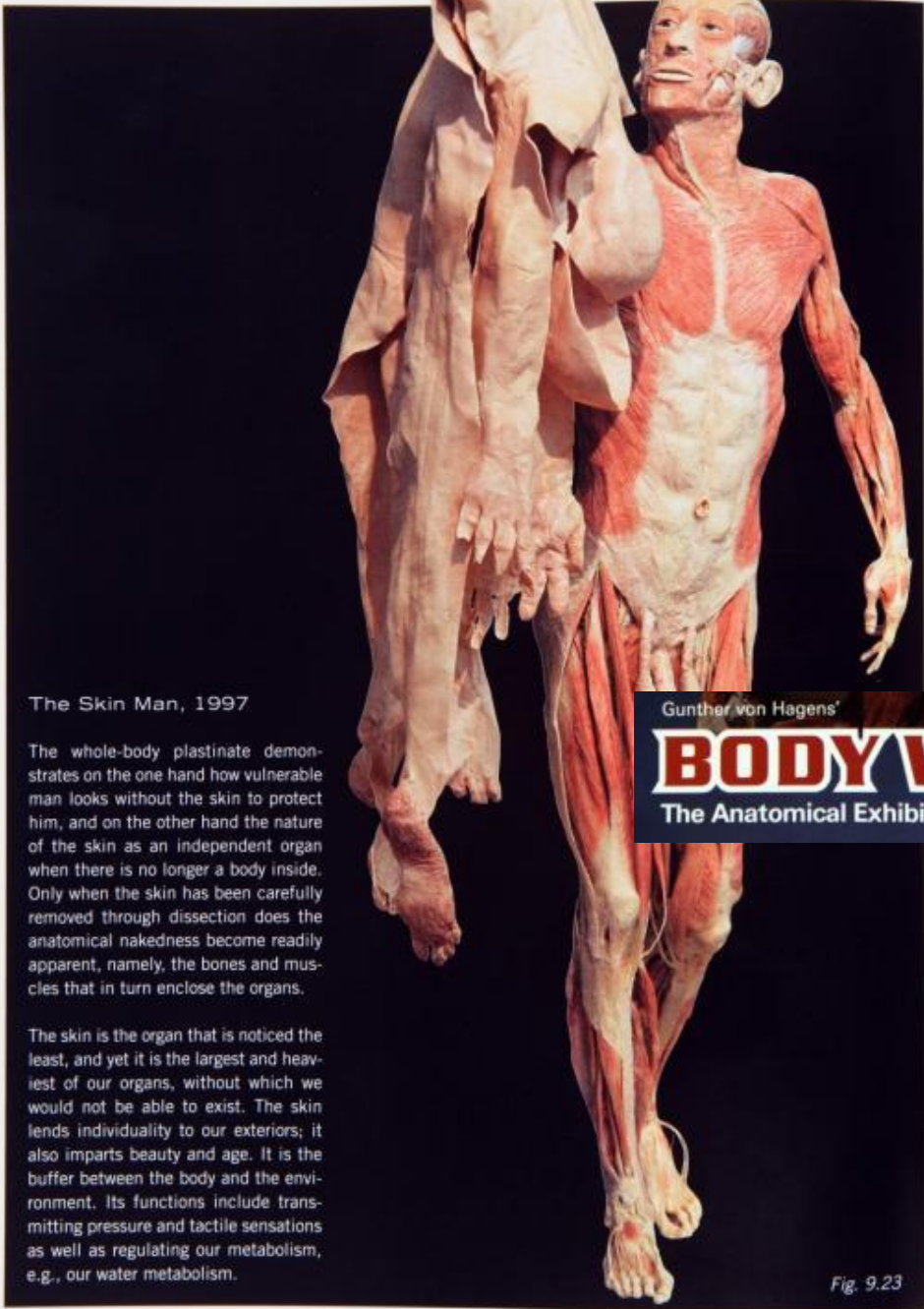




Gunther von Hagens'

# **BODY WORLDS**

The Anatomical Exhibition of Real Human Bodies

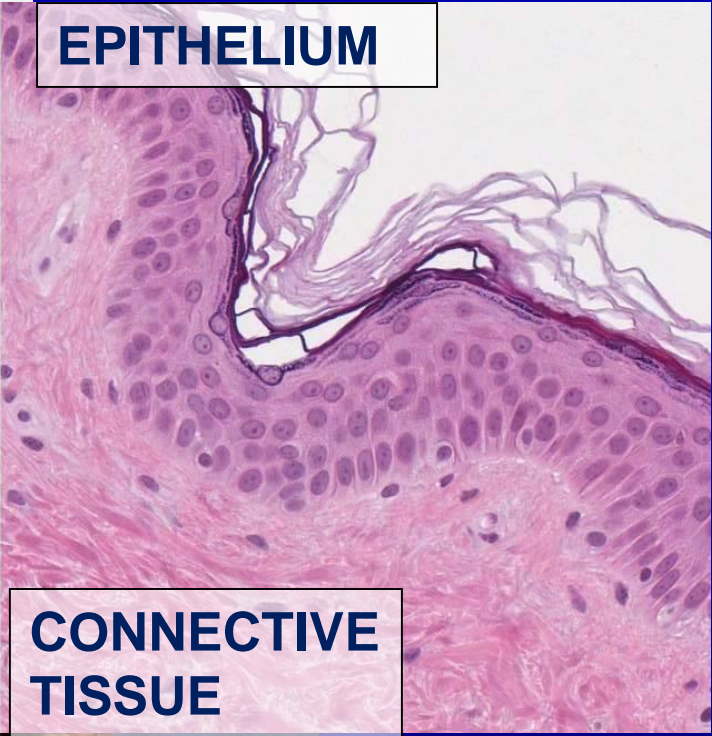


The Skin Man, 1997

The whole-body plastinate demonstrates on the one hand how vulnerable man looks without the skin to protect him, and on the other hand the nature of the skin as an independent organ when there is no longer a body inside. Only when the skin has been carefully removed through dissection does the anatomical nakedness become readily apparent, namely, the bones and muscles that in turn enclose the organs.

The skin is the organ that is noticed the least, and yet it is the largest and heaviest of our organs, without which we would not be able to exist. The skin lends individuality to our exteriors; it also imparts beauty and age. It is the buffer between the body and the environment. Its functions include transmitting pressure and tactile sensations as well as regulating our metabolism, e.g., our water metabolism.

Fig. 9.23



EPITHELIUM

CONNECTIVE TISSUE

Gunther von Hagens' **BODY WORLDS**  
The Anatomical Exhibition of Real Human Bodies

Skin

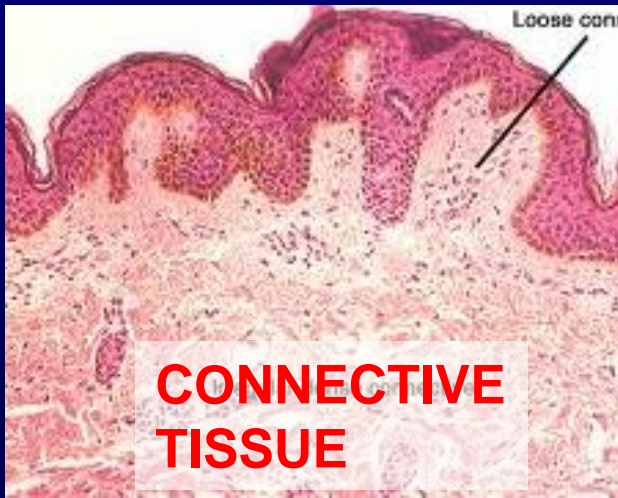
Gunther von Hagens' **BODY WORLDS**



# Connective Tissue

## Function:

the **histological glue** which binds the other tissues together to form organs, specializations include blood, cartilage, and bone.

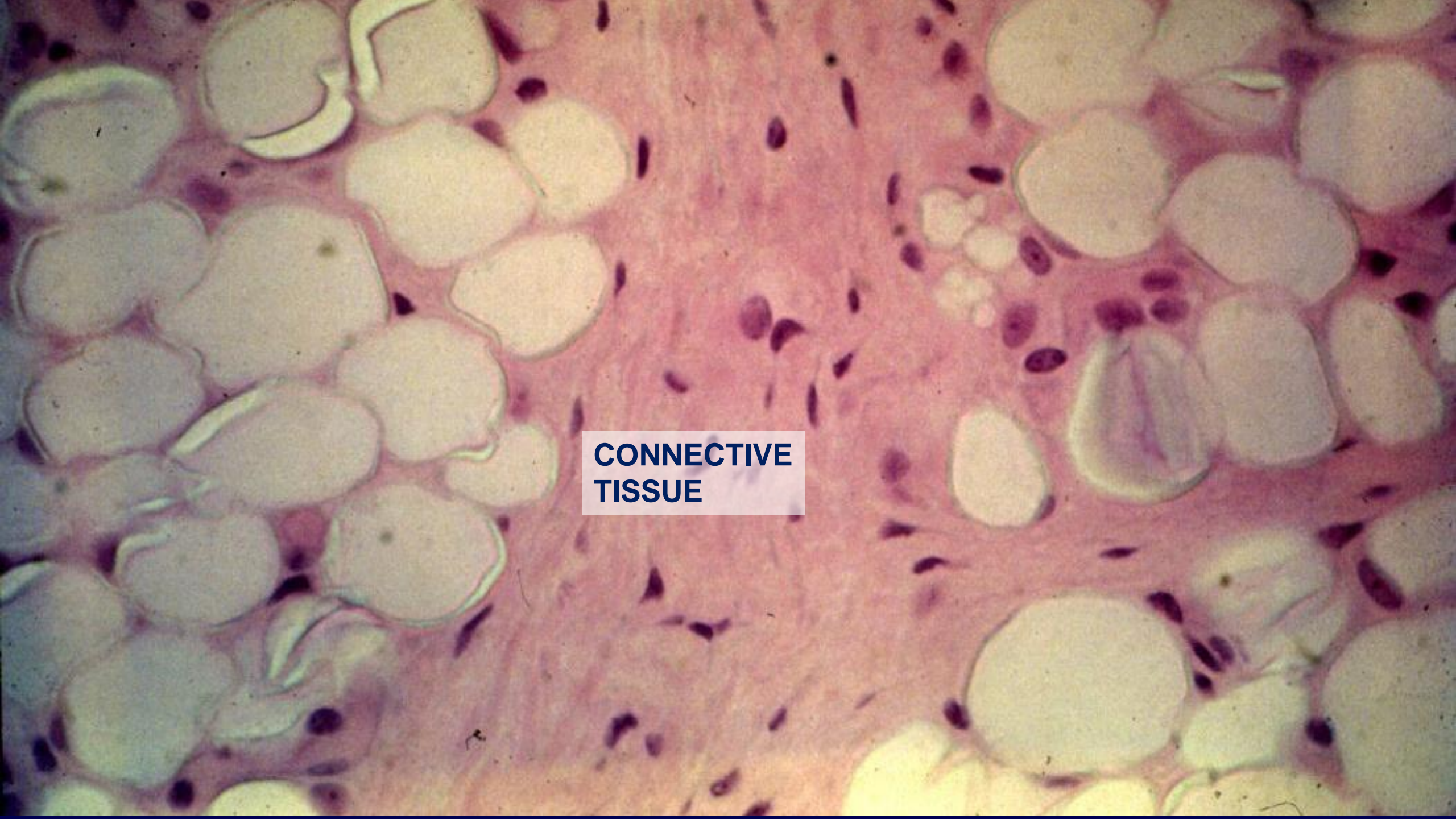


# Connective Tissue

## Distinguishing features and histological identification:

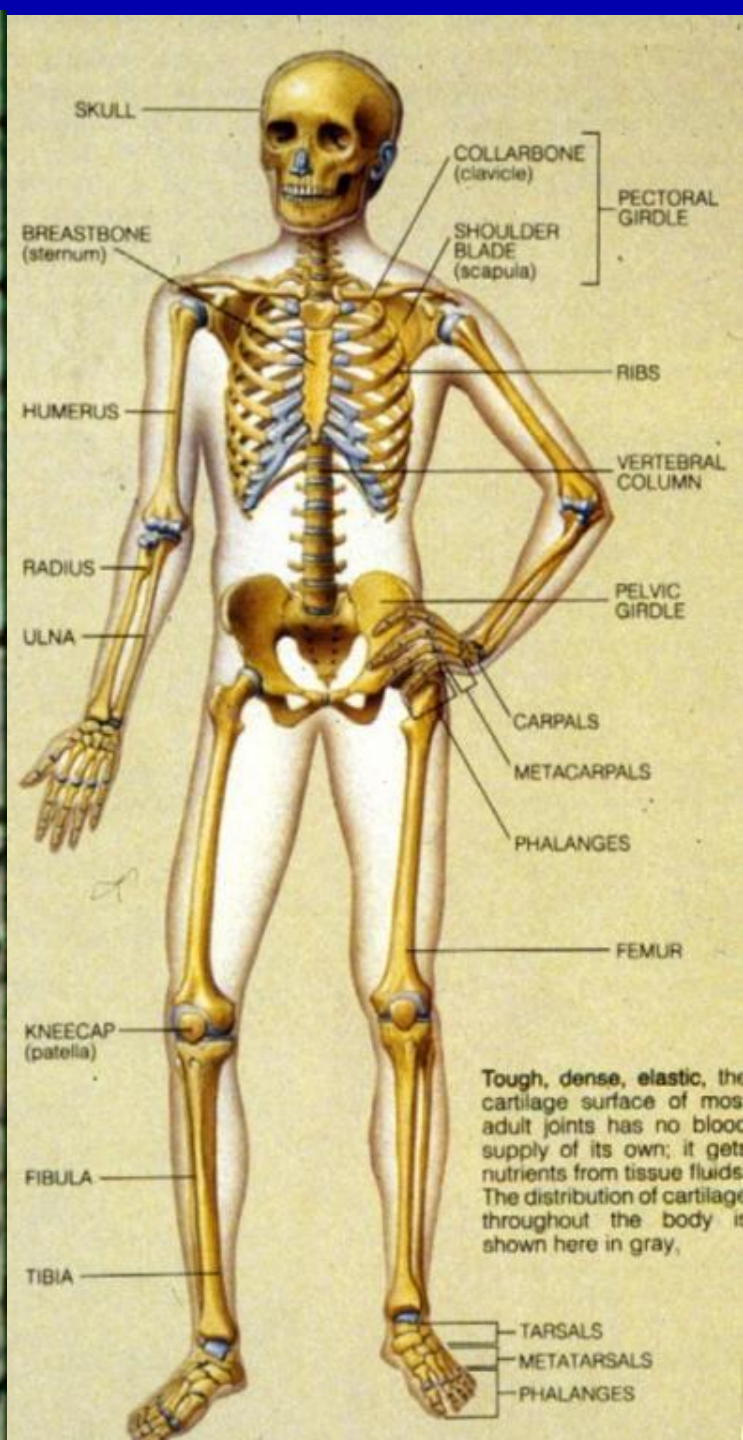
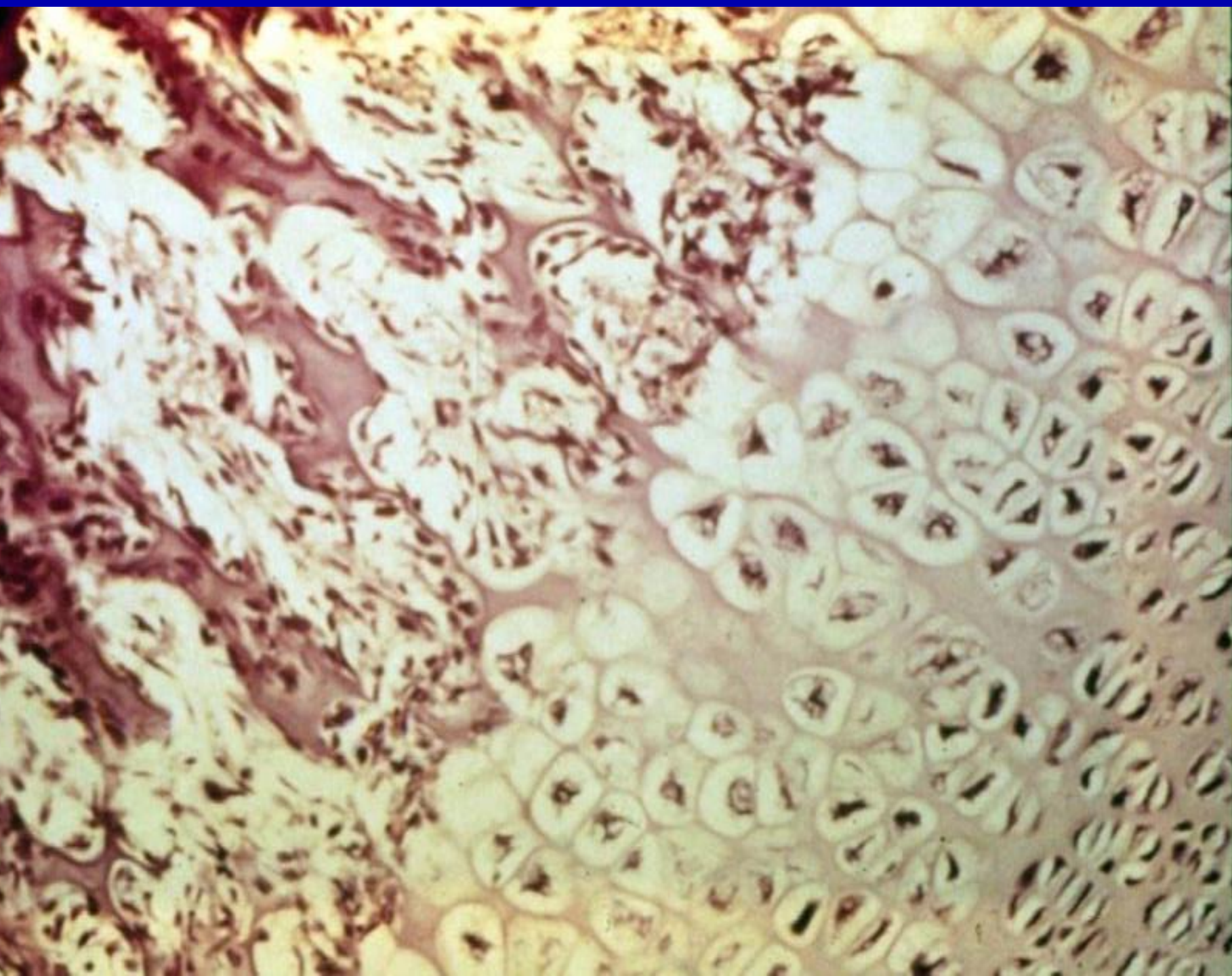
- **Loose connective tissue** – sparse collagen and elastic fibers, plentiful cells including fibroblasts, leukocytes
- **Dense connective tissue** – concentrated collagen, few cell
- **Cartilage** – avascular homogeneous matrix of collagen and protein-polysaccharides with few cell
- **Bone** – calcified collagen matrix with few cells trapped in the caves of bone





**CONNECTIVE  
TISSUE**



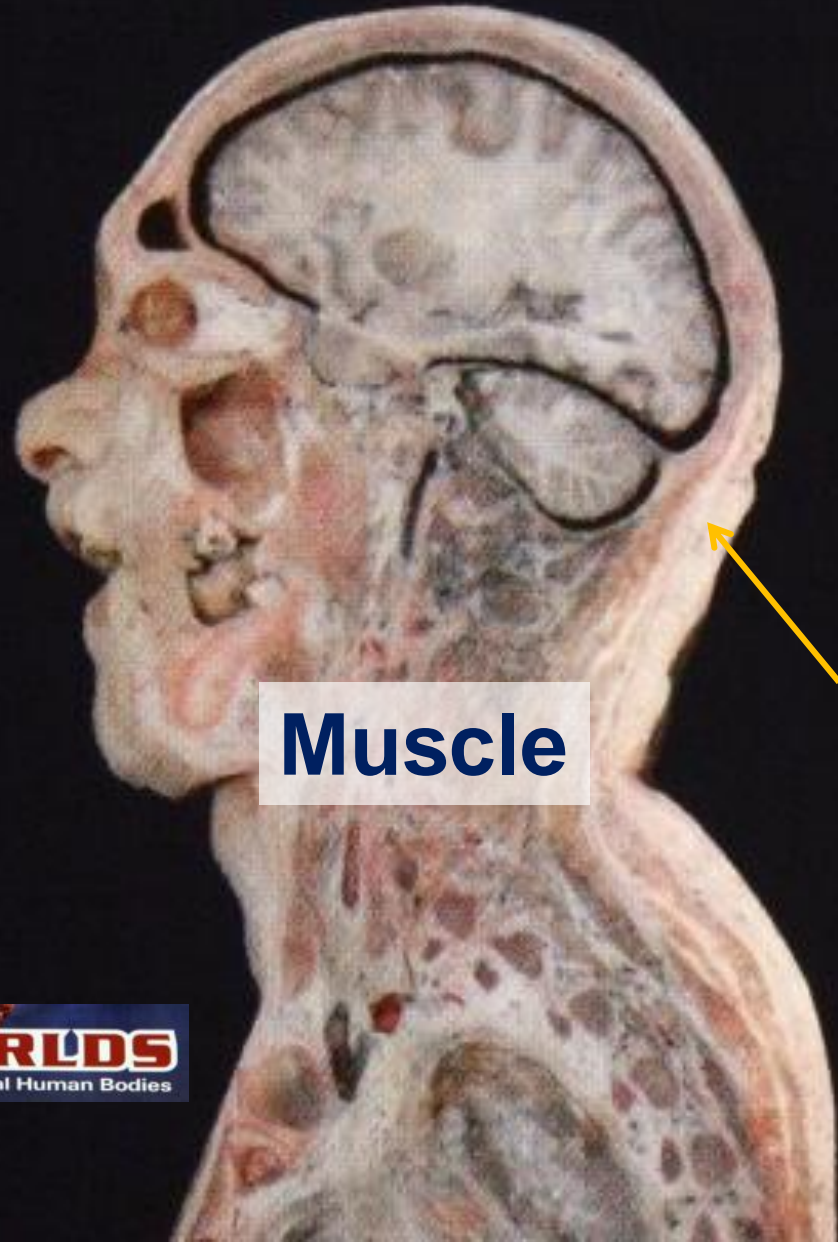






Gunther von Hagens'  
**BODY WORLDS**  
The Anatomical Exhibition of Real Human Bodies

Gunther von Hagens' Body Worlds



Muscle

Fat

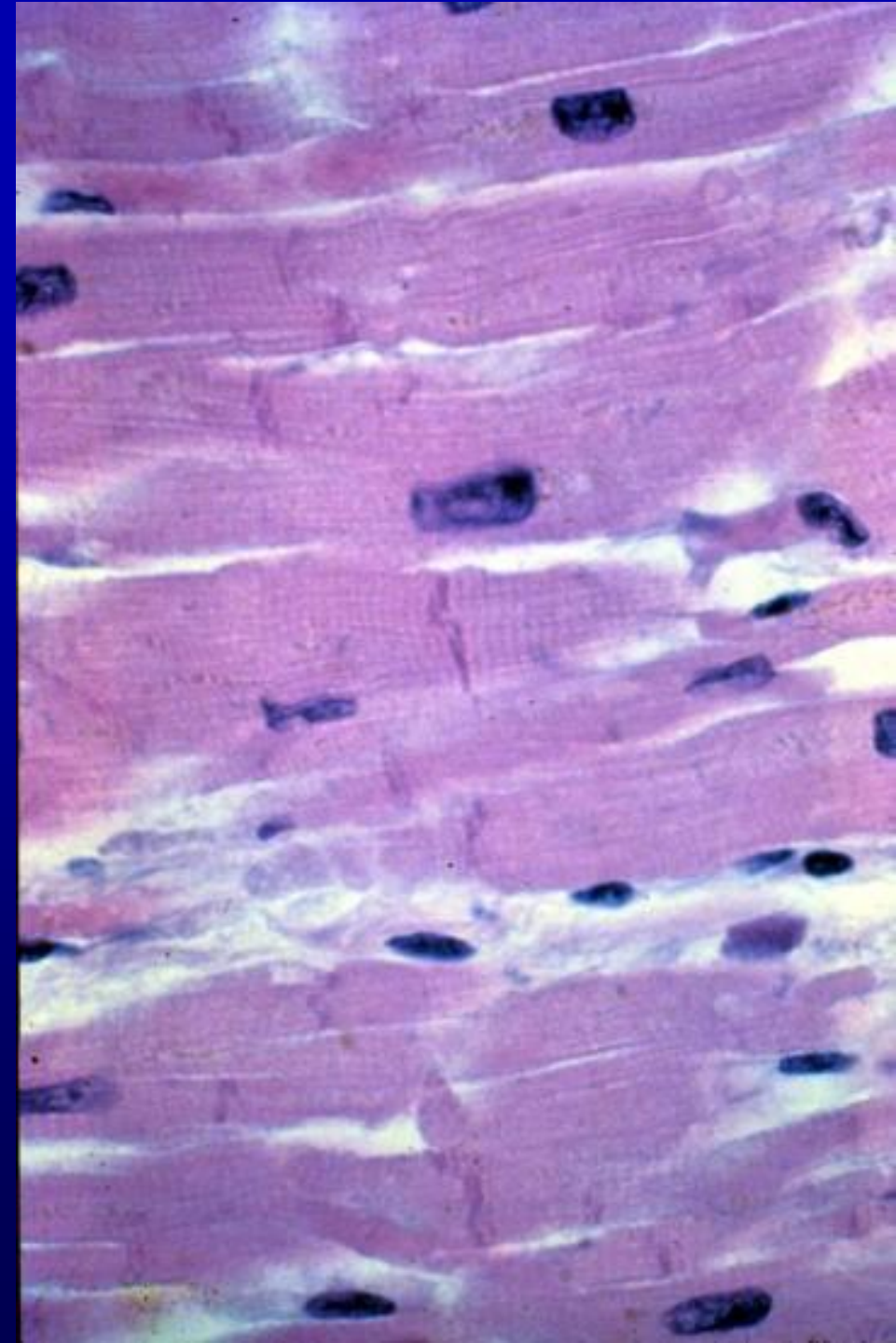
# Muscle

## Function:

generation of **contractile force**.

## Distinguishing features:

- High concentration of contractile proteins **actin** and **myosin** arranged either diffusely in the cytoplasm (**smooth muscle**) or in regular repeating units called **sarcomeres** (**striated muscles**, e.g., cardiac and skeletal muscles).

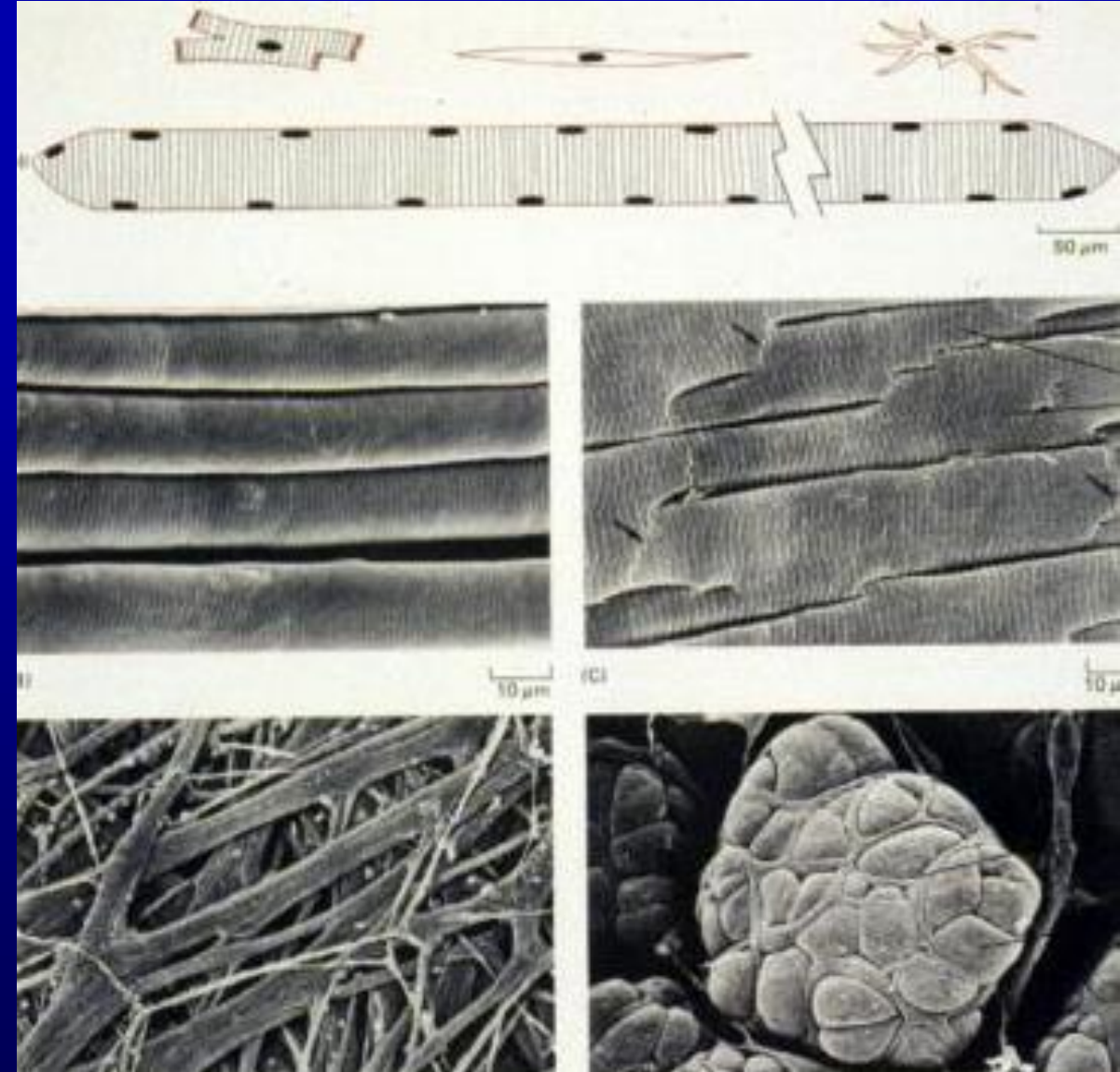




# Muscle

## Histological identification:

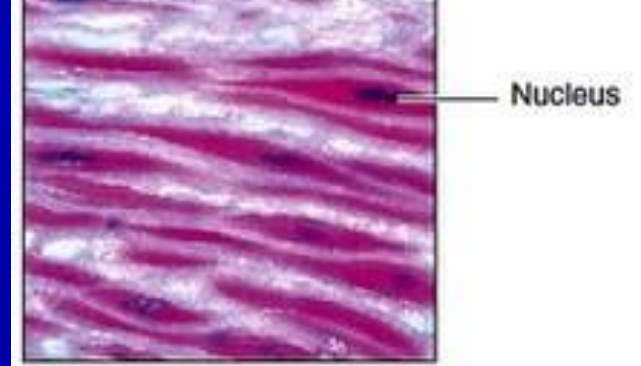
- **Skeletal muscle** – very long cylindrical striated muscle cells with multiple peripheral nuclei
- **Cardiac muscle** – short branching striated muscle cells with one or two centrally located nuclei
- **Smooth muscle** – closely packed spindle-shaped cells with a single centrally placed nucleus and cytoplasm that appears homogeneous by light microscopy



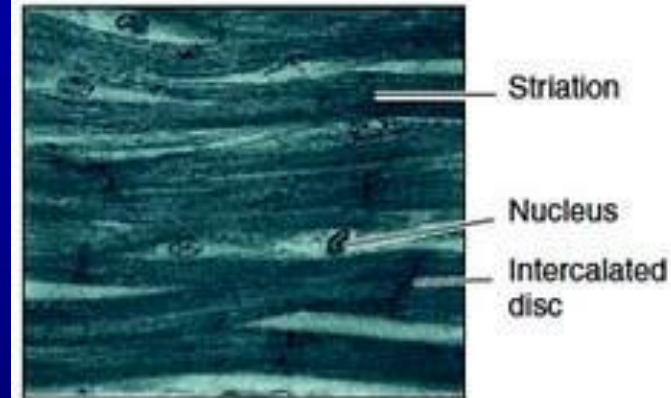
# Muscle

## Distribution:

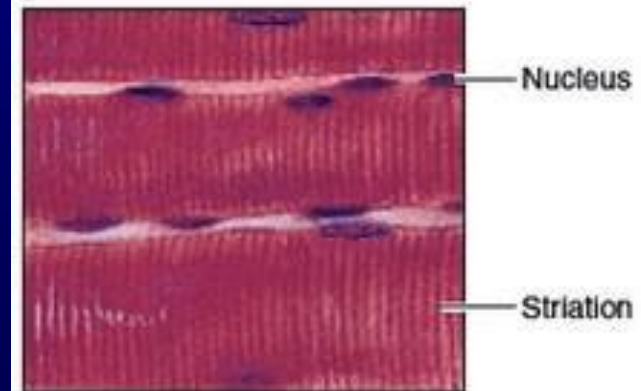
- **Skeletal** – striated muscles mostly associated with the skeleton
- **Cardiac** – striated muscles associated with the heart
- **Smooth** – fusiform cells associated with the viscera, respiratory tract, blood vessels, uterus, etc.



(a) Smooth muscle tissue

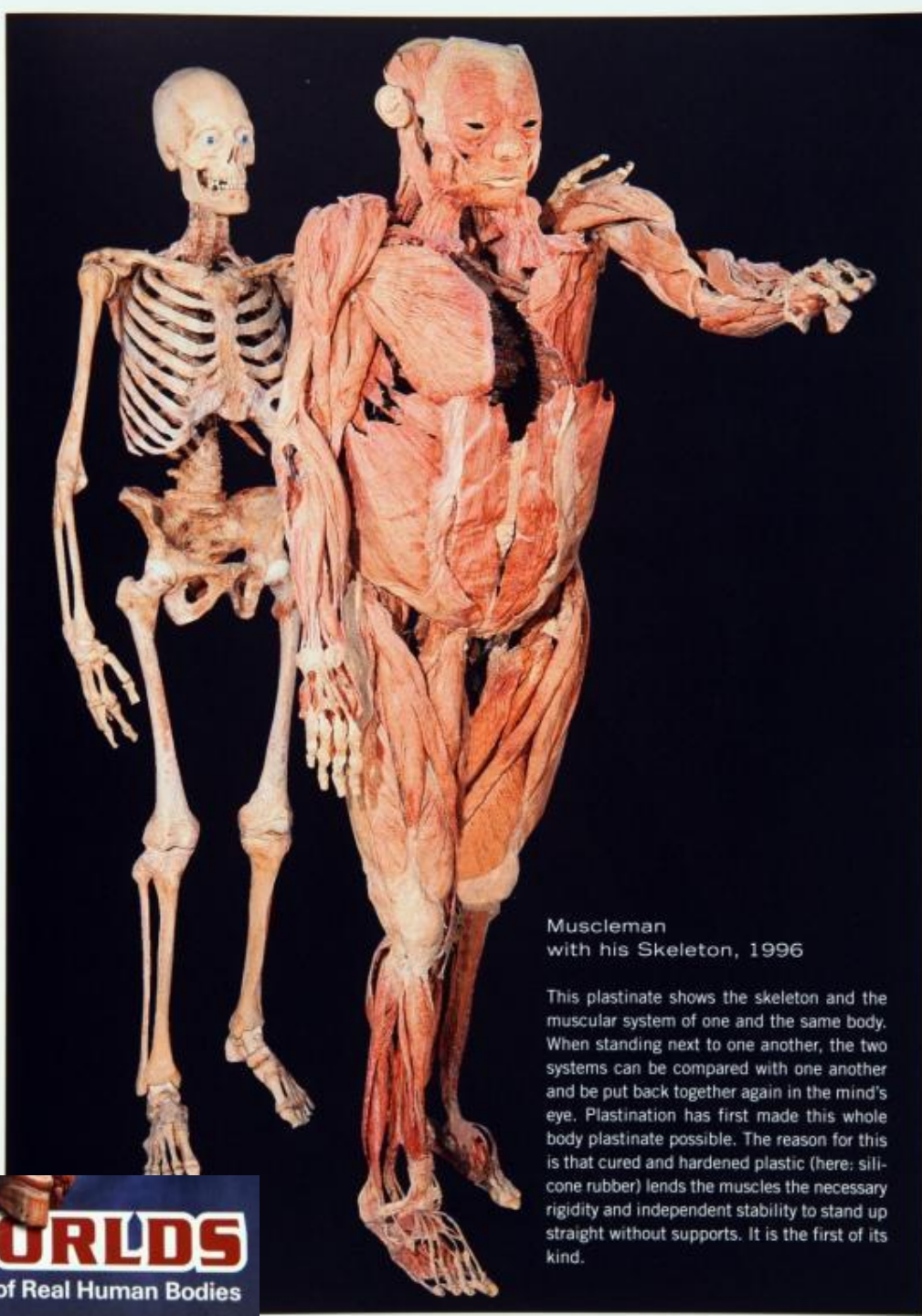


(b) Cardiac muscle tissue



(c) Skeletal muscle tissue





Muscleman  
with his Skeleton, 1996

This plastinate shows the skeleton and the muscular system of one and the same body. When standing next to one another, the two systems can be compared with one another and be put back together again in the mind's eye. Plastination has first made this whole body plastinate possible. The reason for this is that cured and hardened plastic (here: silicone rubber) lends the muscles the necessary rigidity and independent stability to stand up straight without supports. It is the first of its kind.

Smooth muscle

NERVOUS  
TISSUE

Gunther von Hagens'

**BODY WORLDS**

The Anatomical Exhibition of Real Human Bodies

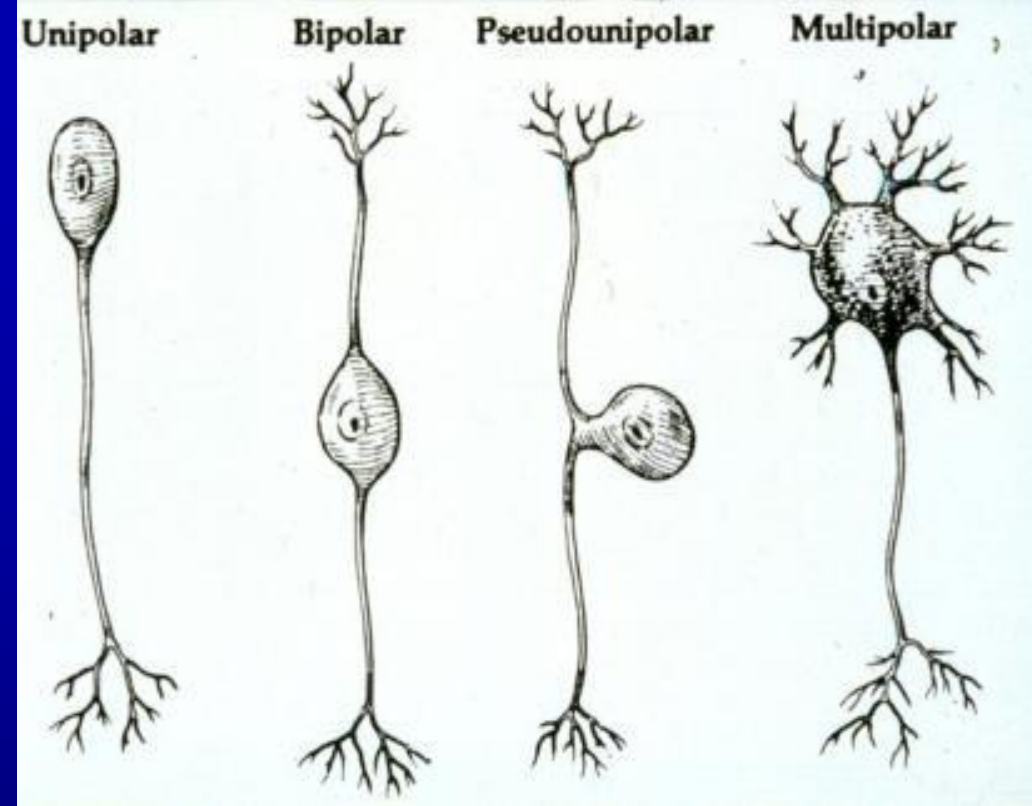
# Nervous Tissue

## Functions:

specialized for the **transmission**, **reception**, and **integration** of electrical impulses

## Distinguishing features:

- **neurons** – very large excitable cells with long processes called **axons** and **dendrites**. The axons make contact with other neurons or muscle cells at a specialization called a synapse where the impulses are either electrically or chemically transmitted to other neurons or various target cells (e.g., muscle). Others secrete hormones.



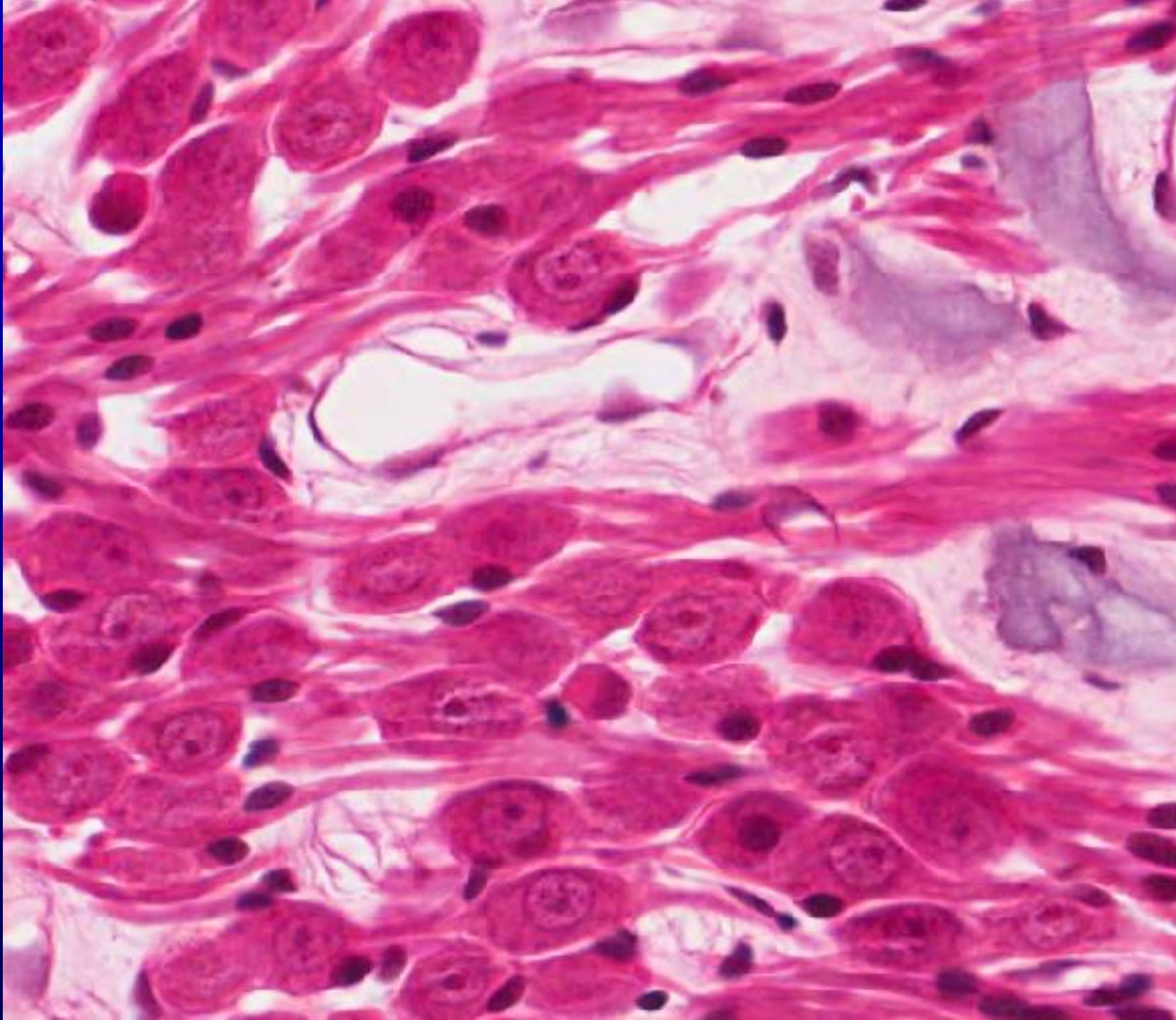


# Nervous Tissue

Bipolar



Bipolar  
neurons  
of inner ear

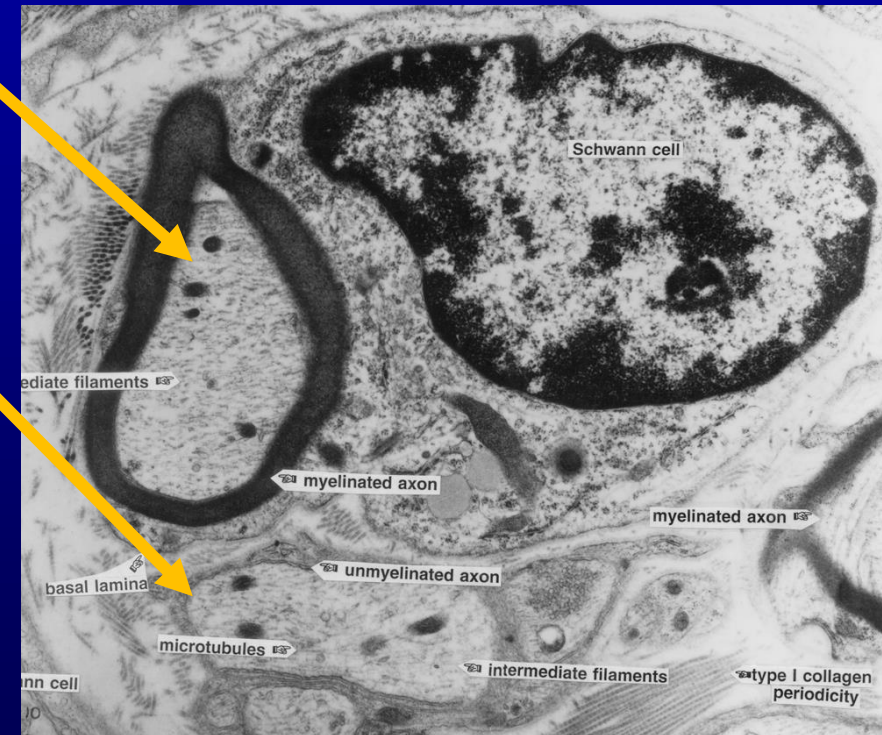
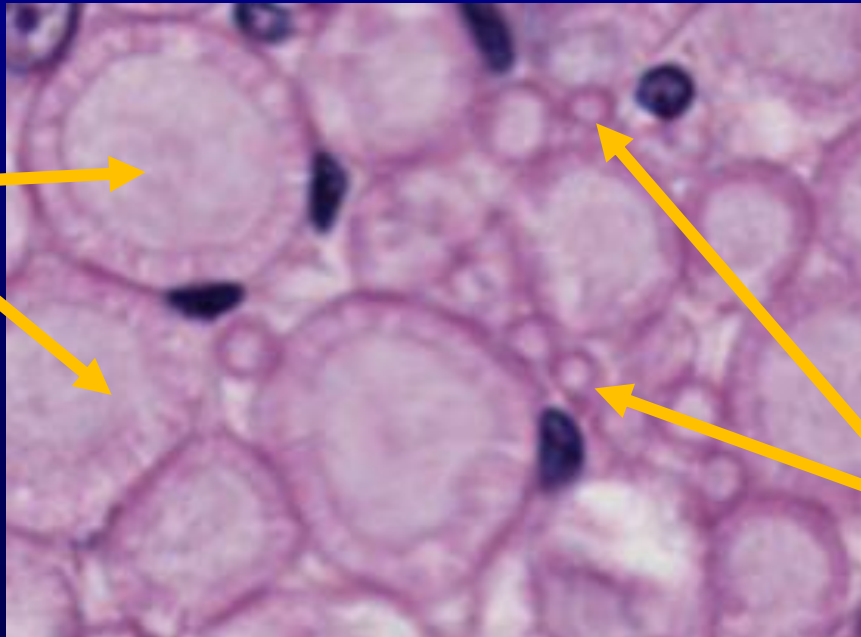


# Nervous Tissue

**Glial cells** – the supporting cells of nervous tissue.

**Nerves** – collections of neuronal processes bound together by connective tissue. Axons may be coated by a myelin sheath (“**myelinated**”) or simply protected by being cradled in an indentation of a glial cell (“**unmyelinated**”).

**Myelinated**

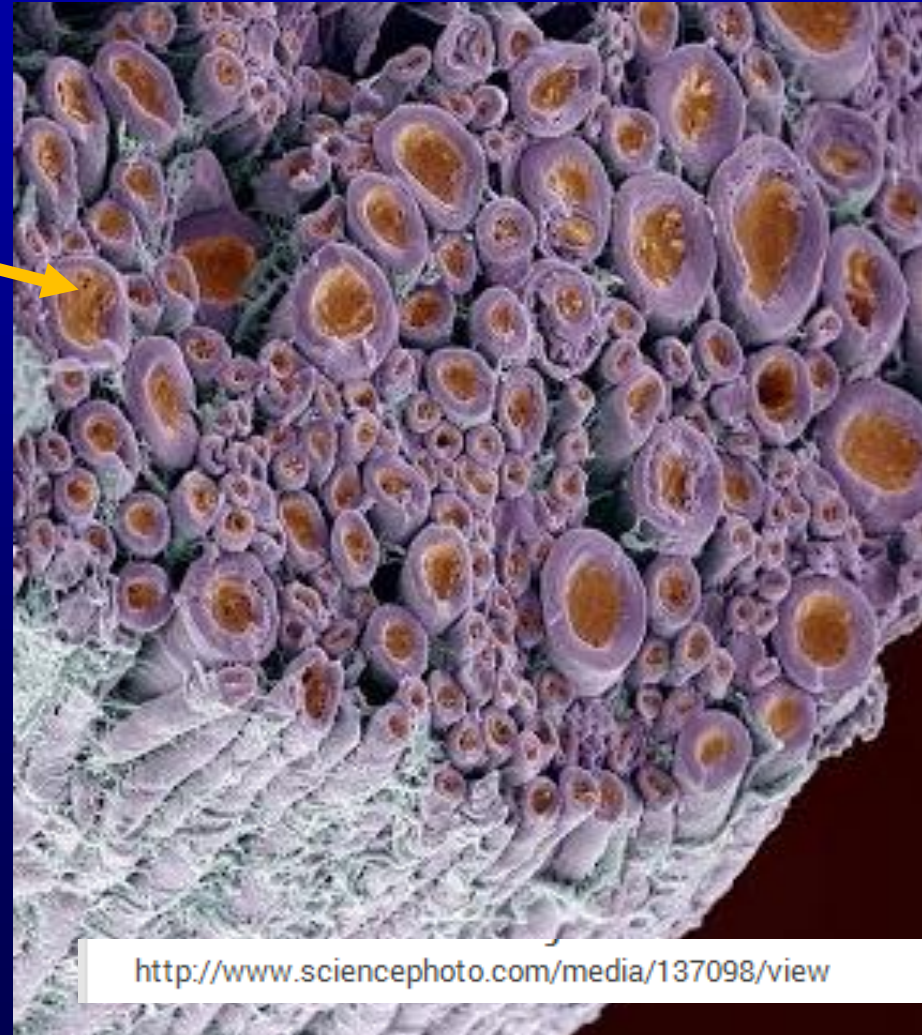
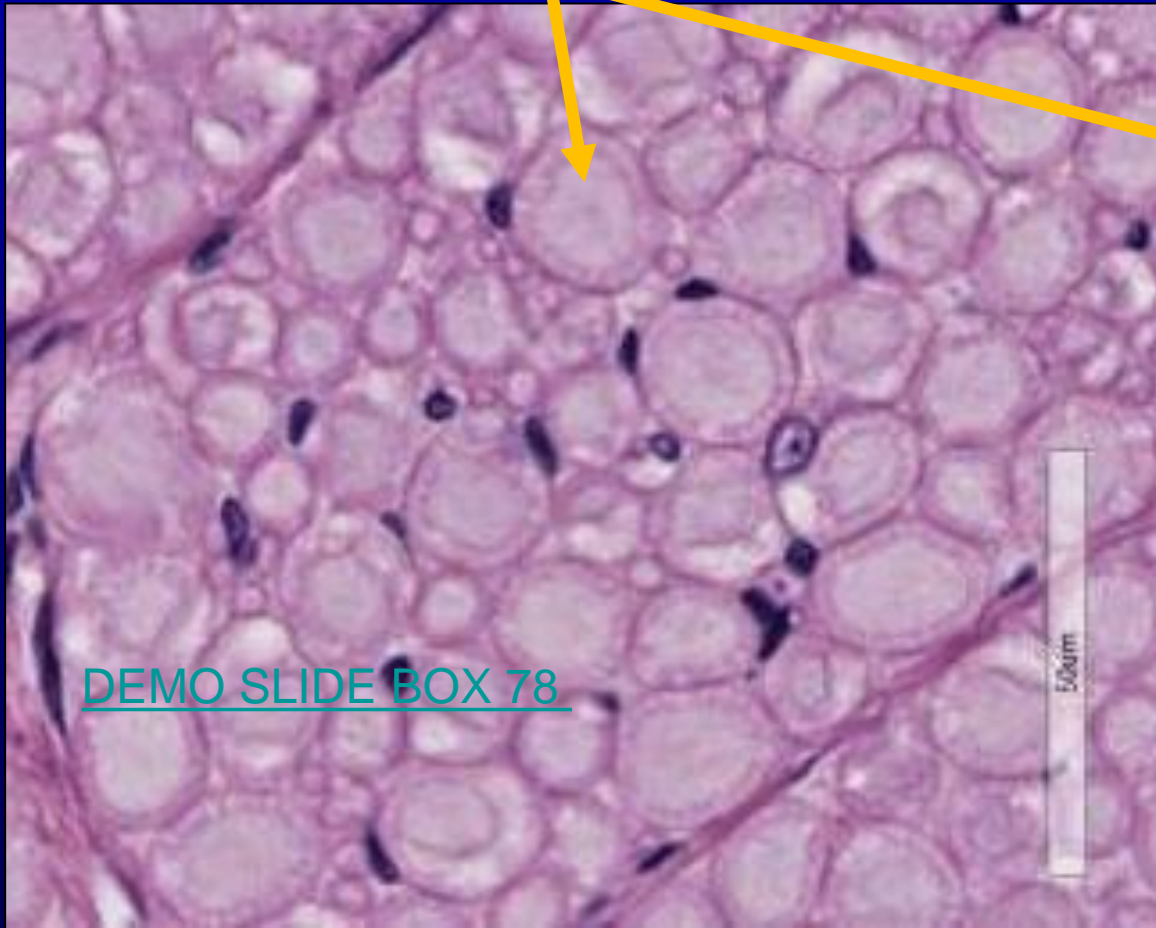


**Unmyelinated?**



# Nervous Tissue

**Nerves** – collections of neuronal processes bound together by connective tissue. Axons may be coated by a myelin sheath (“**myelinated**”)

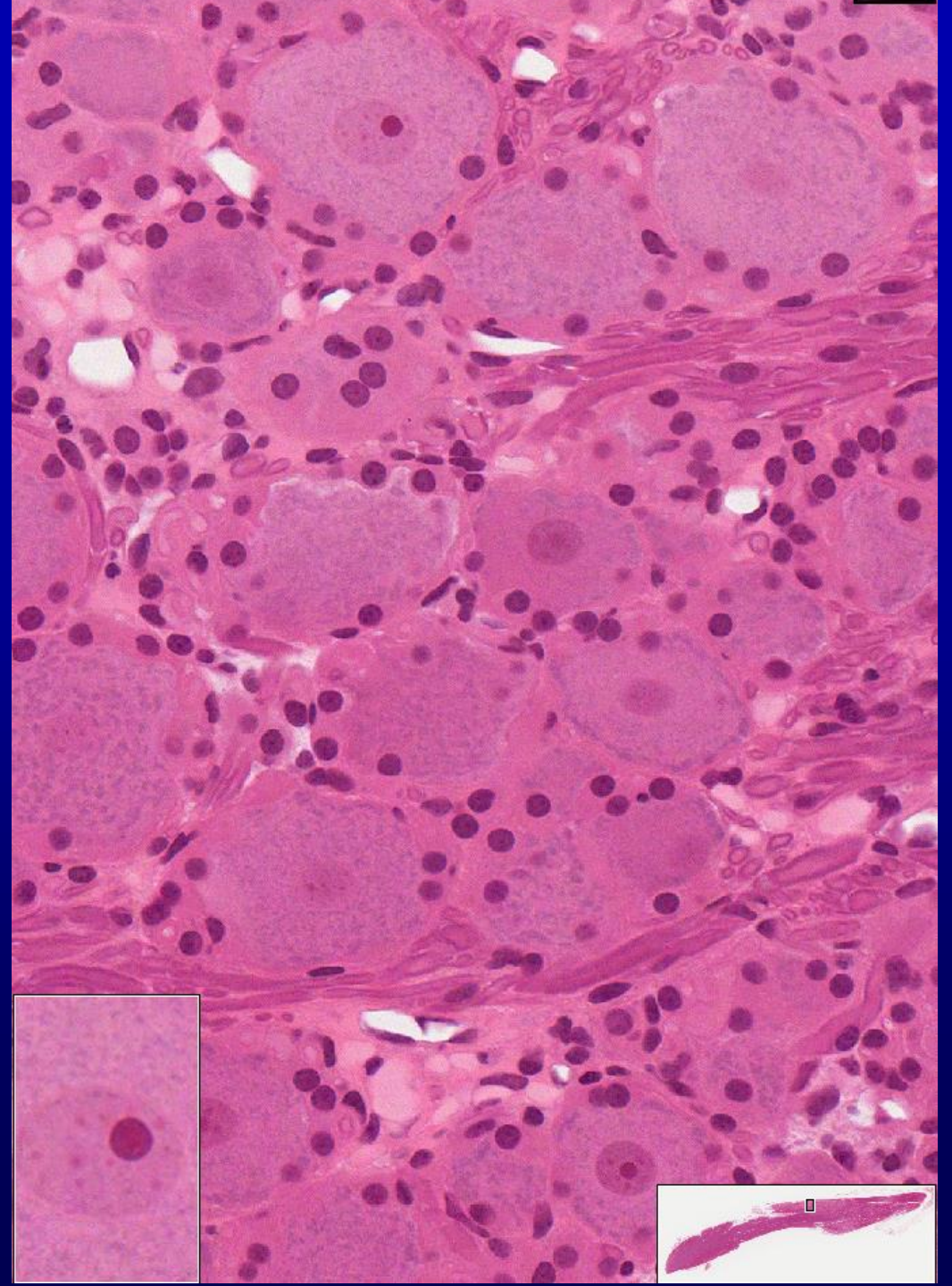




# Nervous Tissue

## Distribution:

- Comprise the **central nervous system**
- Individual **peripheral nerves** are found throughout the body
- Individual neurons and clusters of neurons (called ganglia) are found in most **organs**





# Gunther von Hagens' Body Worlds

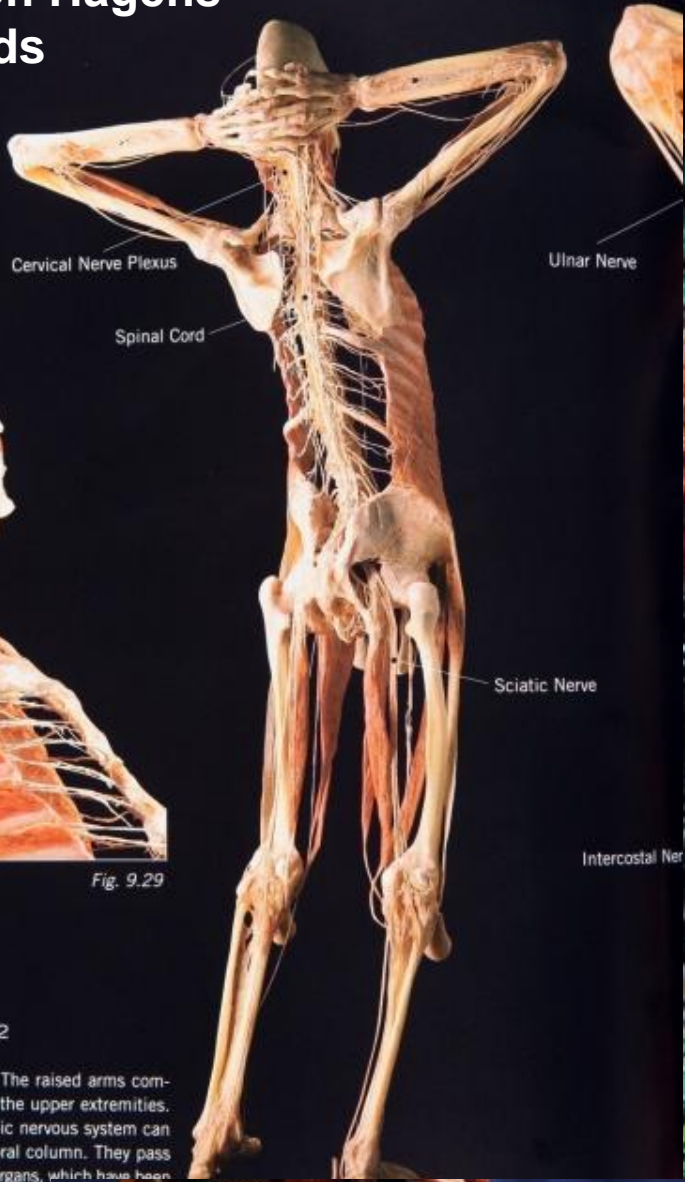
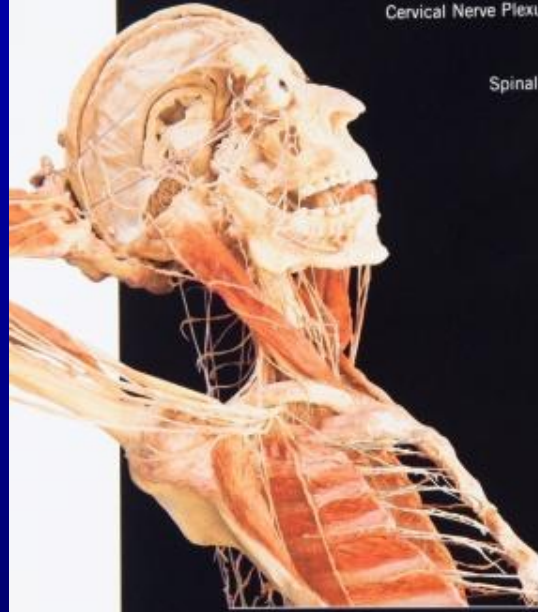


Fig. 9.29

Man at Leisure, 2002

All major nerves are shown. The raised arms completely expose the nerves of the upper extremities. Some nerves of the autonomic nervous system can be seen in front of the vertebral column. They pass their signals to the abdominal organs, which have been removed in this specimen.

Gunther von Hagens

**BODY WORLDS**  
The Anatomical Exhibition of Real Human Bodies



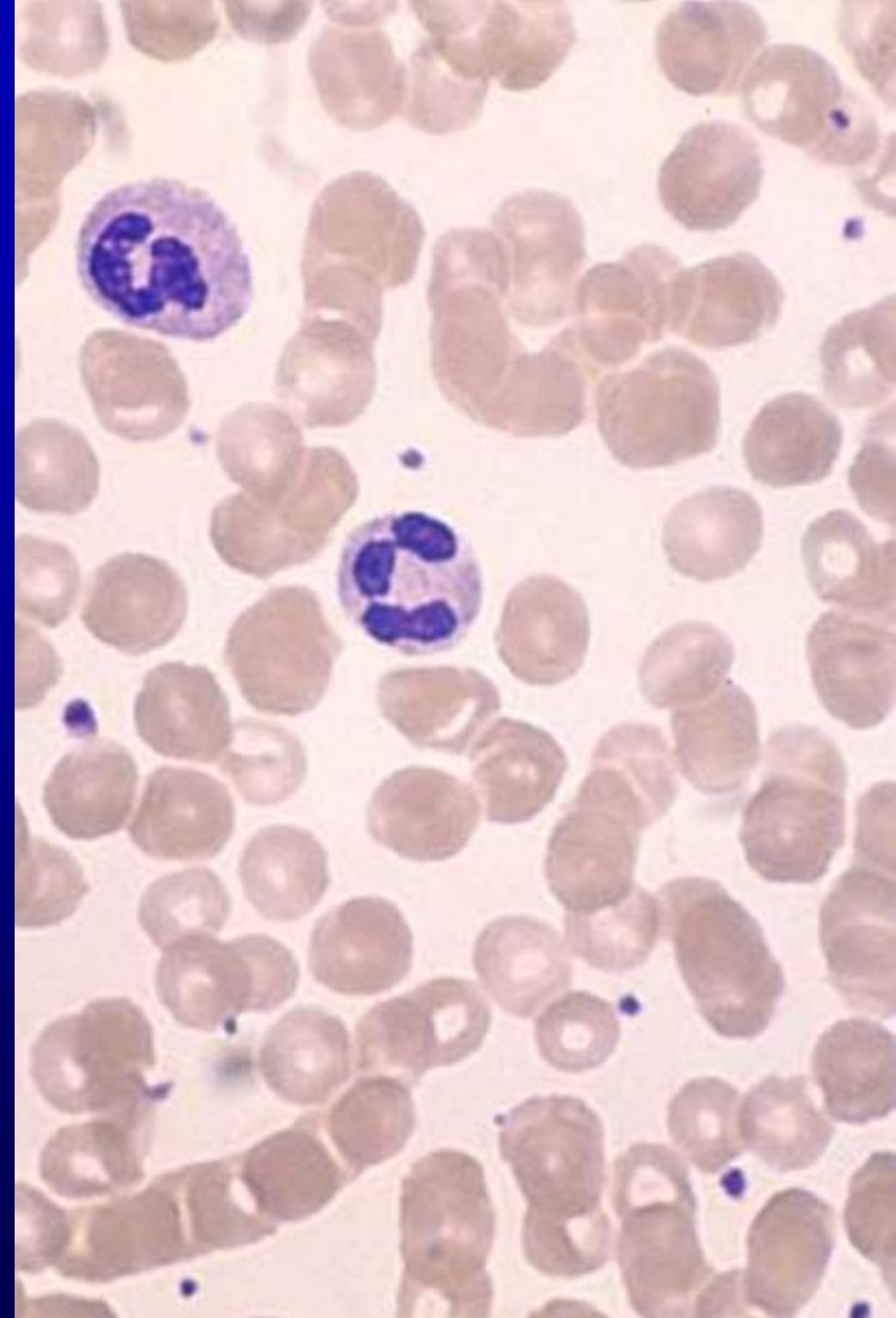


# Blood cells -functions

(classified as connective tissue)

## Functions:

- **Red cells** – carry oxygen to the tissues and CO<sub>2</sub> from tissues
- **White cells** – transient inhabitants of the blood which are manufactured in bone marrow and pass through the blood to connective tissue where they participate in defense against biological and chemical invaders
- **Platelets** – blood clotting



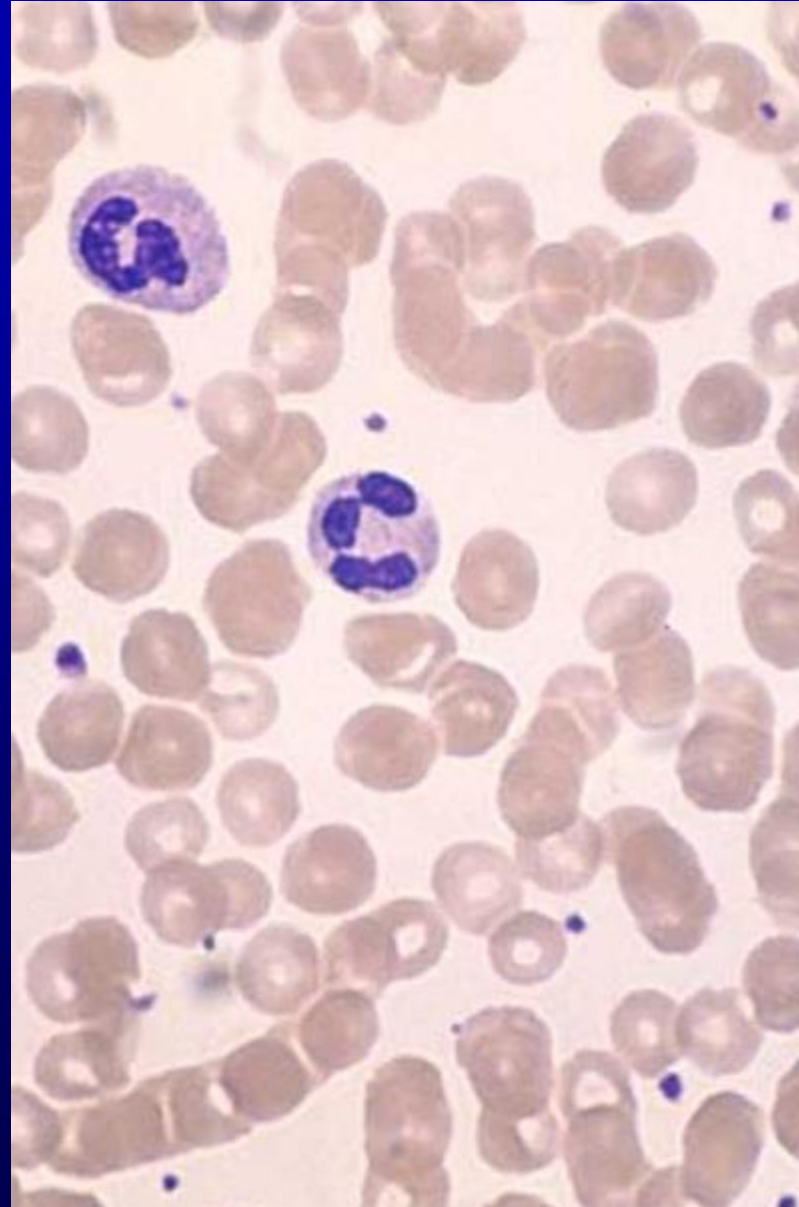


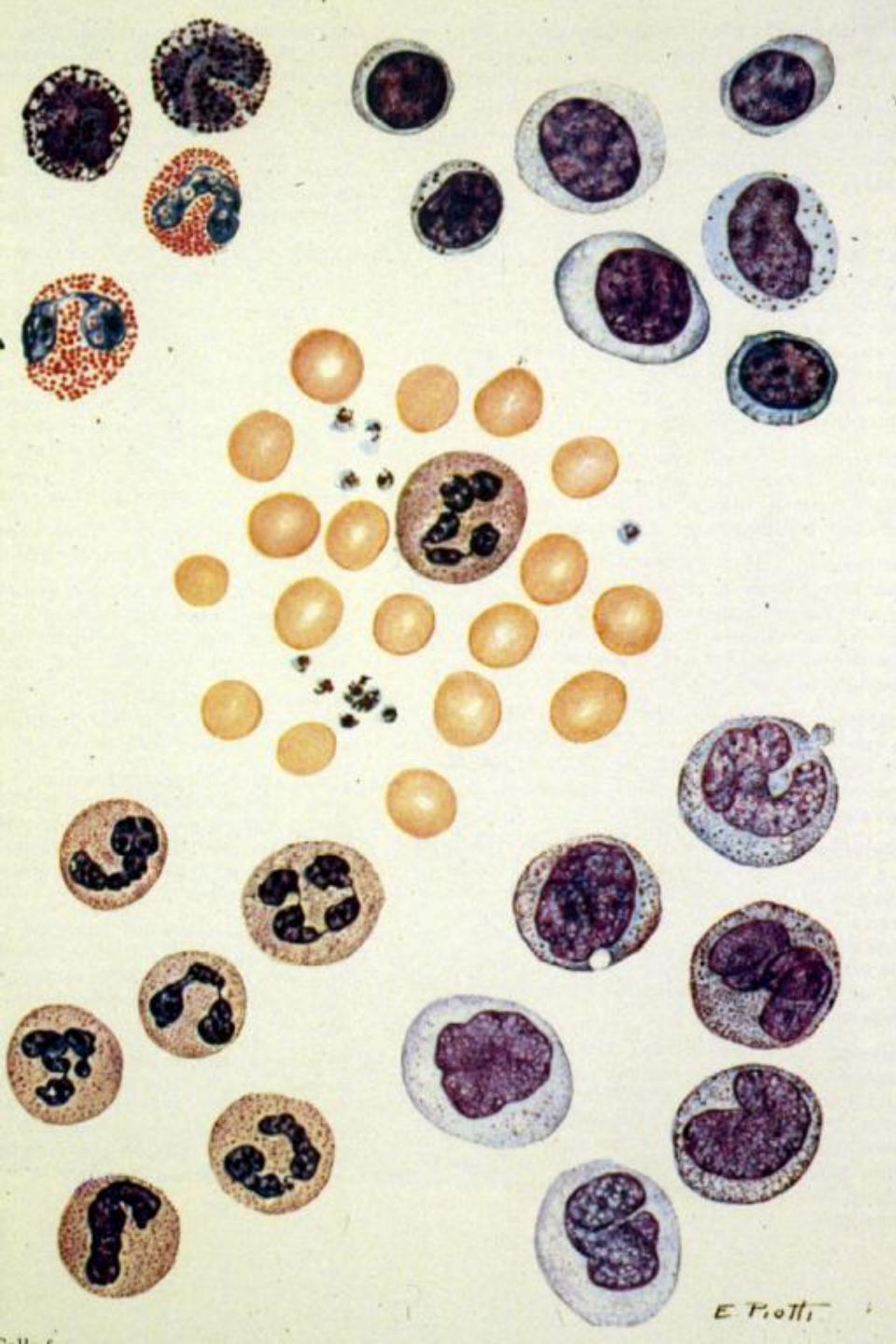
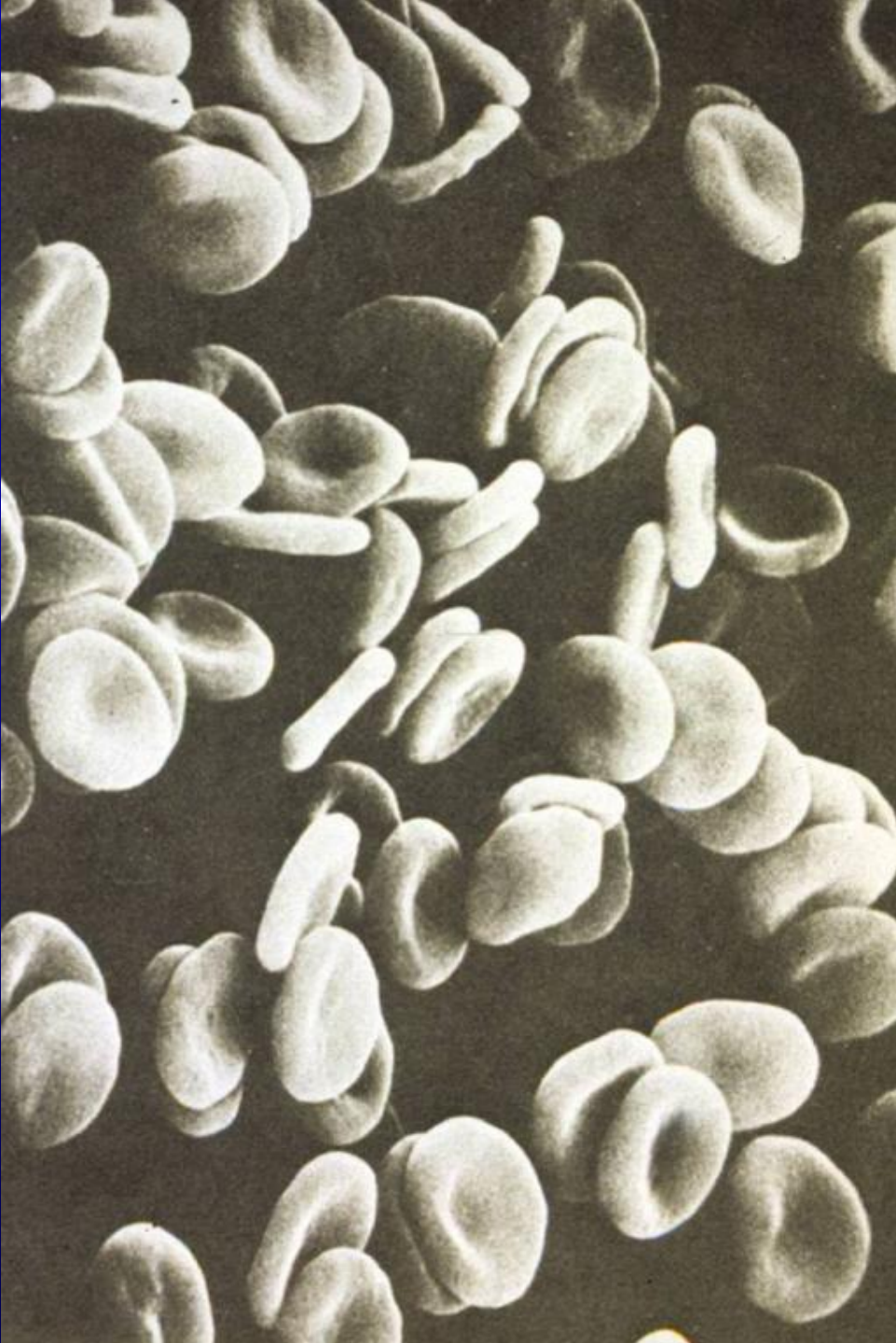
# Blood cells

(classified as connective tissue)

## Distinguishing features and histological identification:

- **red cells** – biconcave discs containing hemoglobin
- **white cells** – granulocytes have one of three different types of granules and lobed nuclei; lymphocytes and monocytes have few granules and round or indented nuclei
- **platelets** – anucleate cell fragments produced by megakaryocytes in the bone marrow



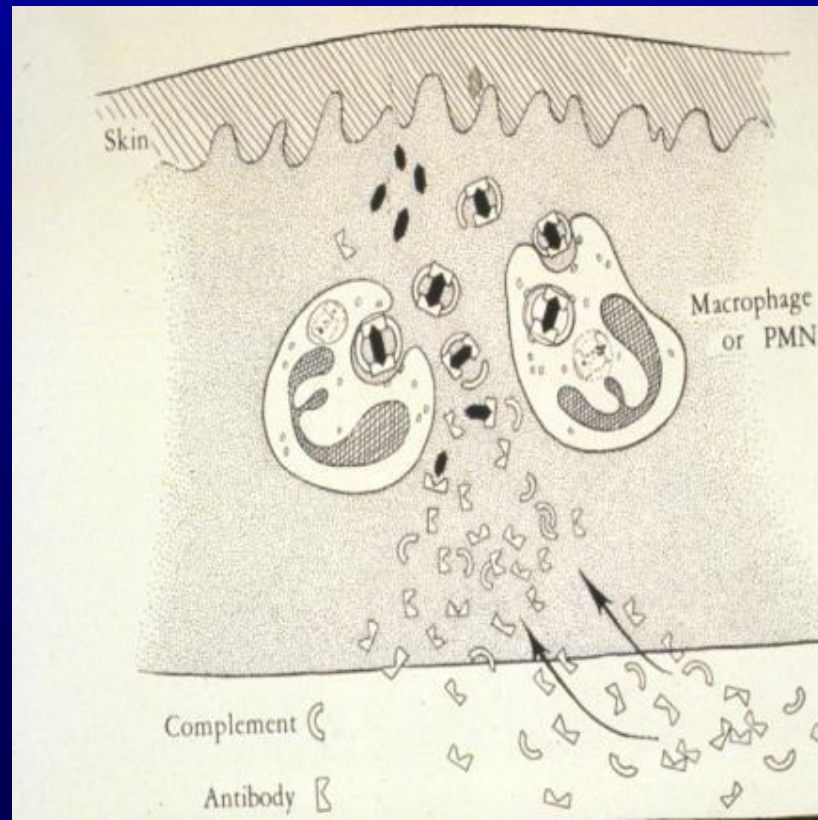




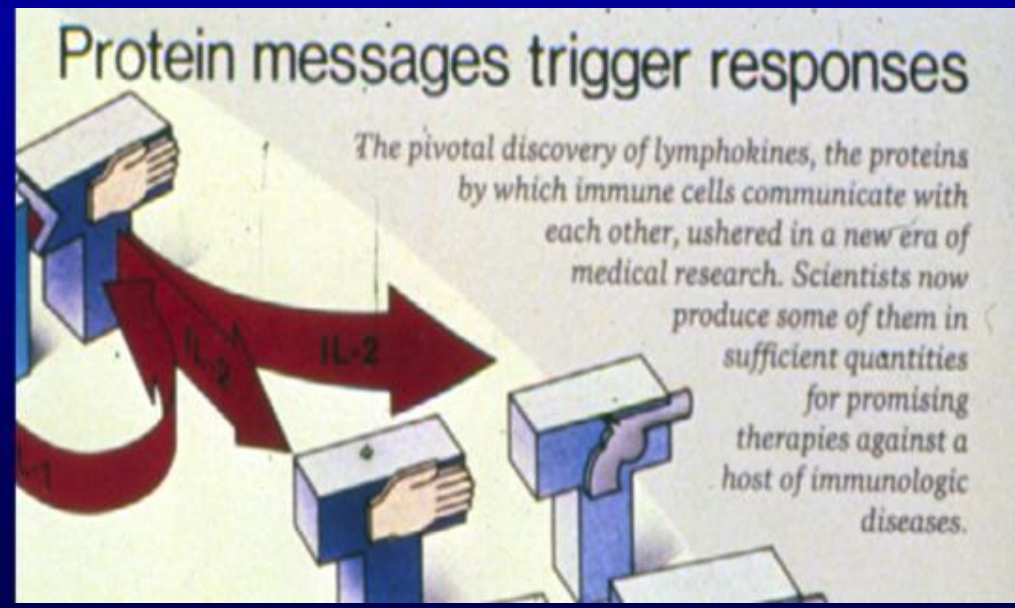
# LYMPHOID TISSUE

## Functions:

responsible for the “immune response” to foreign invaders which is mediated by either antibody produced by the cells or by the cells themselves.



Amplification of the immune response: factors (lymphokines) and other cell types involved

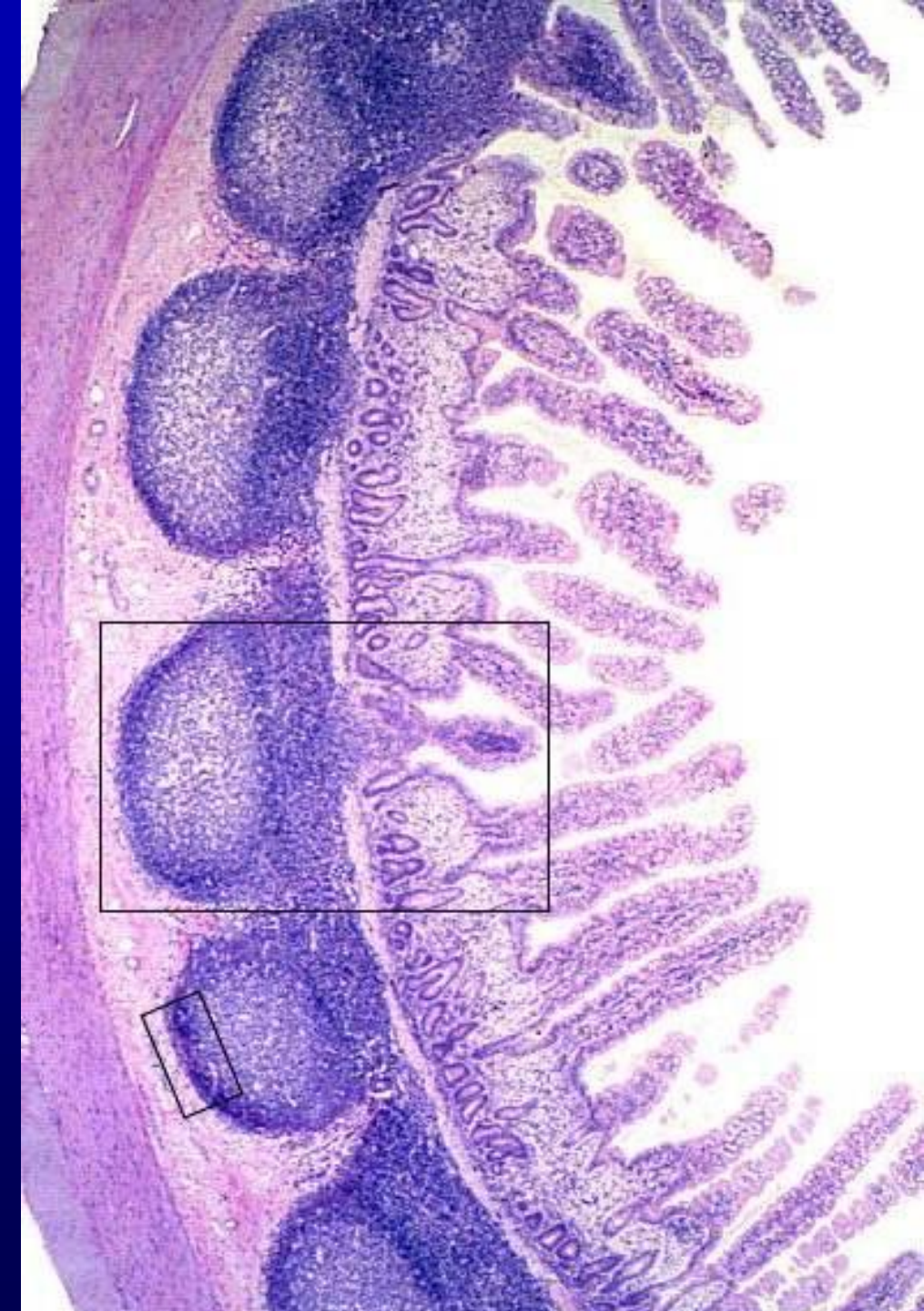


# Lymphoid Tissue

Distinguishing features and histological identification: clusters of **lymphocytes free** in the connective tissue or surrounded by a connective tissue capsule as in a **lymph node**

## Distribution:

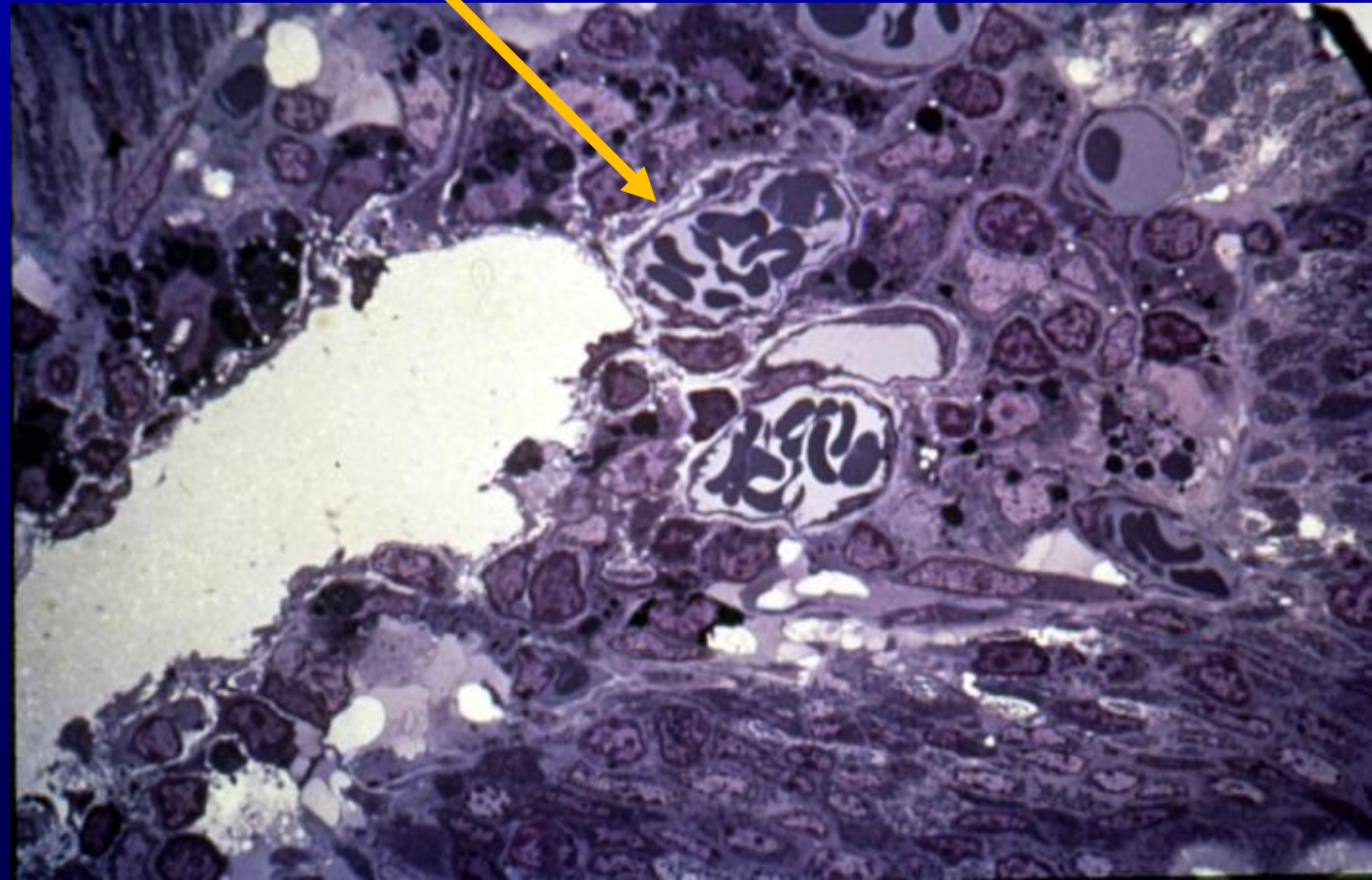
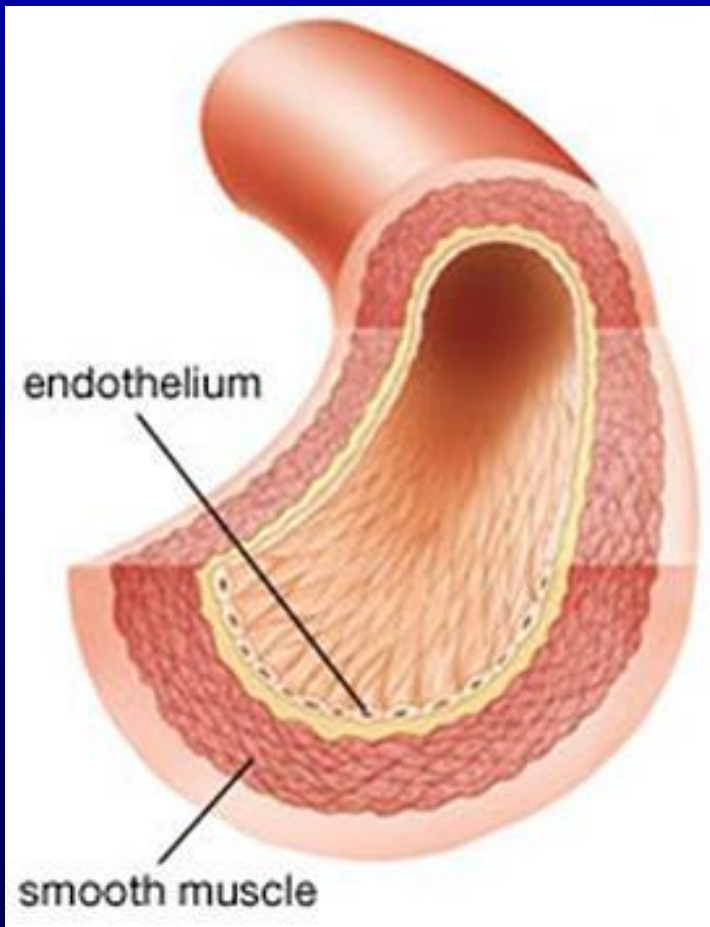
in primary **lymphoid organs** (spleen, thymus, lymph nodes); along alimentary canal and respiratory passages.





# Blood Vessels

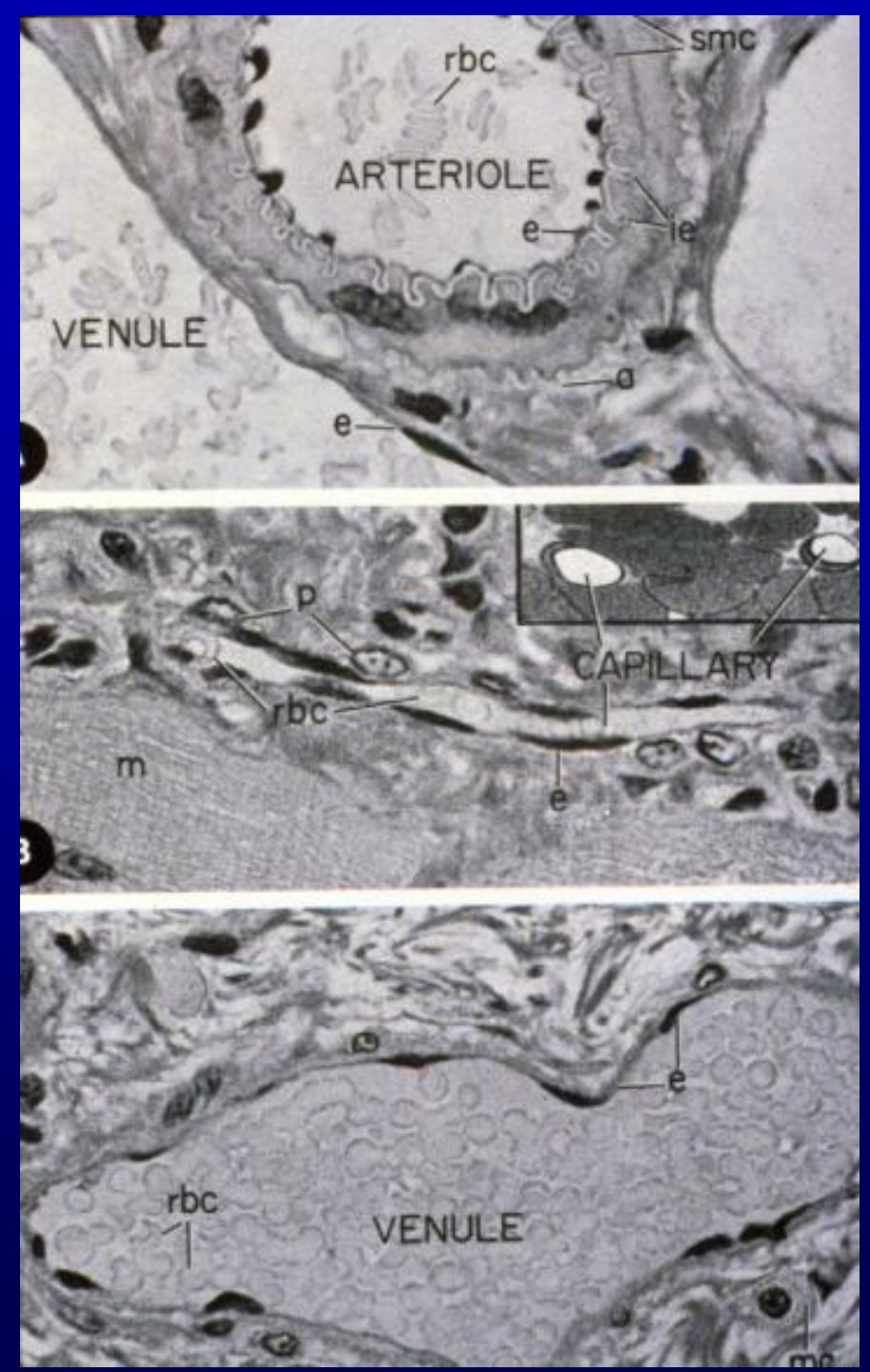
Not one of the four basic tissues, but necessary to learn at this stage; All blood and lymph vessels are lined with endothelium (a simple squamous epithelium)



# Blood Vessels

## Histological identification:

- **Artery** – thick wall composed of smooth muscle plus some connective tissue surrounding a small lumen
- **Capillary** – narrow tube lined with a single endothelial cell
- **Vein** – large lumen relative to thickness of connective tissue and smooth muscle wall
- **Lymphatic** – small thin walled vessels which carry lymph





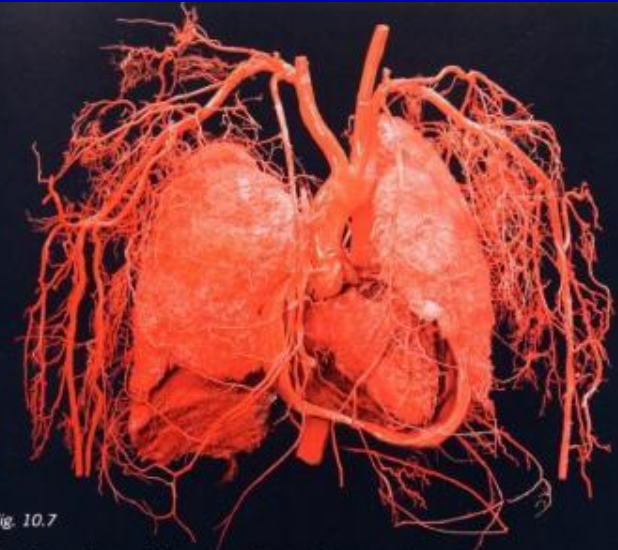
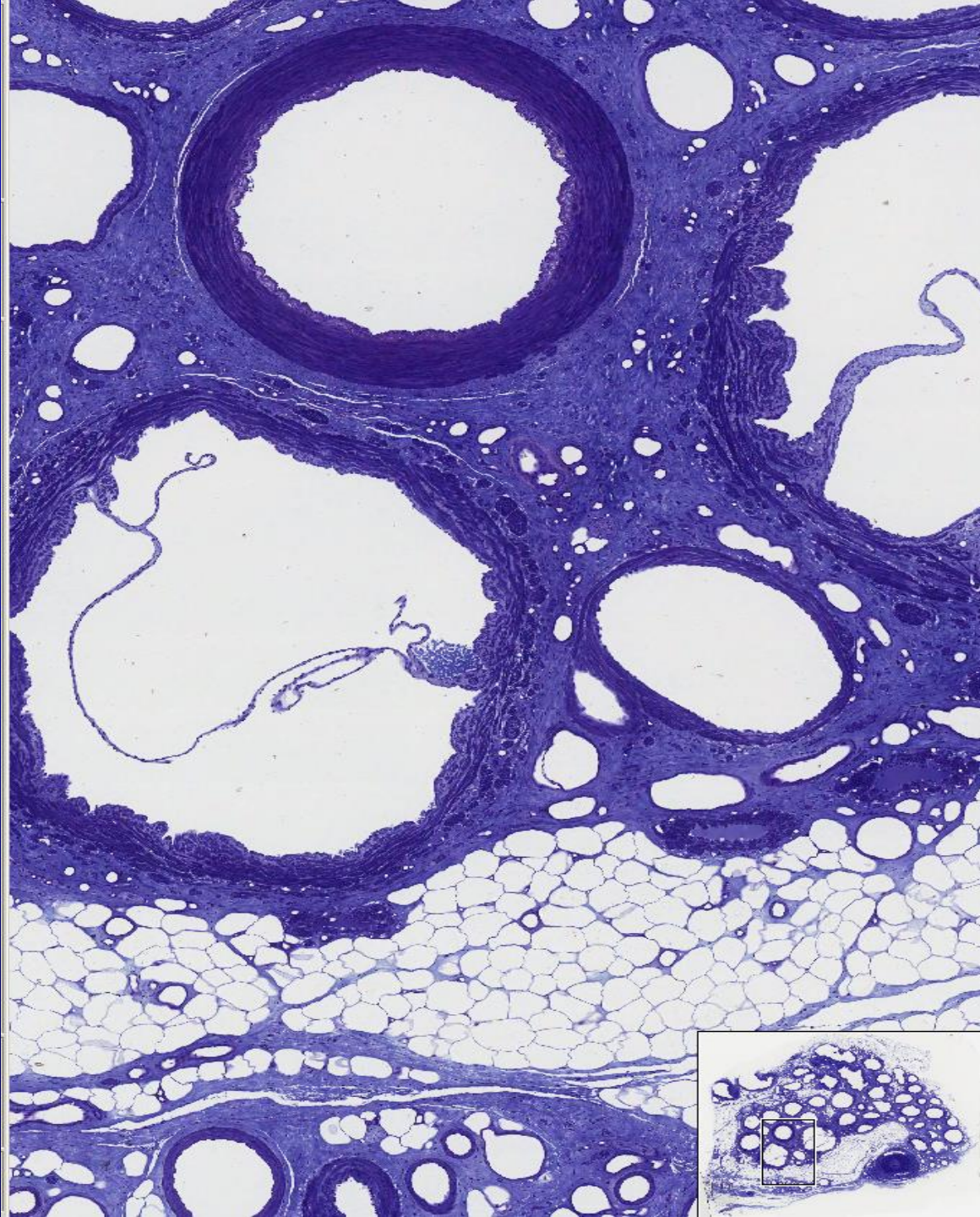


Fig. 10.7

Configuration of Arteries of the Thoracic Organs

In the area of the aortic arch, the arteries diverge to supply blood to the head, the upper extremities and the upper half of the torso.

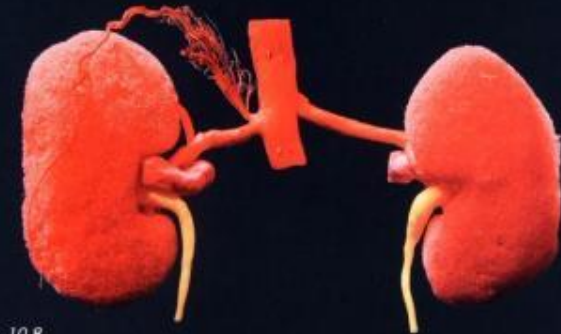


Fig. 10.8

Configuration of the Renal Arteries

Shown are the renal arteries (view from the rear) and the ureters that drain urine from the kidneys (yellow). The renal arteries flow into extremely fine capillary tufts or glomeruli (Malpighian or renal corpuscles) in the kidney tissue, which then filter the incoming blood.

Gunther von Hagens'

**BODY WORLDS**

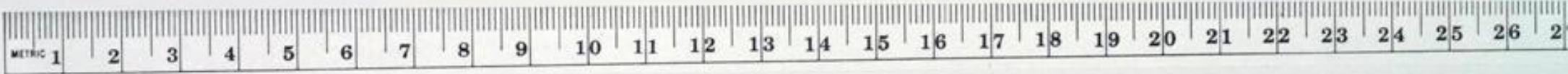
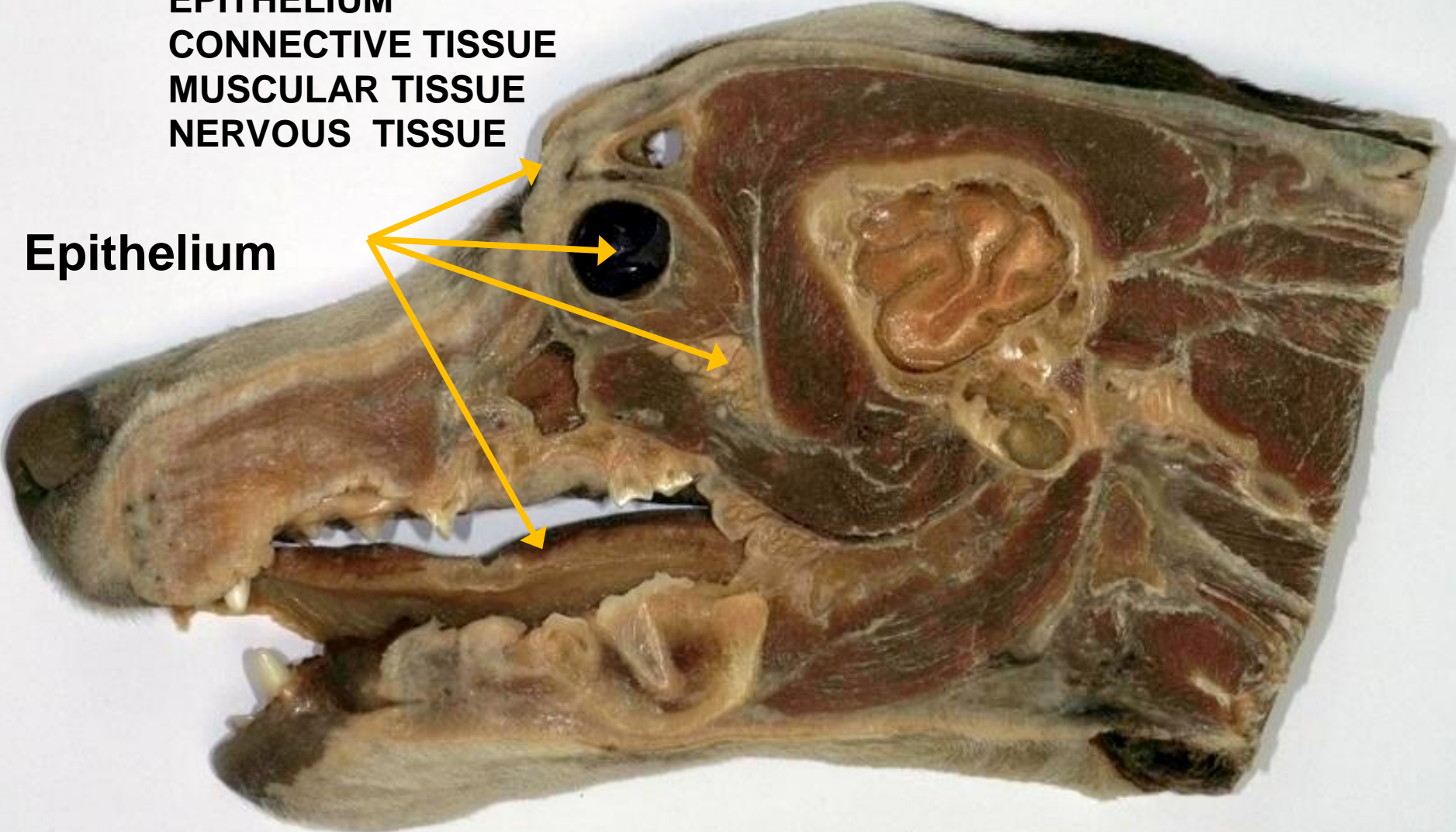
The Anatomical Exhibition of Real Human Bodies



**Where are these basic tissues located?**

- EPITHELIUM**
- CONNECTIVE TISSUE**
- MUSCULAR TISSUE**
- NERVOUS TISSUE**

**Epithelium**

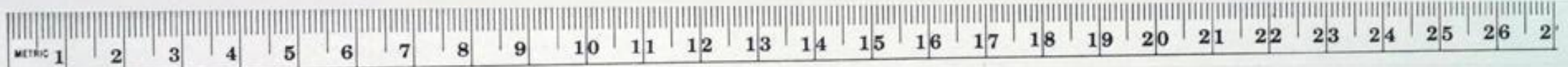
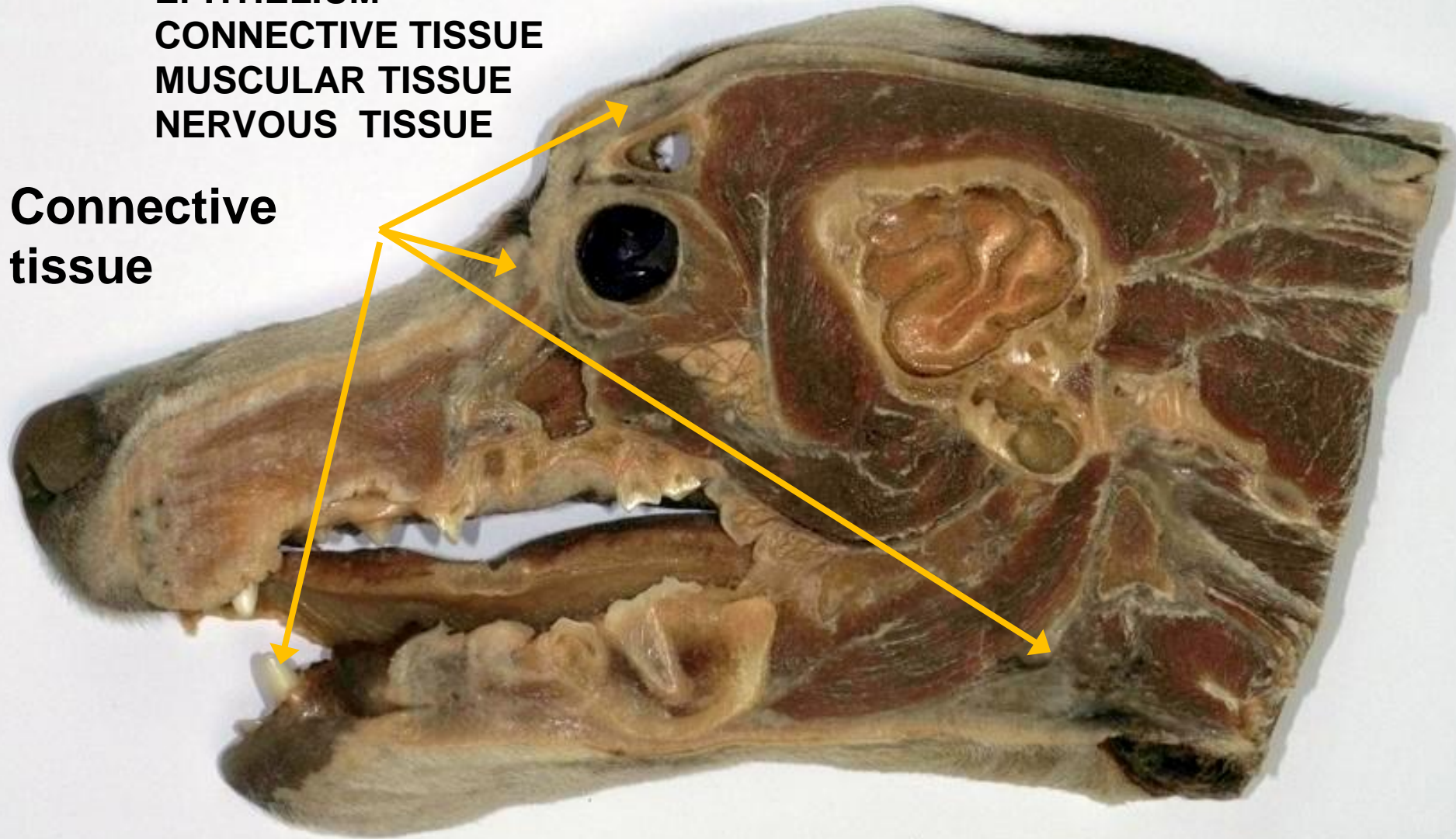




# Where are these basic tissues located?

- EPITHELIUM
- CONNECTIVE TISSUE
- MUSCULAR TISSUE
- NERVOUS TISSUE

**Connective  
tissue**

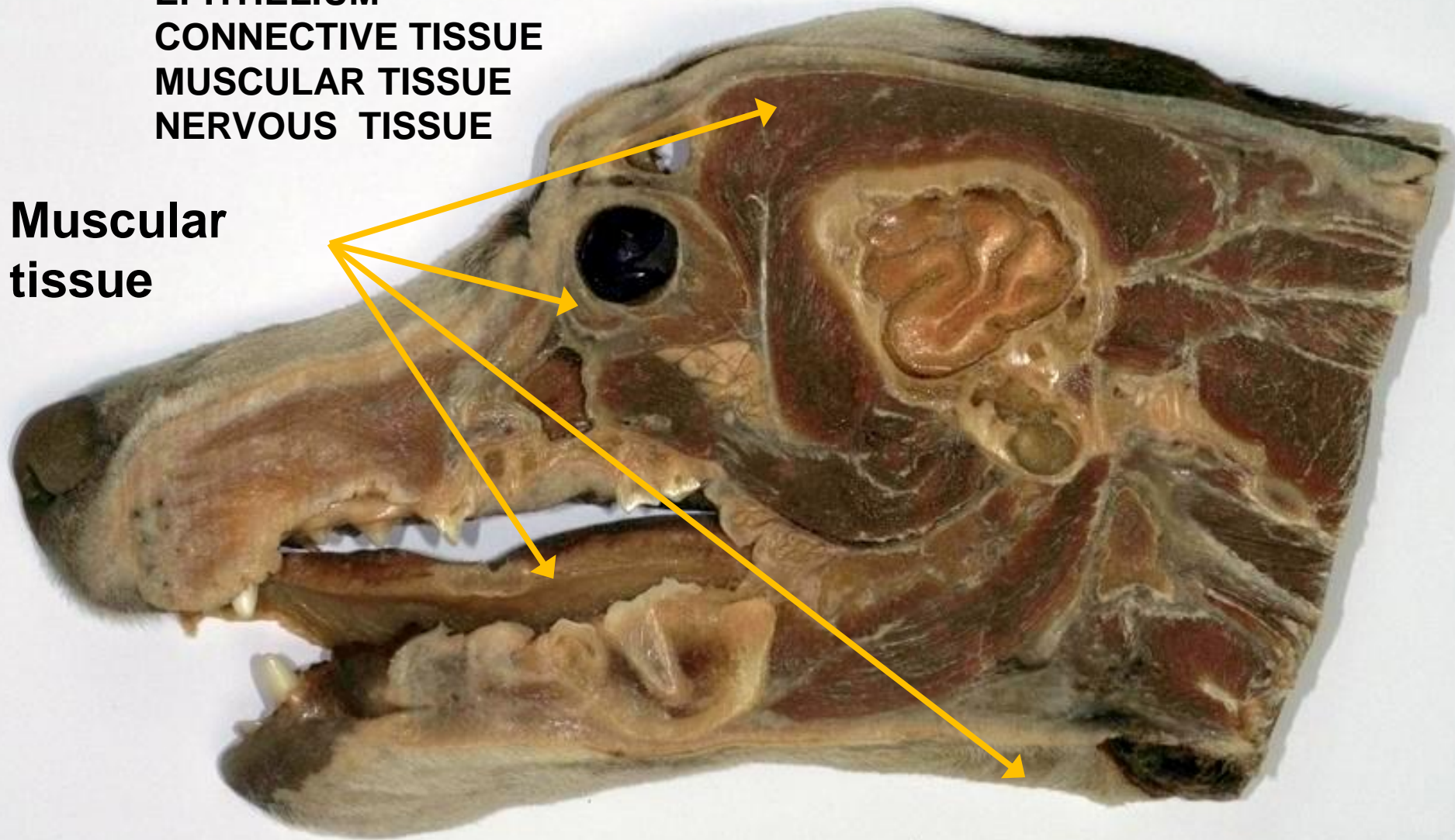




# Where are these basic tissues located?

- EPITHELIUM
- CONNECTIVE TISSUE
- MUSCULAR TISSUE
- NERVOUS TISSUE

**Muscular tissue**

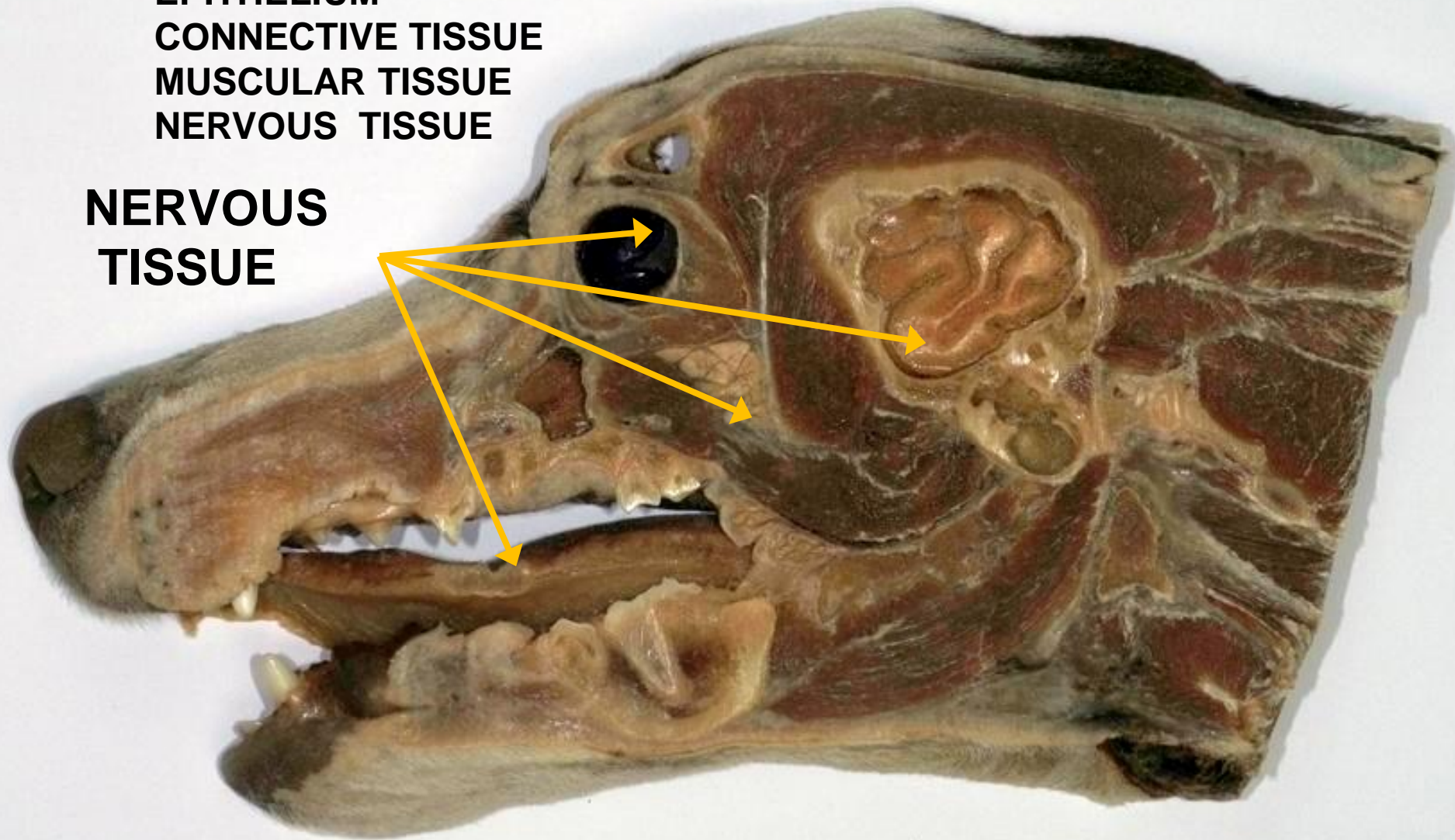


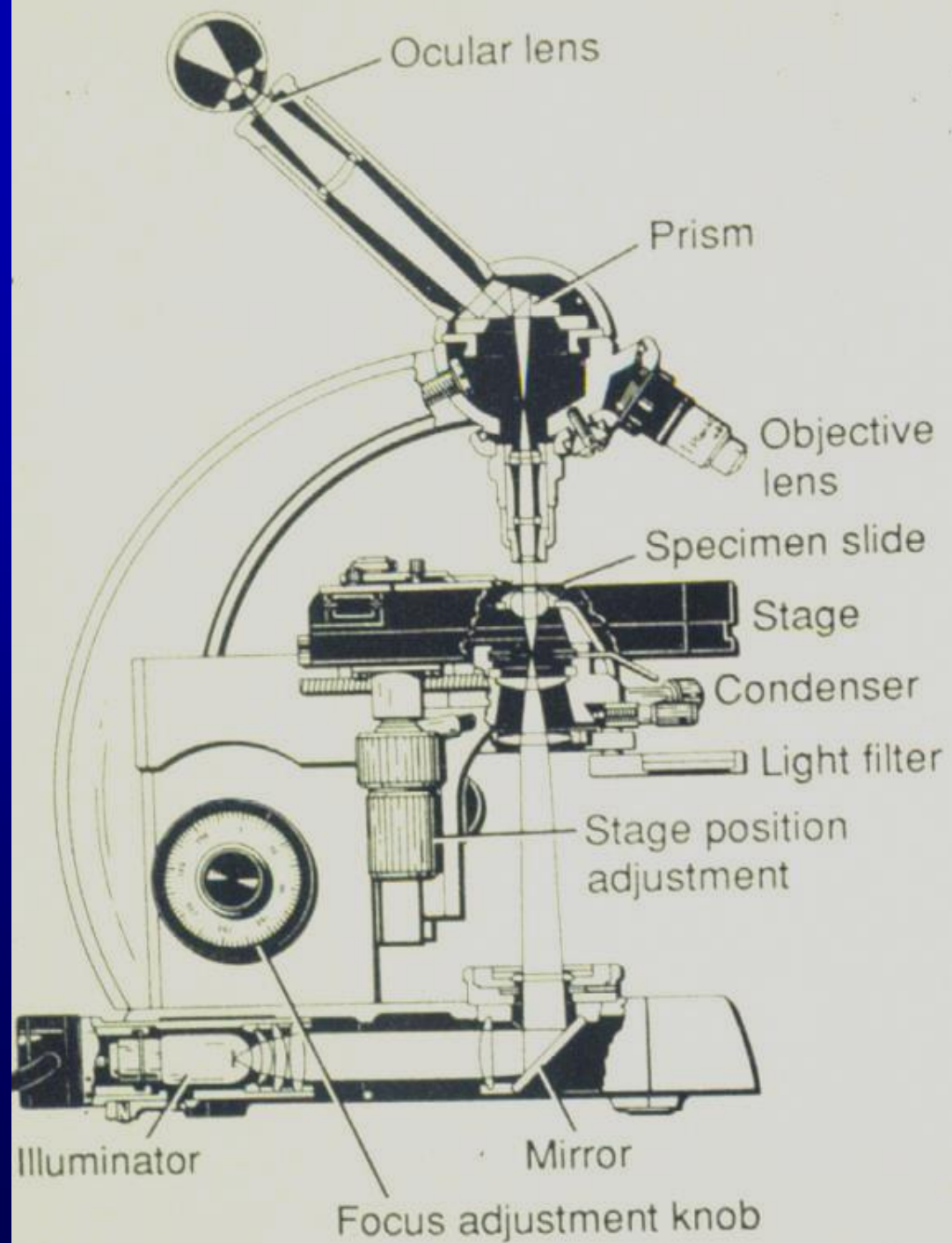


# Where are these basic tissues located?

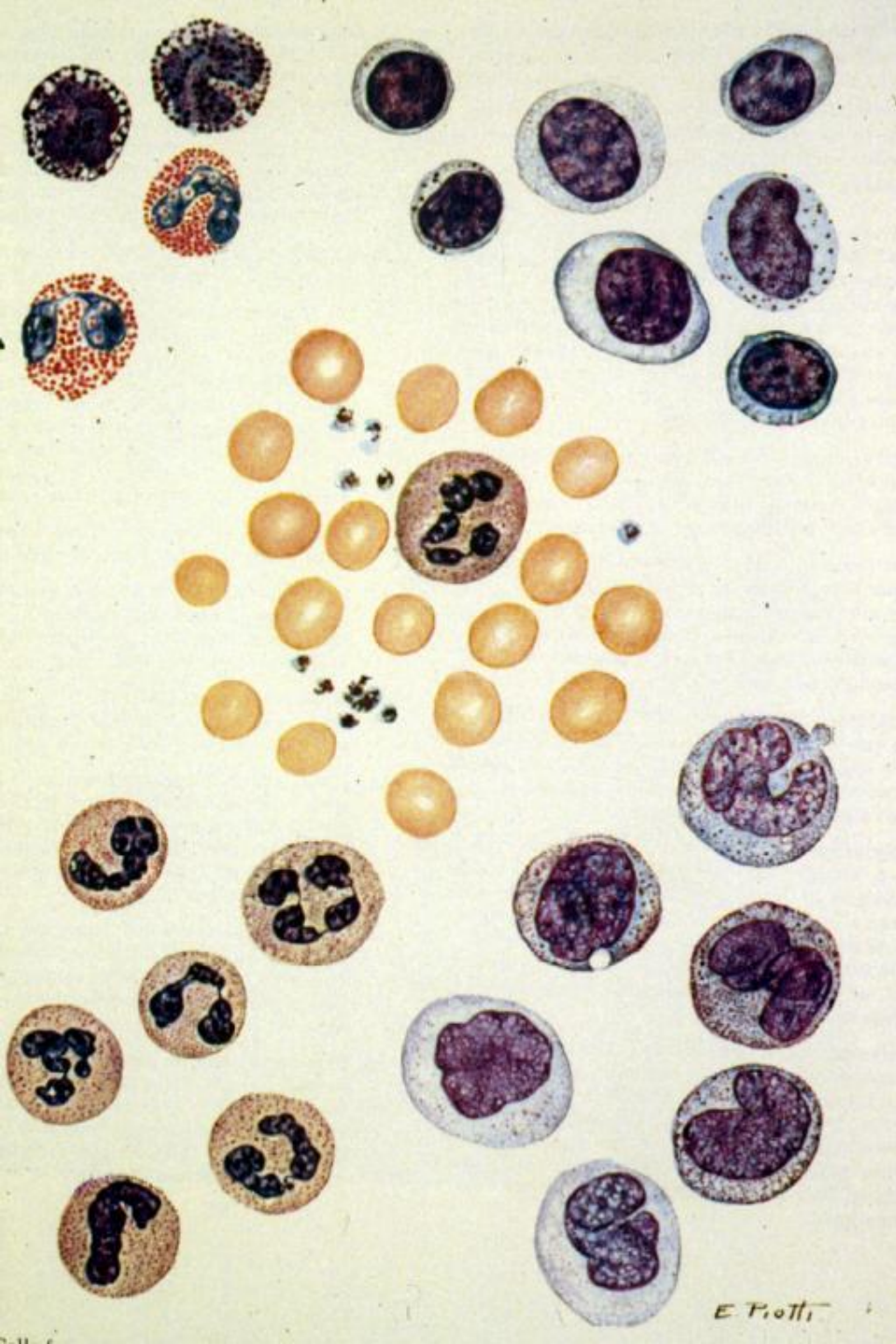
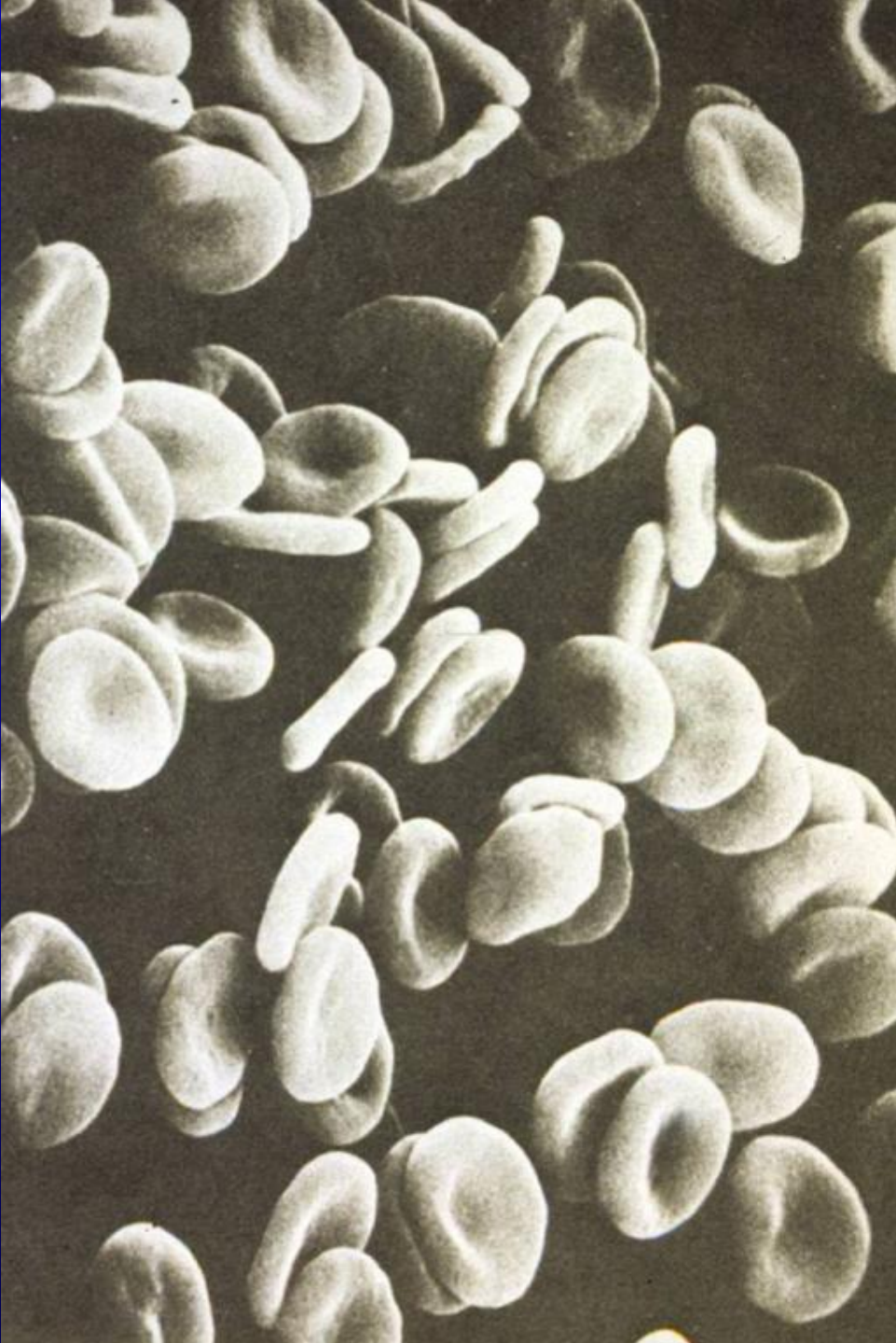
- EPITHELIUM
- CONNECTIVE TISSUE
- MUSCULAR TISSUE
- NERVOUS TISSUE

**NERVOUS  
TISSUE**











# Magnification vs. Resolution

1. **Magnification** - increase in image size
2. **Resolution** - smallest distance between two points that can be seen (distinguished)





# Magnification vs. Resolution

1. magnification - increase in image size
2. resolution - smallest distance between two points that can be seen (distinguished)

Calculated by:

$$0.61 \text{ (wavelength)} / \text{numerical aperture}$$

0.25  $\mu\text{m}$  for light microscope

0.1 nm for electron microscope

# Sample Preparation

1. Fixation
2. Embedding
  - A. Paraffin
  - B. Plastic



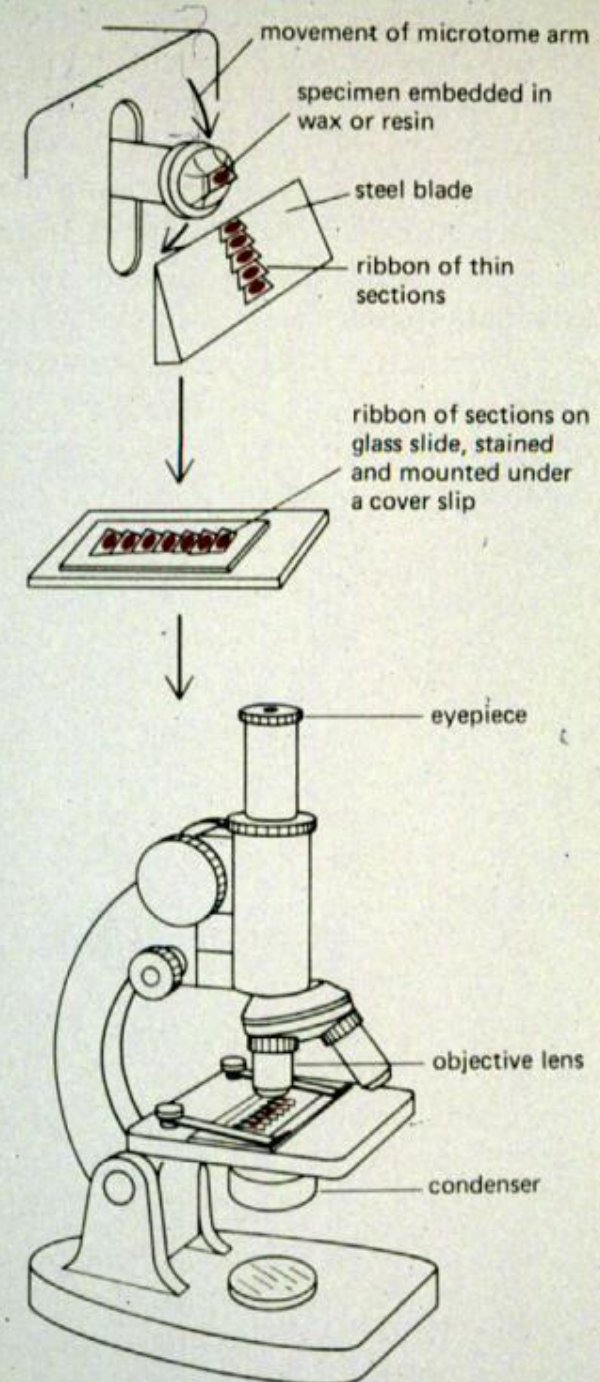
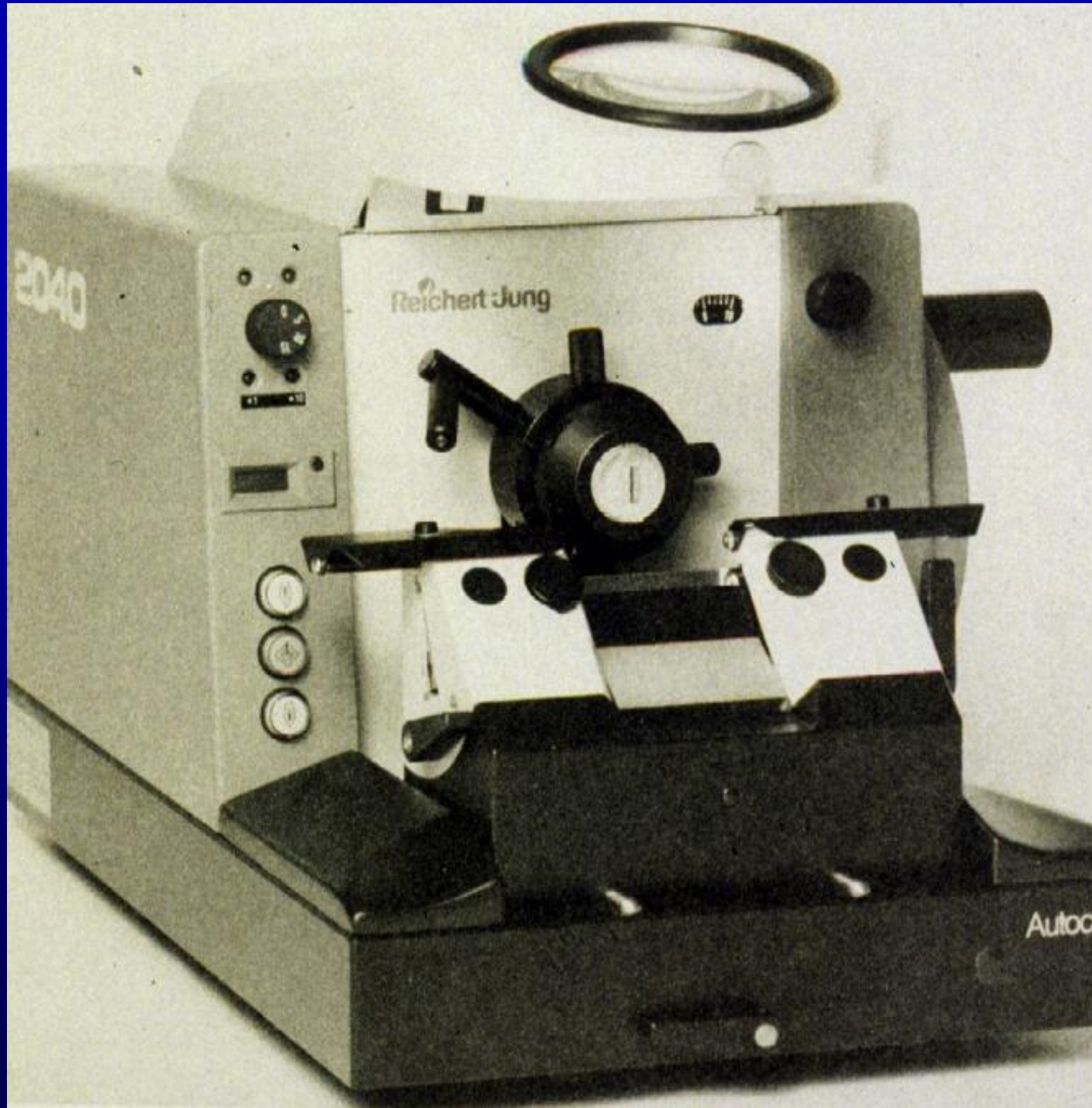


# Sample Preparation

1. Fixation
2. Embedding
  - A. Paraffin
  - B. Plastic
3. Sectioning
  - A. 0.5  $\mu\text{m}$  for Light Microscopy
  - B. 60-80 nm for Electron Microscopy

**Table 1-2.** Typical sequence of procedures in preparing tissues for observation under the light microscope. Following embedding in paraffin blocks, the tissues can be sectioned with a microtome (Fig 1-1).

Stage	Purpose	Duration
1. Fixation in simple or compound fixatives (Bouin's fluid, Zenker's formalin)	To preserve tissue morphology and molecular composition	About 12 h, according to the fixative and the size of the piece of tissue
2. Dehydration in graded concentrated ethyl alcohol (70% up to 100% alcohol)	To replace tissue water with organic solvents	6-24 h
3. Clearing in benzene, xylene, or toluene	To impregnate the tissues with a paraffin or a plastic resin solvent	1-6 h
4. Embedding in melted paraffin at 60 °C or plastic resin at room temperature	Paraffin or resin penetrates all intercellular spaces and even into the cells, making the tissues more resistant to sectioning	1-3 h



EXAMINATION WITH LIGHT MICROSCOPE



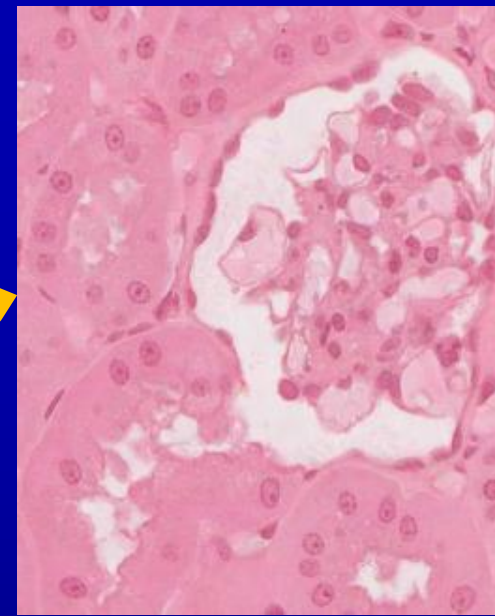
# Sample Preparation



# Staining

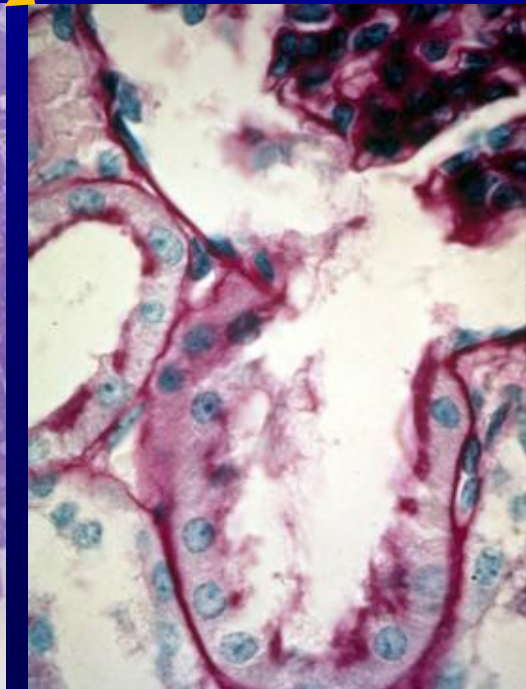
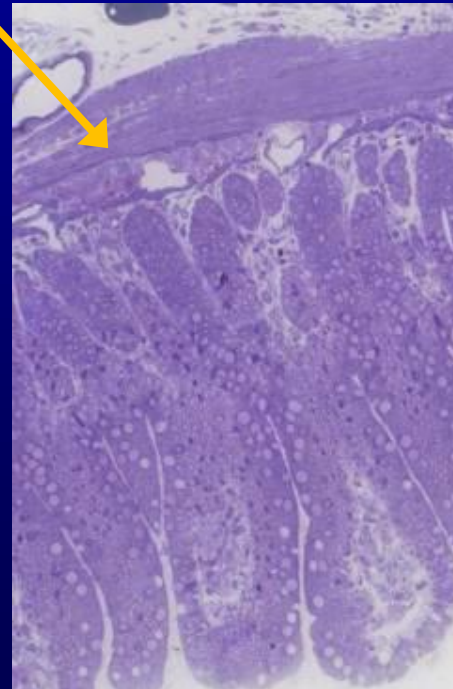
## 1. Light Microscopy

- A. Hematoxylin and Eosin (H&E)
- B. Periodic Acid/Shiff (PAS)
- C. Toluidine Blue



## 2. Electron Microscopy (TEM)

- A. Osmium
- B. Lead Citrate



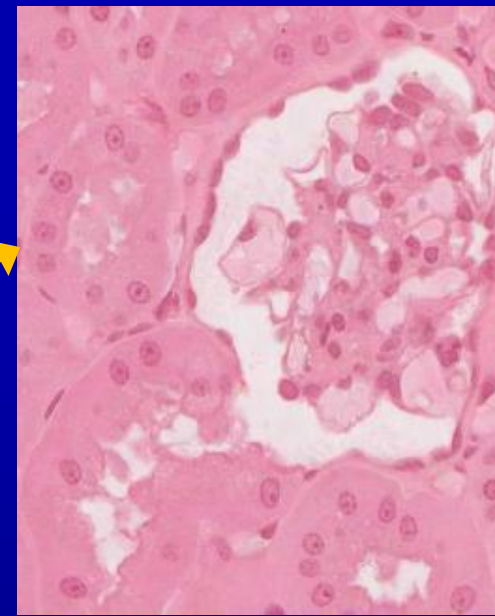


# Staining

## 1. Light Microscopy

### A. Hematoxylin and eosin (H&E)

### B. Periodic Acid/Shiff (PAS)



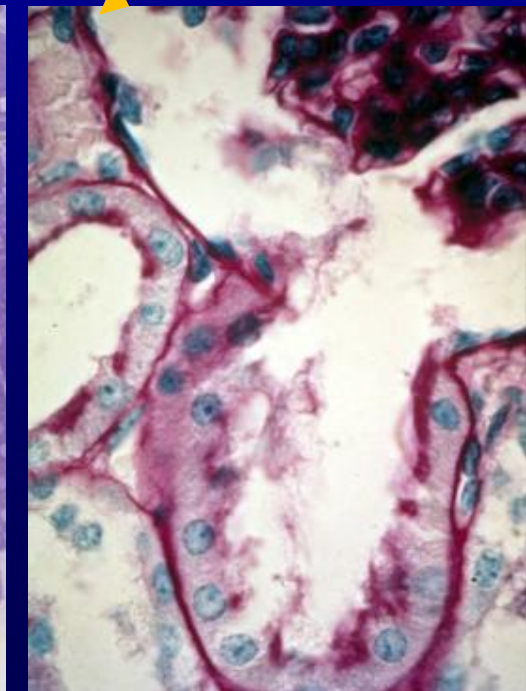
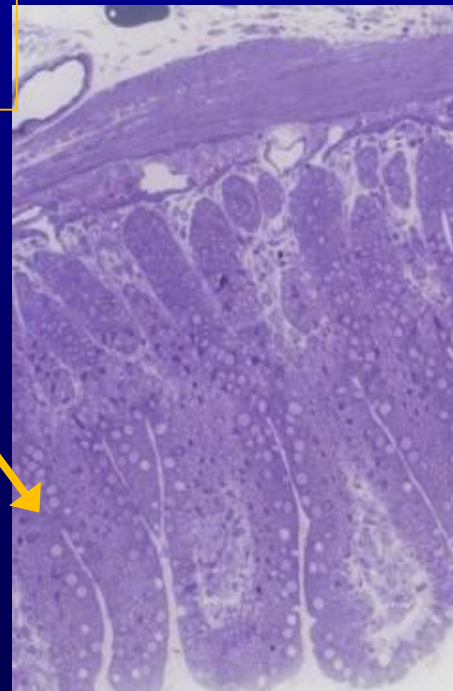
Color provides clues

### C. Toluidine Blue

Shape

Size

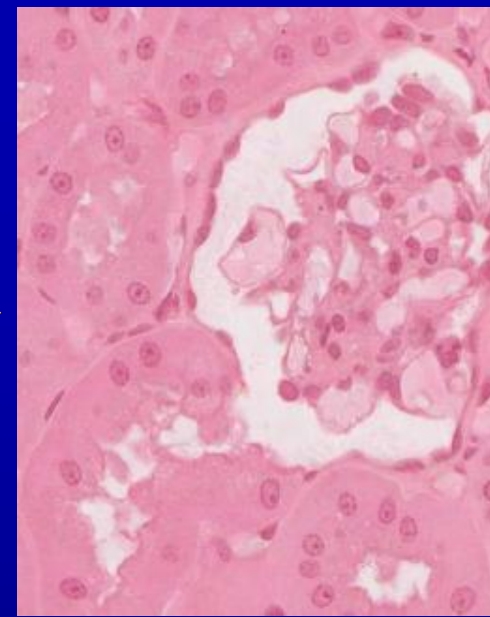
Intensity of staining



# Staining

## 1. Light Microscopy

- A. Hematoxylin and Eosin (H&E)
- B. Periodic Acid/Shiff (PAS)
- C. Toluidine Blue



## 2. Electron Microscopy (TEM)

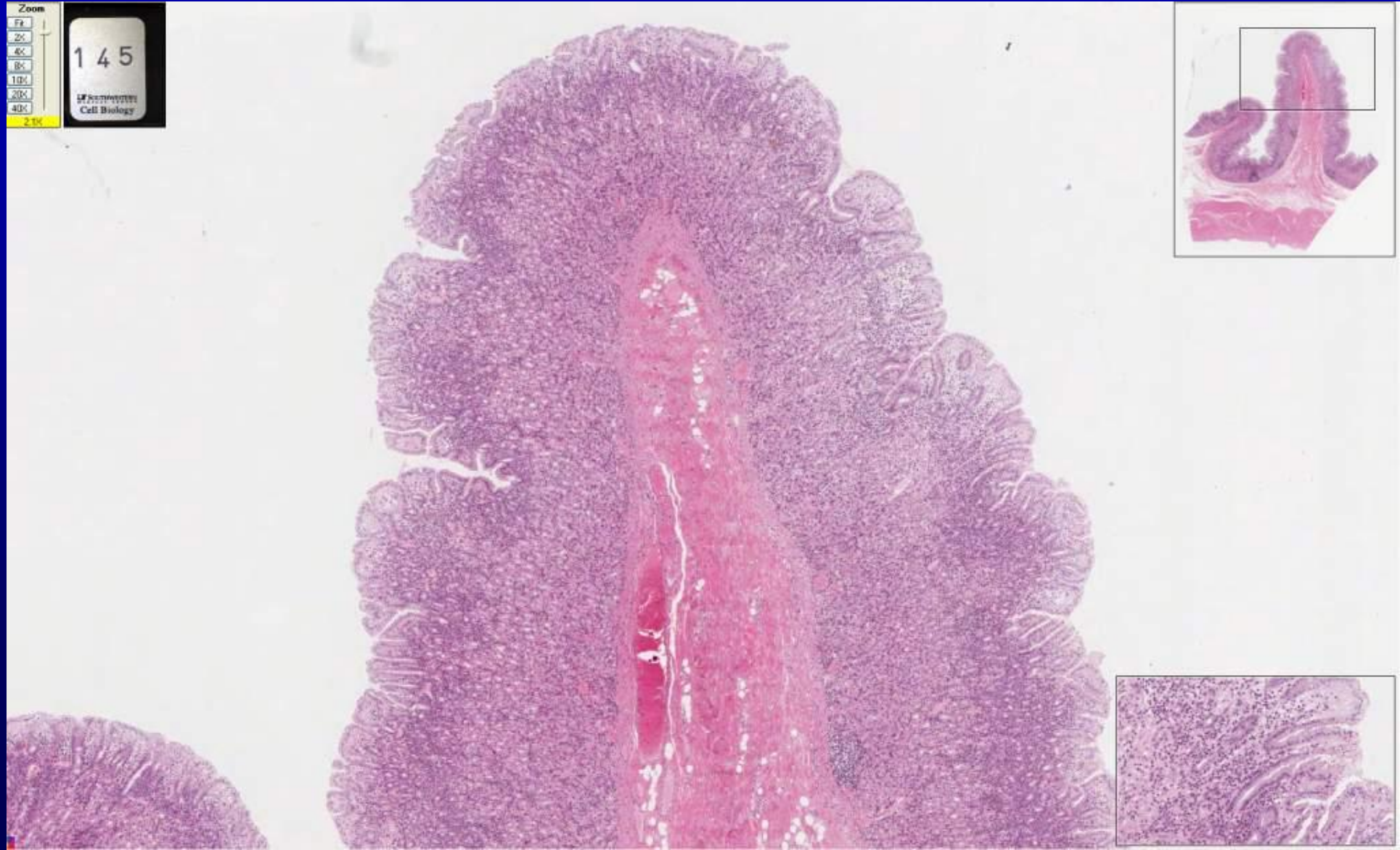
- A. Osmium
- B. Lead Citrate





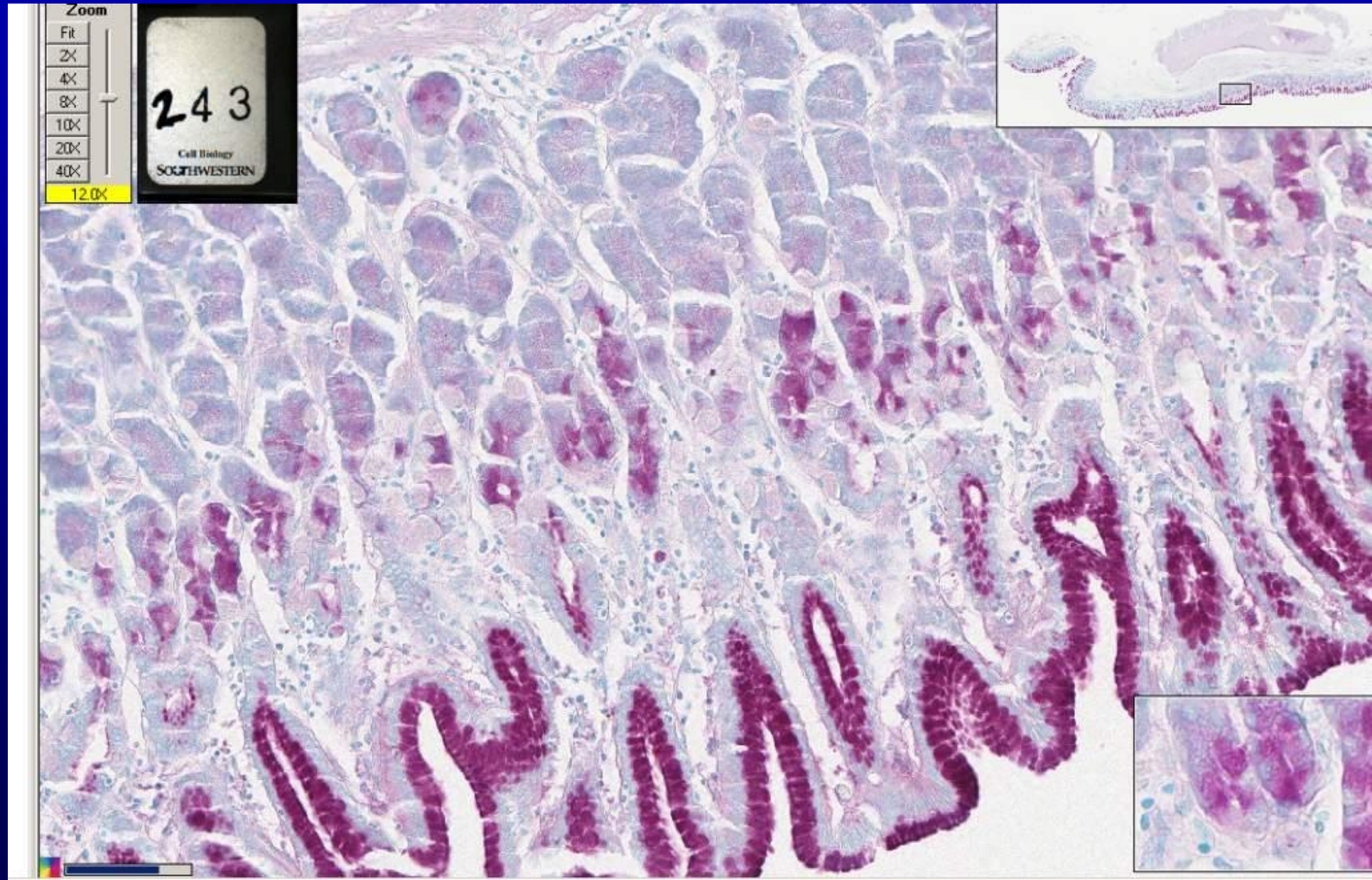
145

# Fundic stomach (H&E)



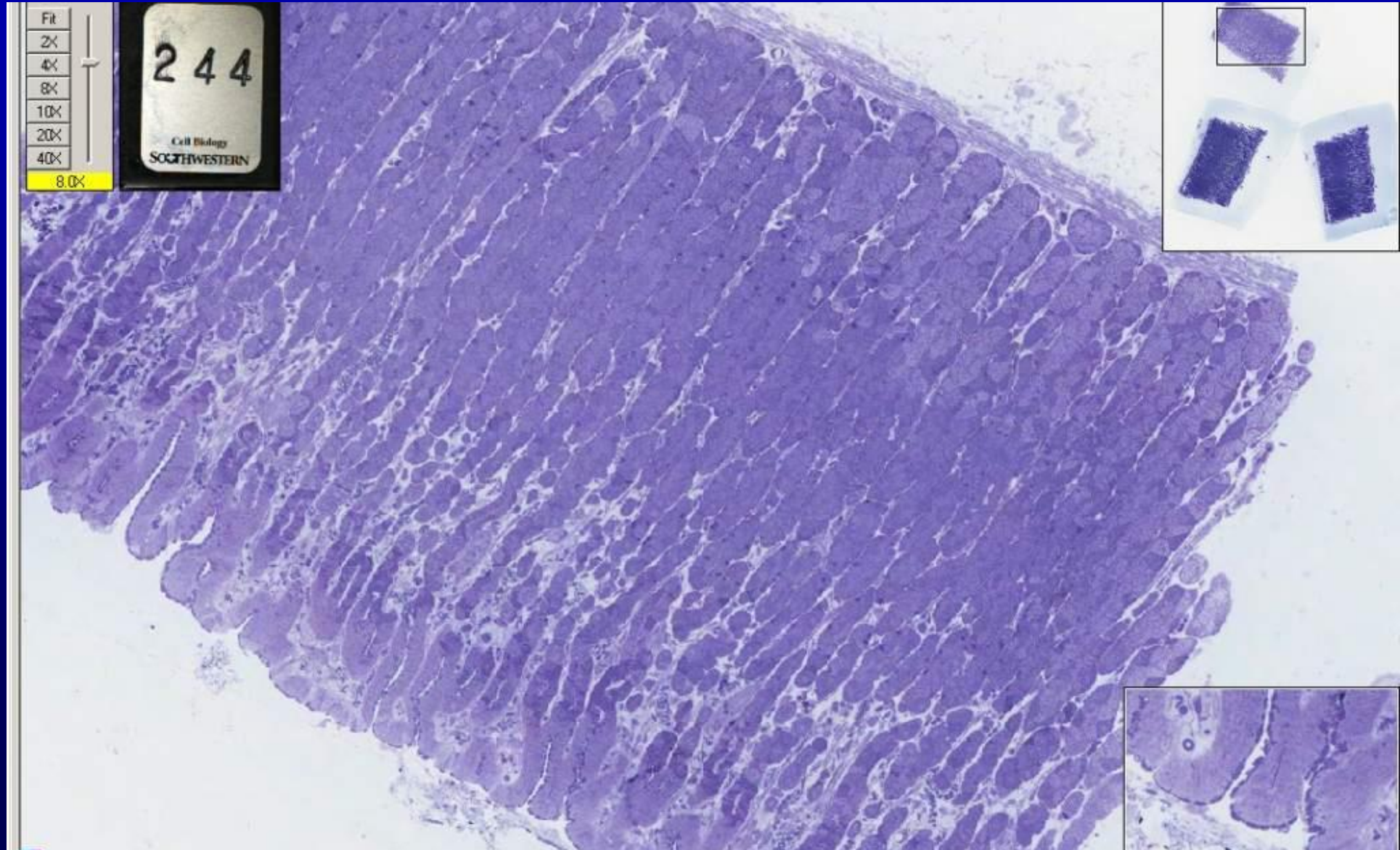


# 243 Fundic stomach, monkey (PAS)





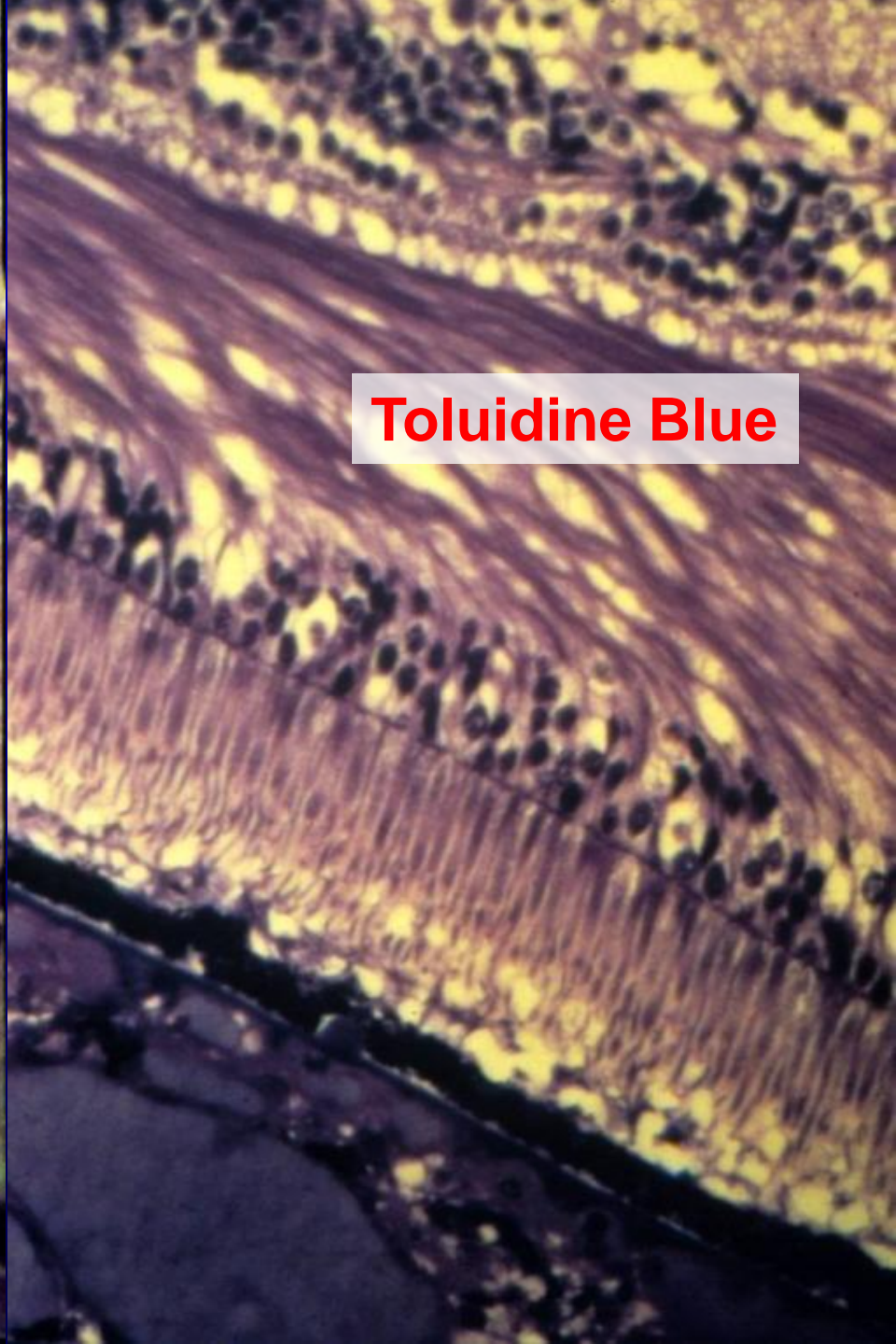
# 244 Surface mucus cells of Fundic stomach, rabbit (toluidine blue)





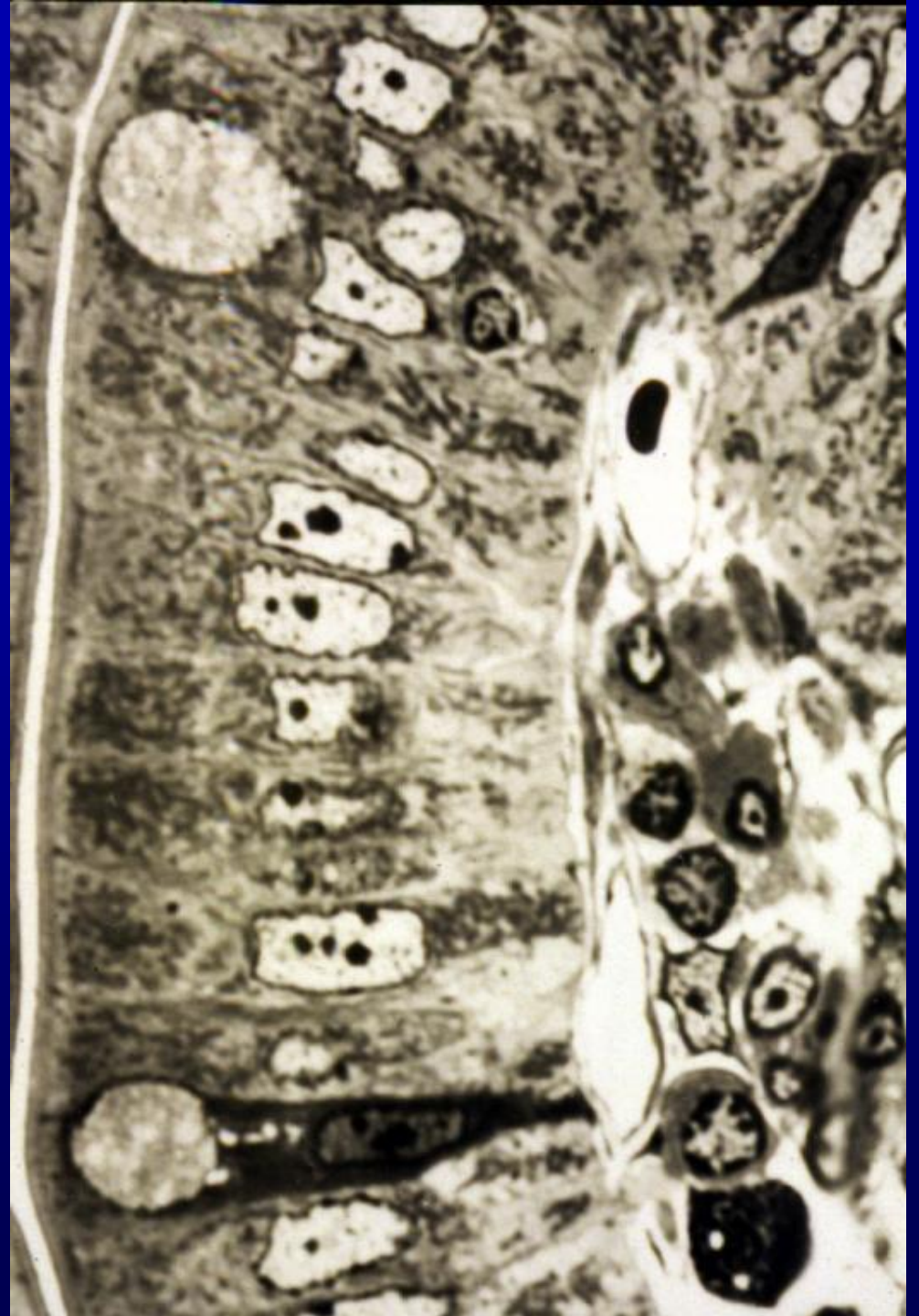
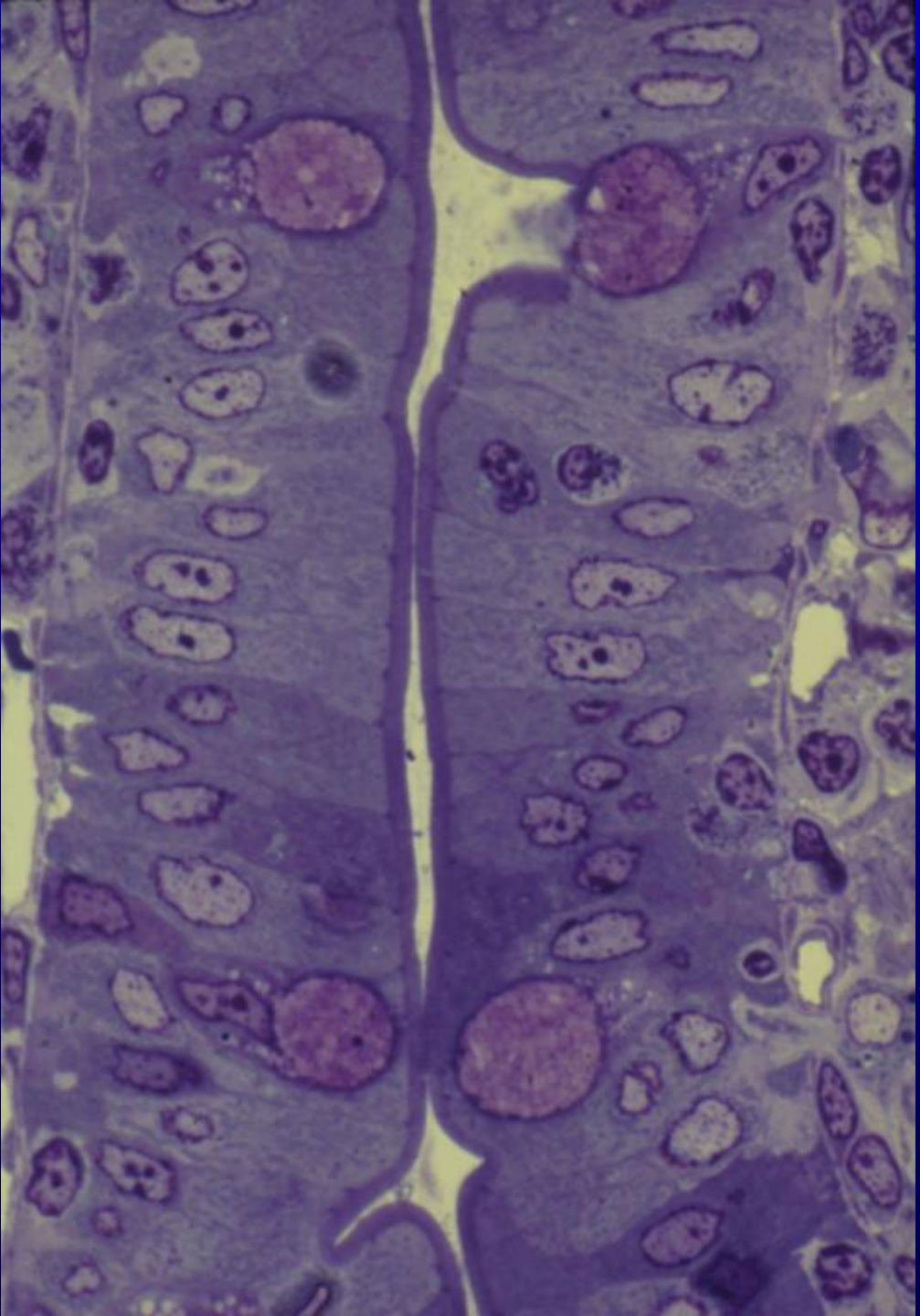


**Eosin (H&E) of retina**

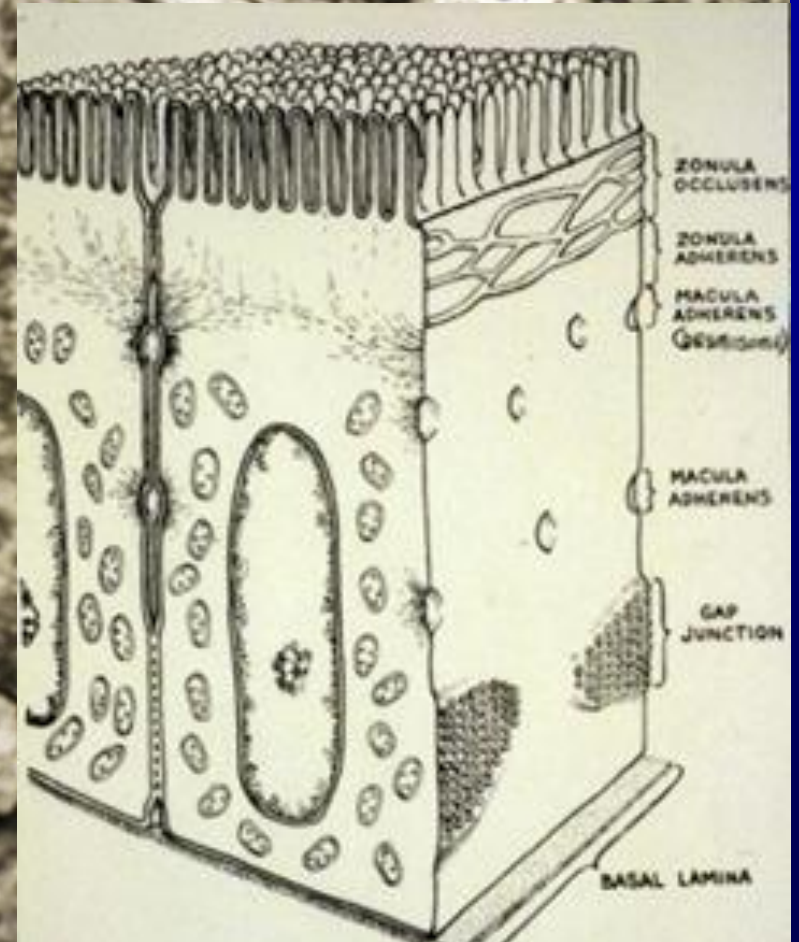
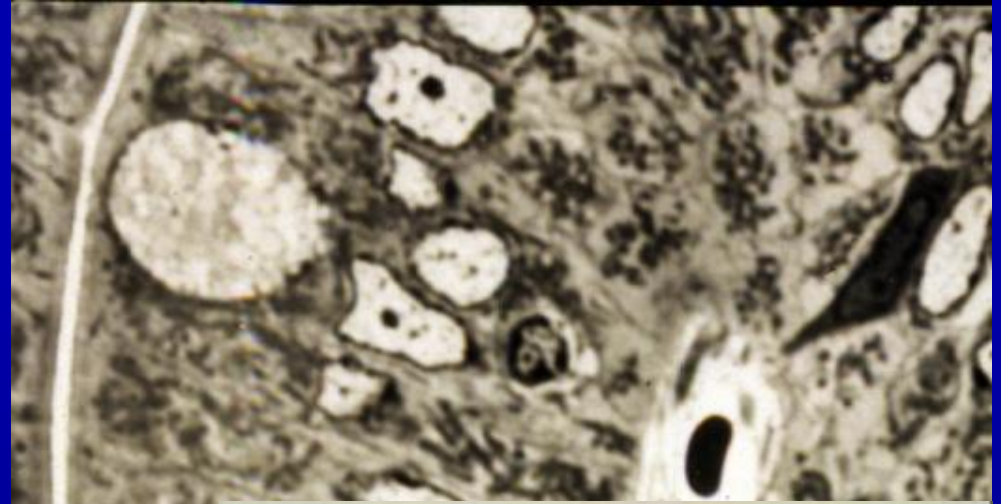
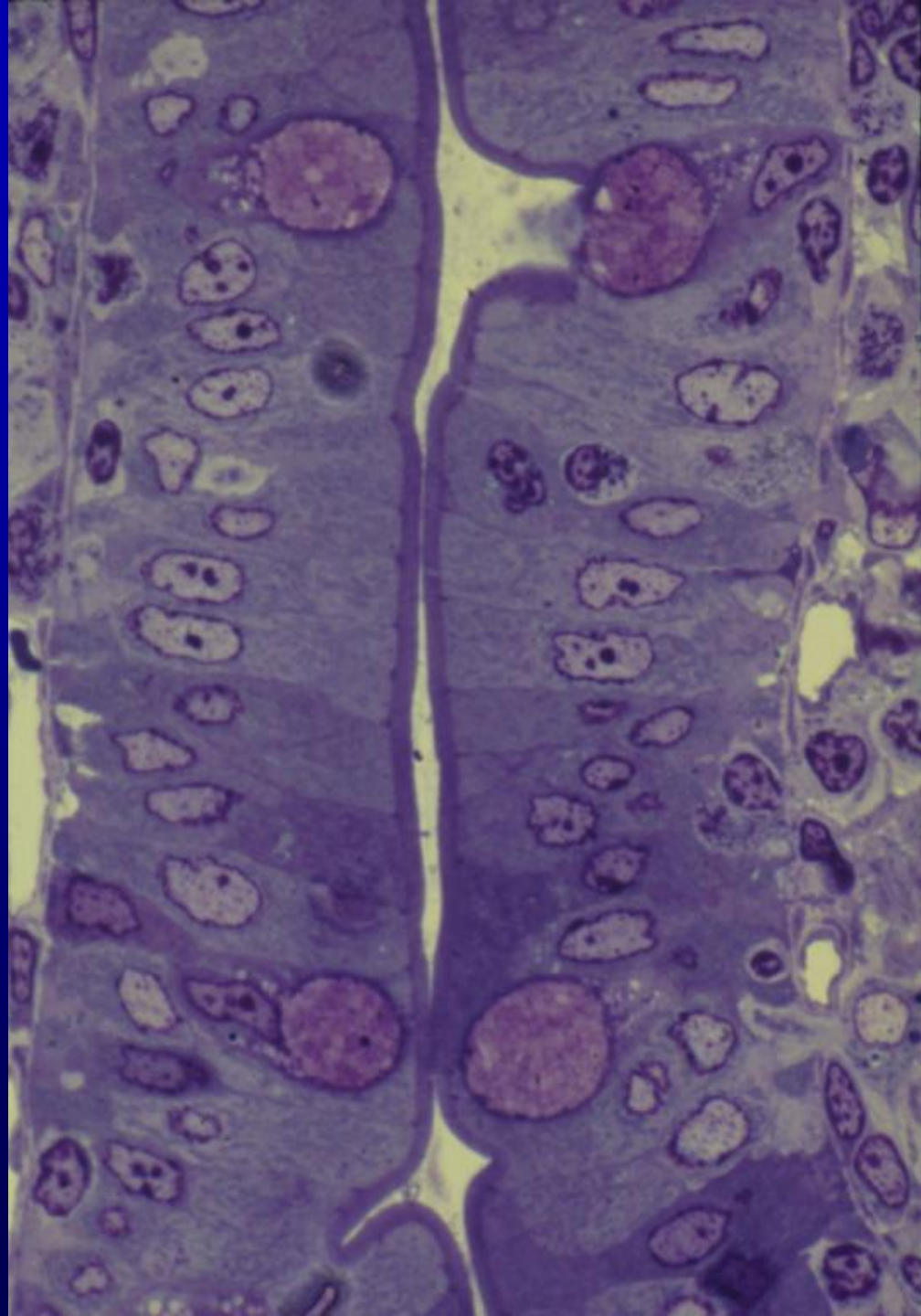


**Toluidine Blue**

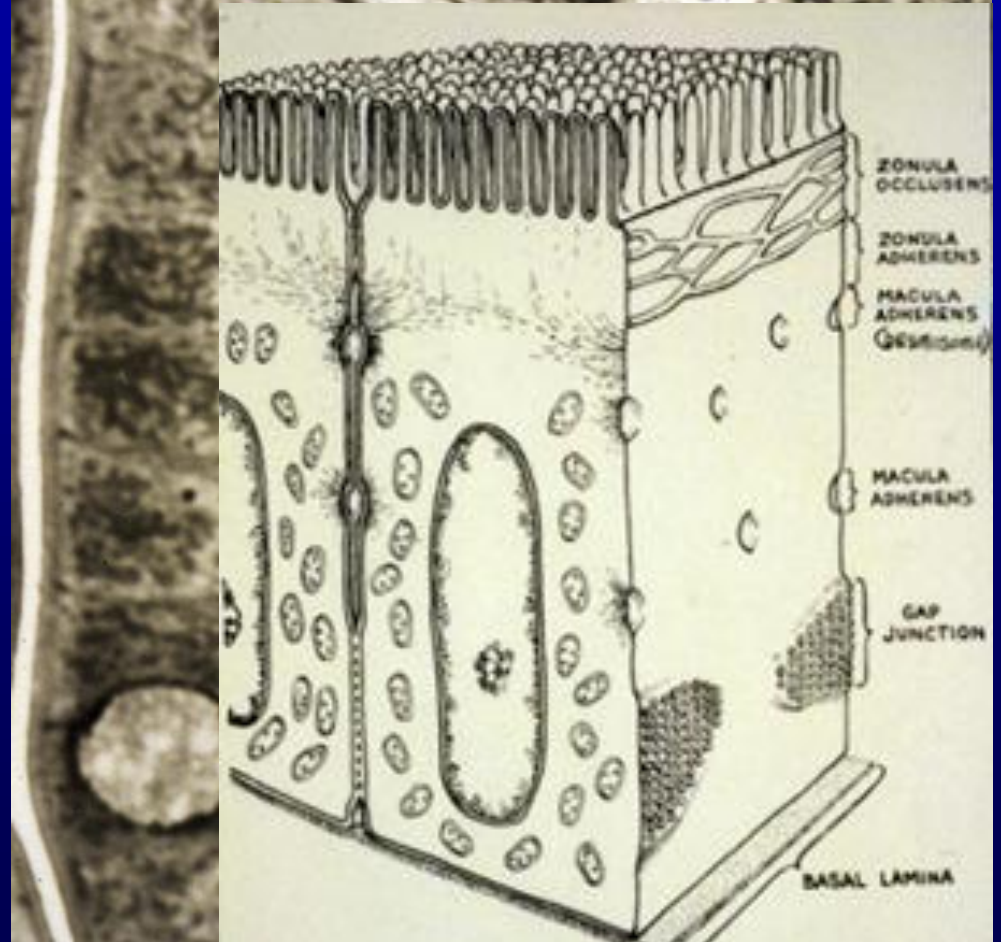
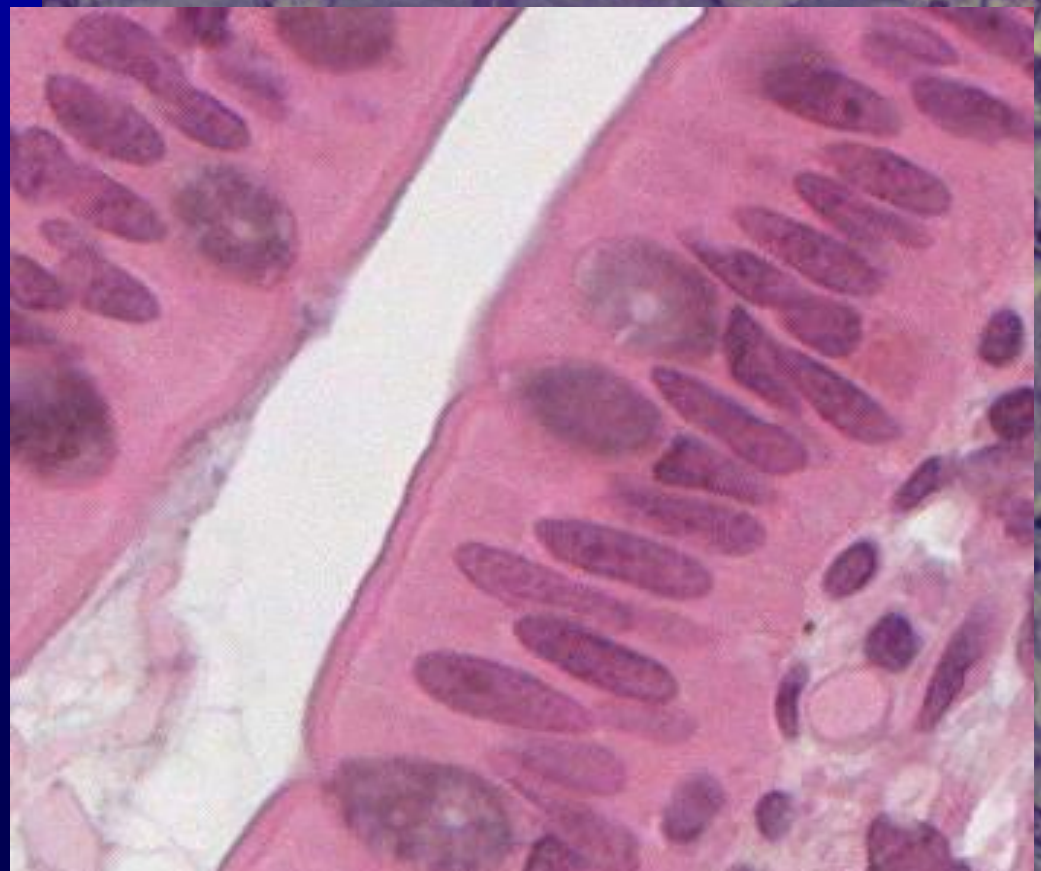
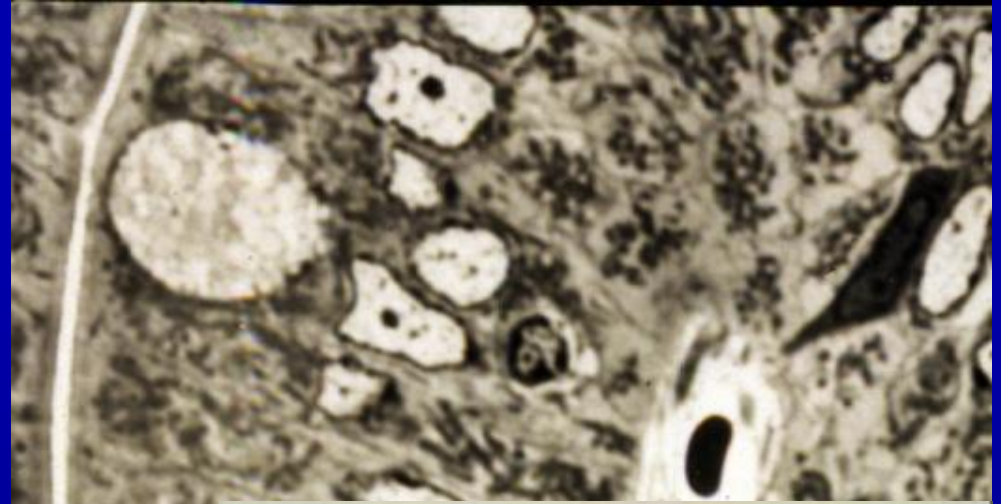
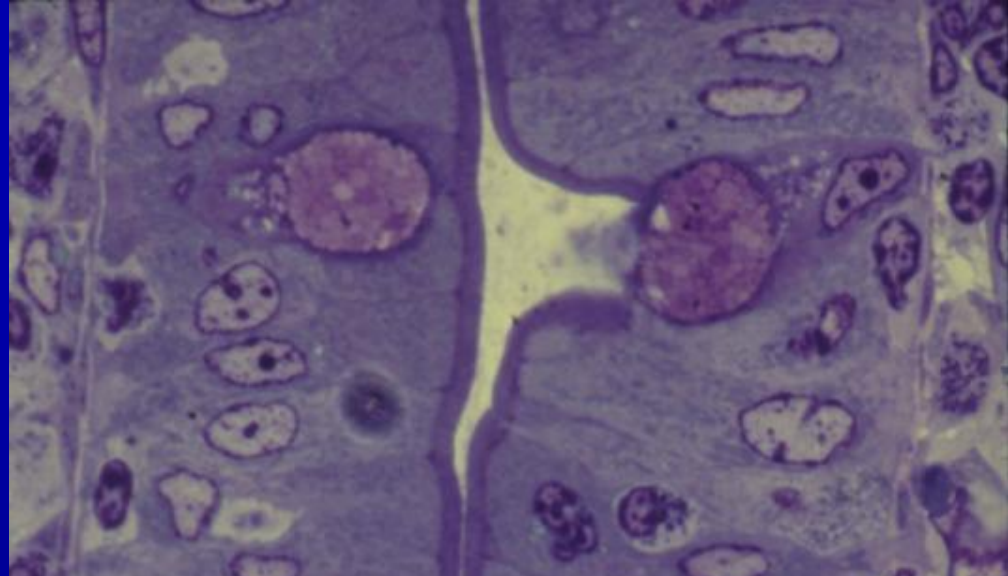




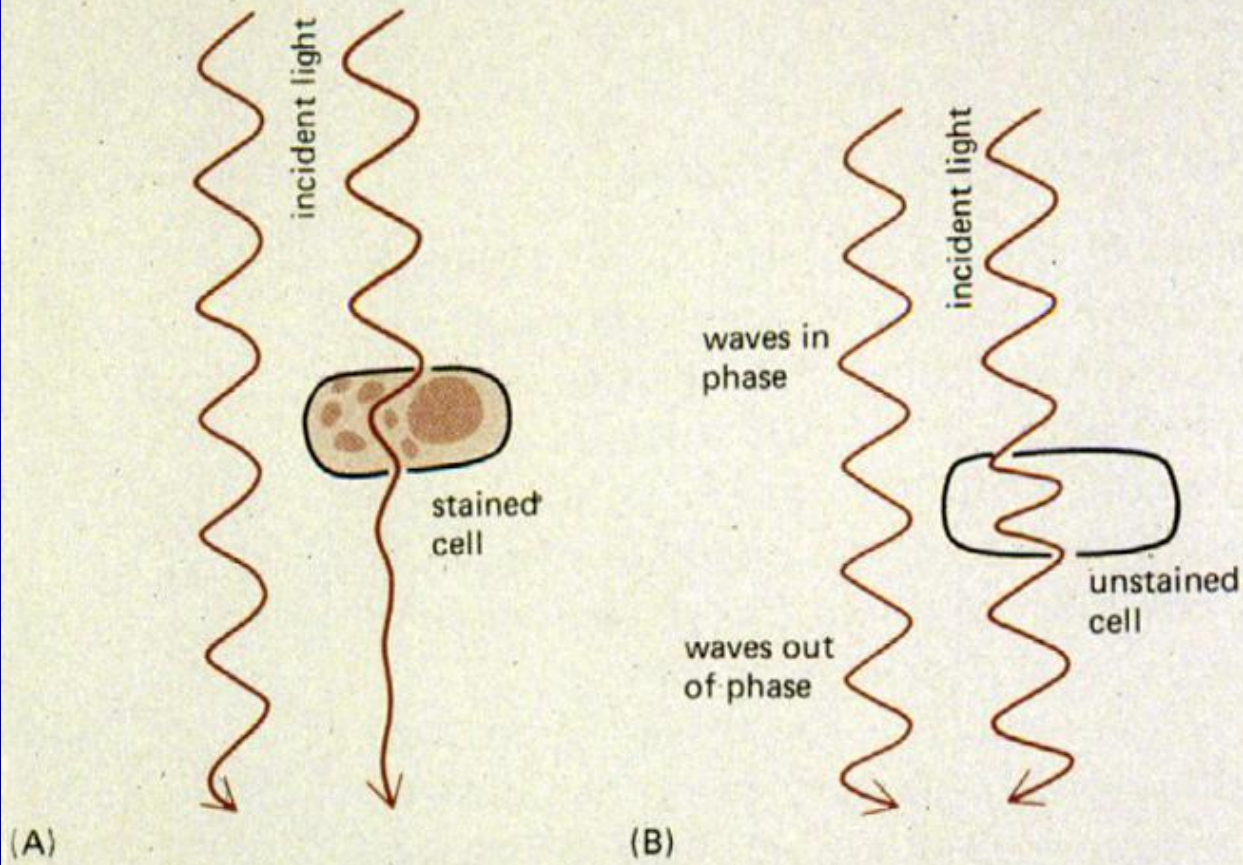




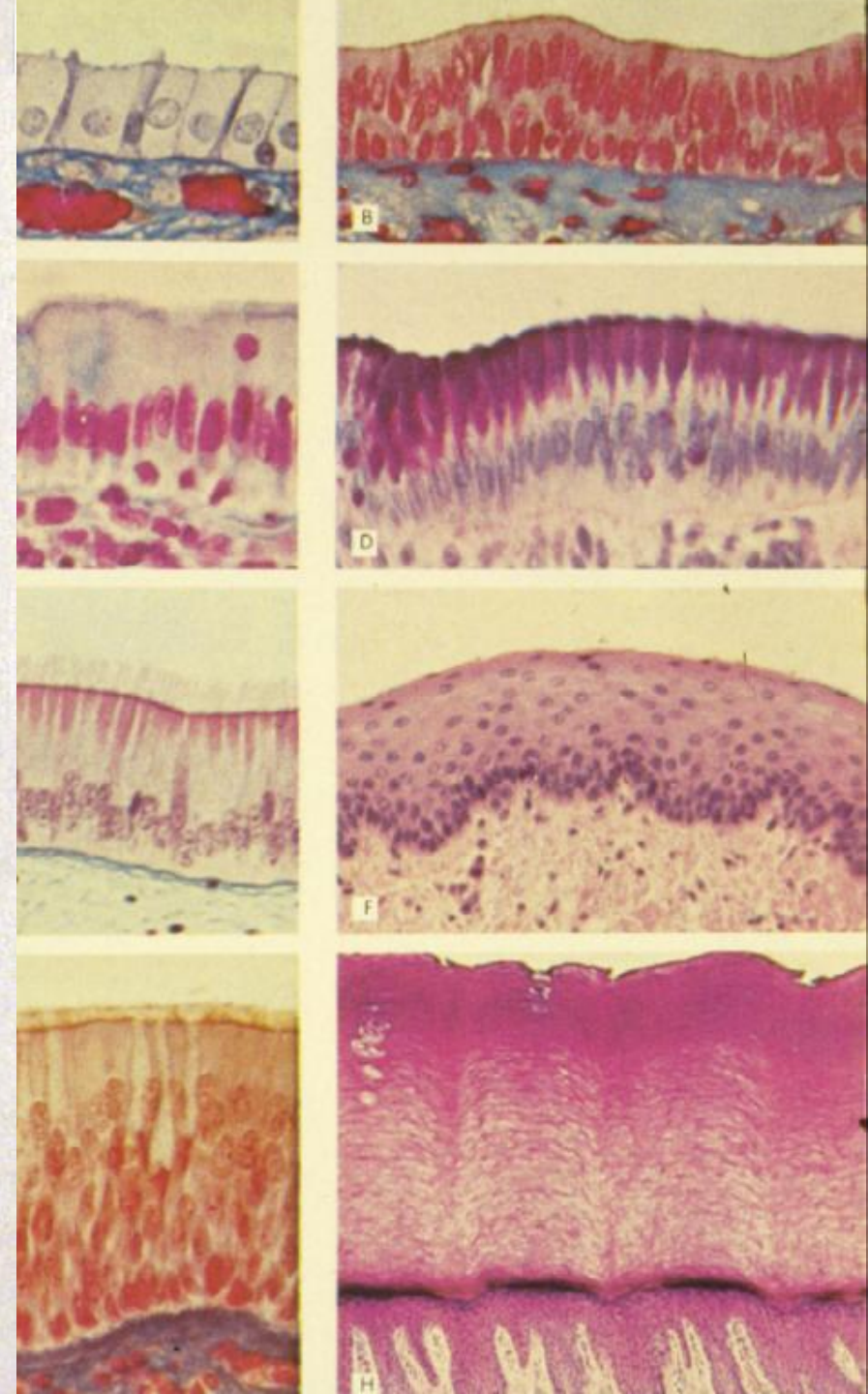






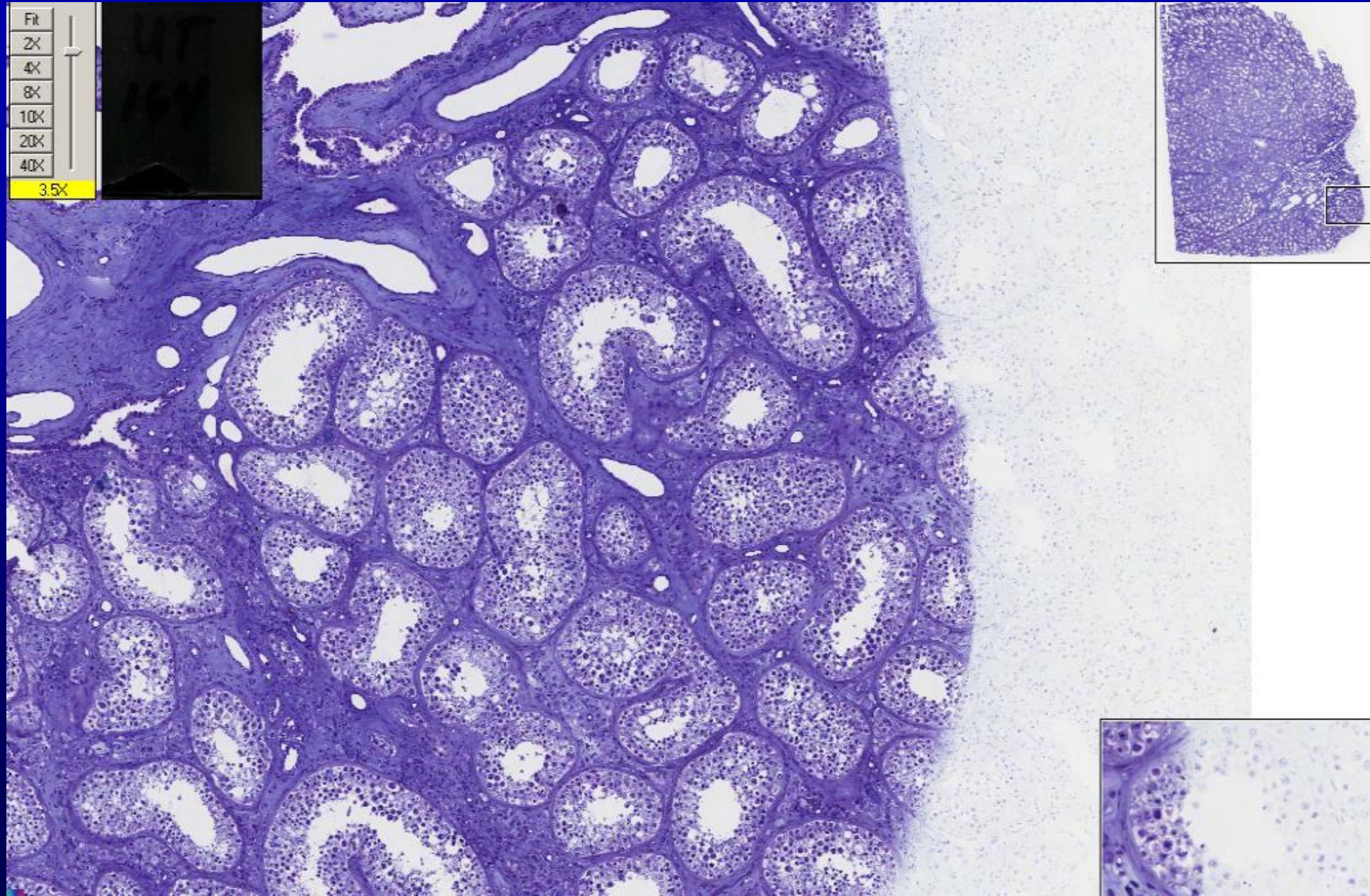


**Figure 4-5** The stained portions of the cell in (A) reduce the amplitude of light waves of particular wavelengths passing through them. A colored image of the cell is thereby obtained that is visible by direct observation. Light passing through the unstained, living cell (B) does not undergo a major change in amplitude, and many details cannot, therefore, be seen directly; however, changes occur in the phase of this light that are exploited in phase-contrast and differential-interference-contrast microscopy to produce a high-contrast image.



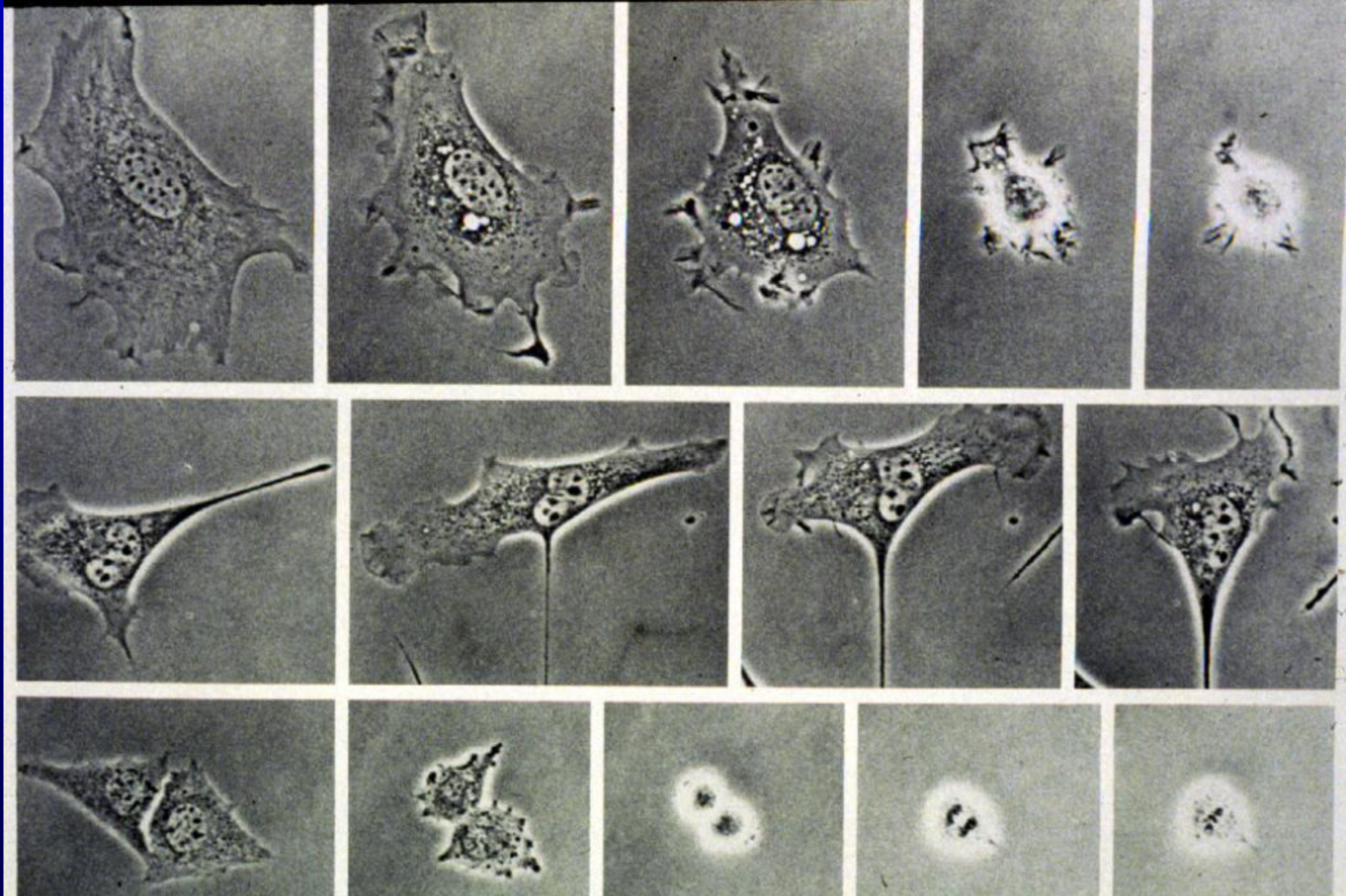


# 19709 Transparency of unstained tissue





# Phase Contrast





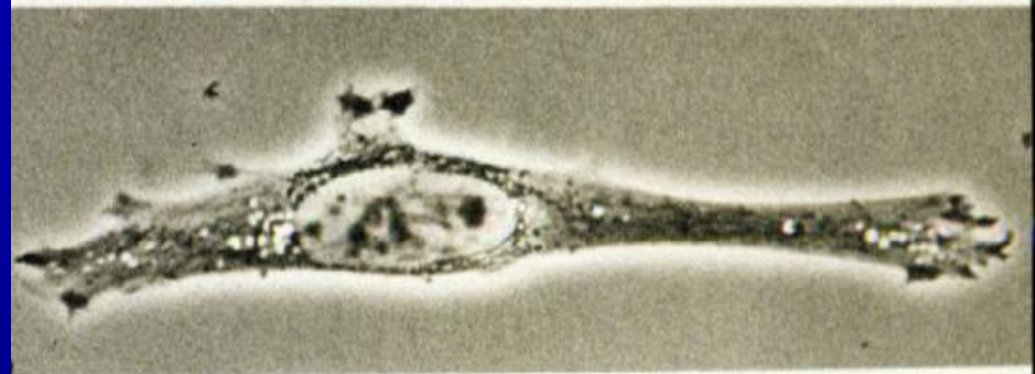
Dead stained cells

Bright Field

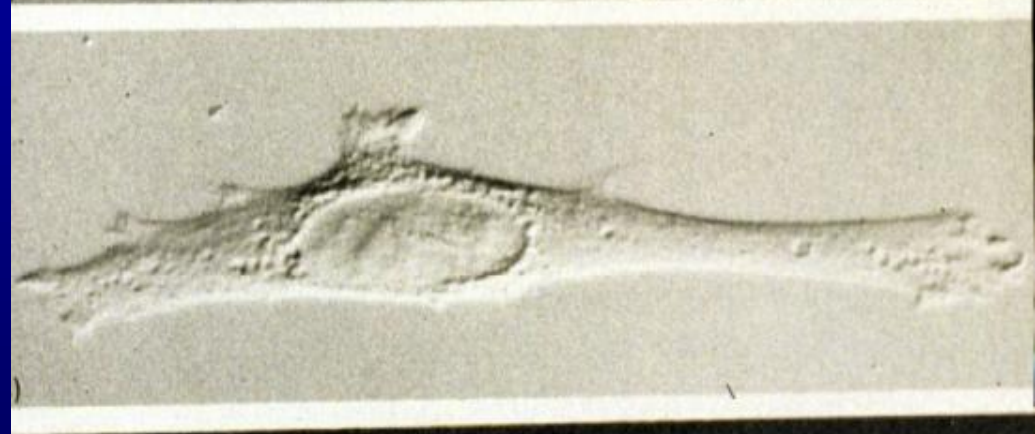


Live unstained cells

Phase Contrast

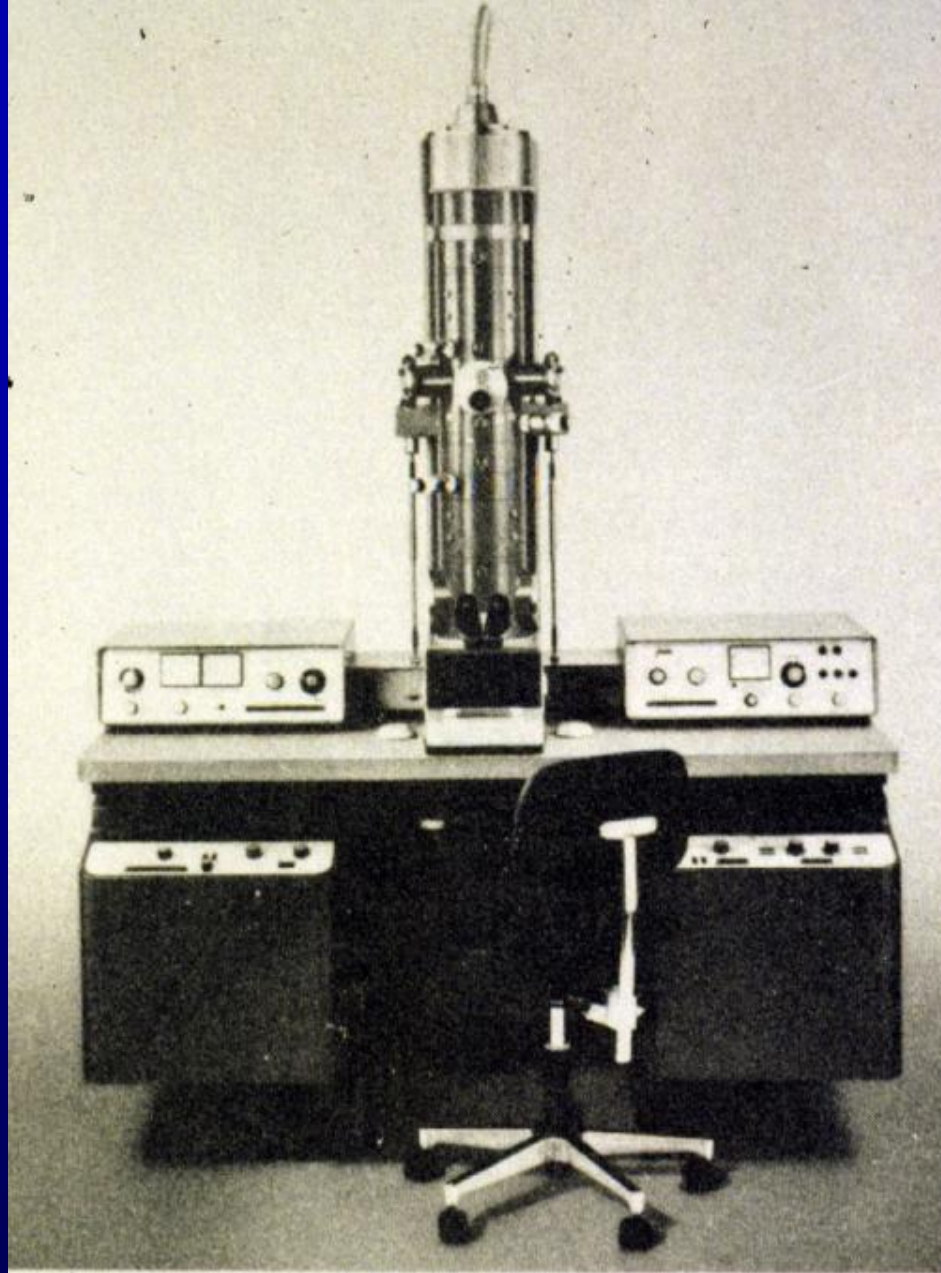


Nomarski  
“differential interference  
contrast”

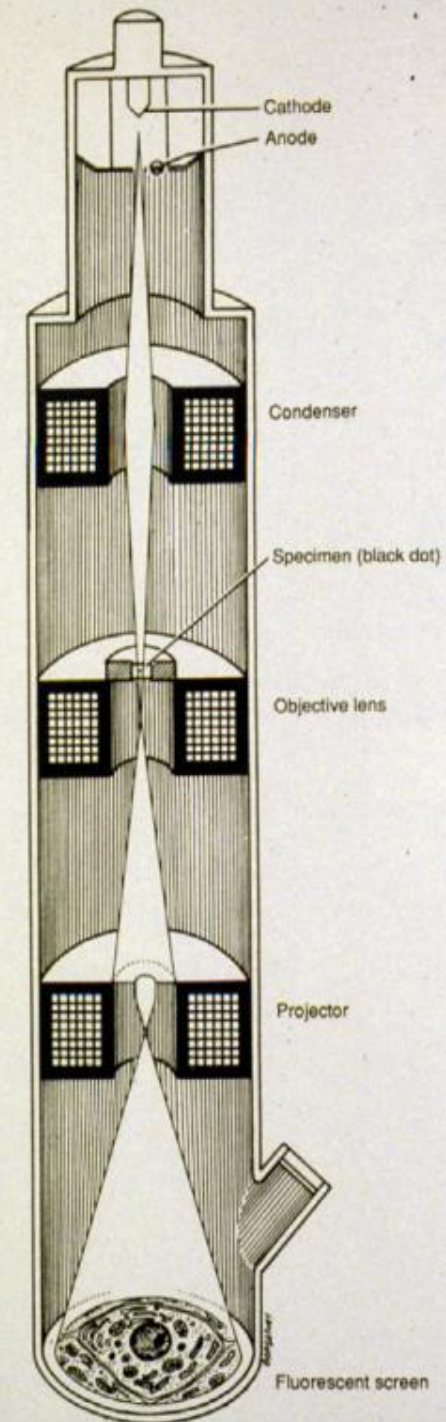


Dark Field

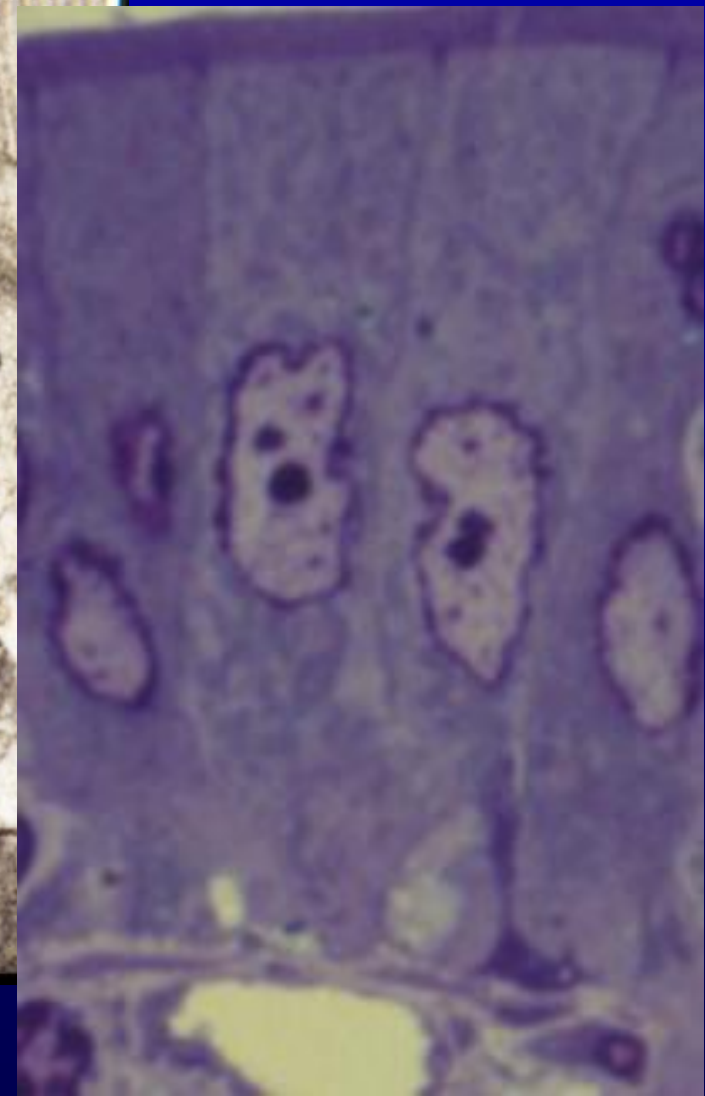
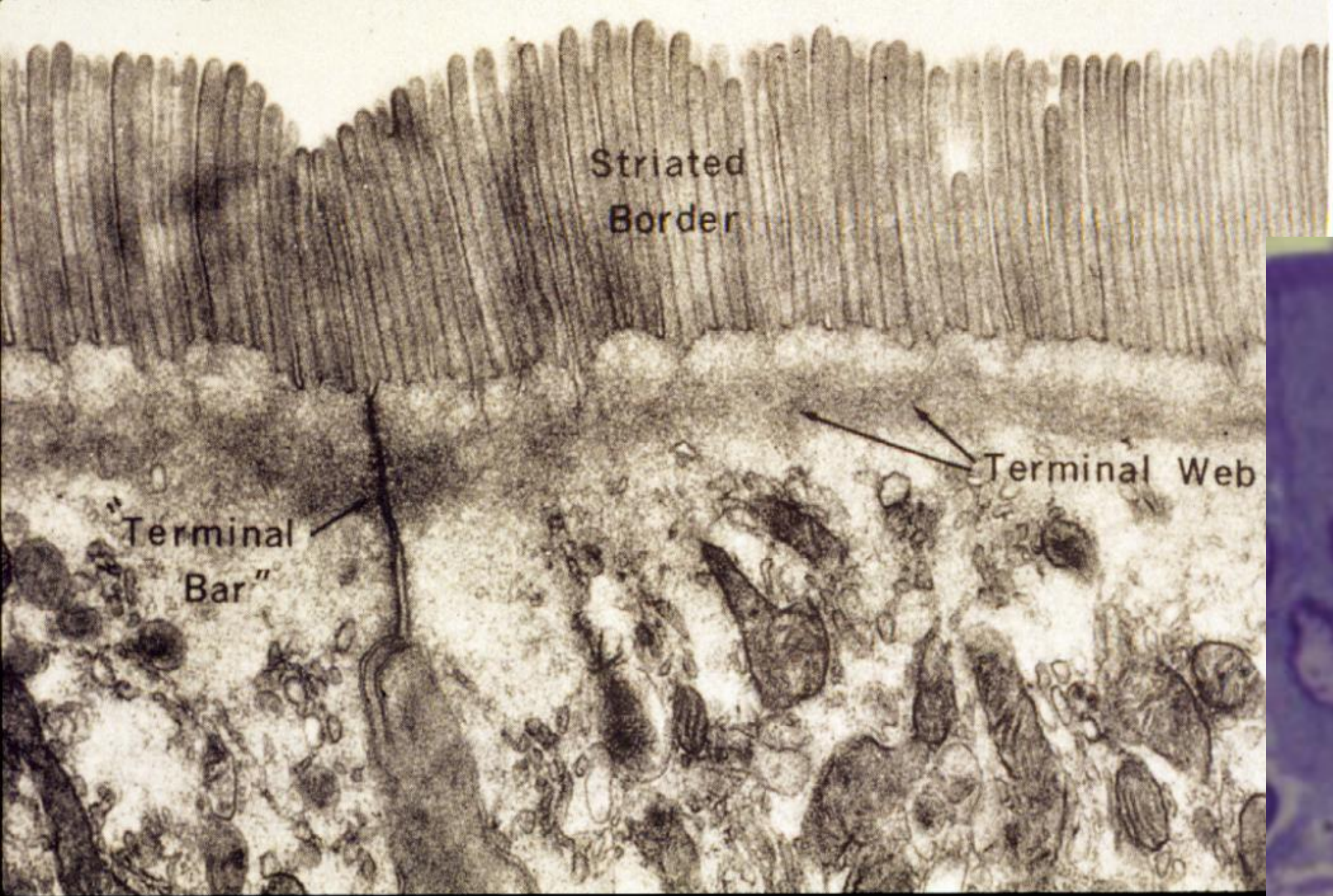




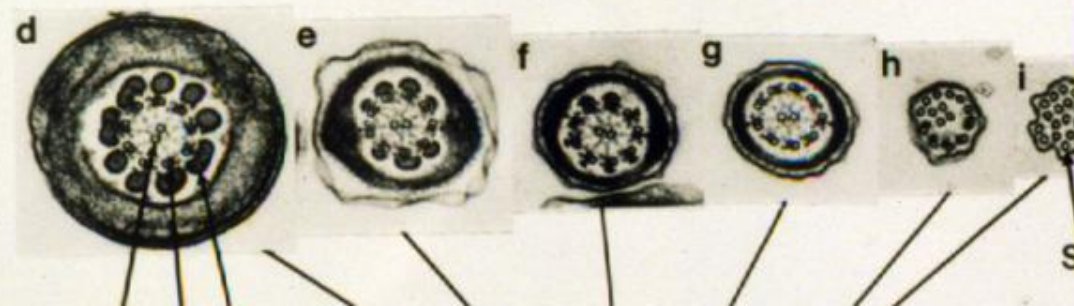
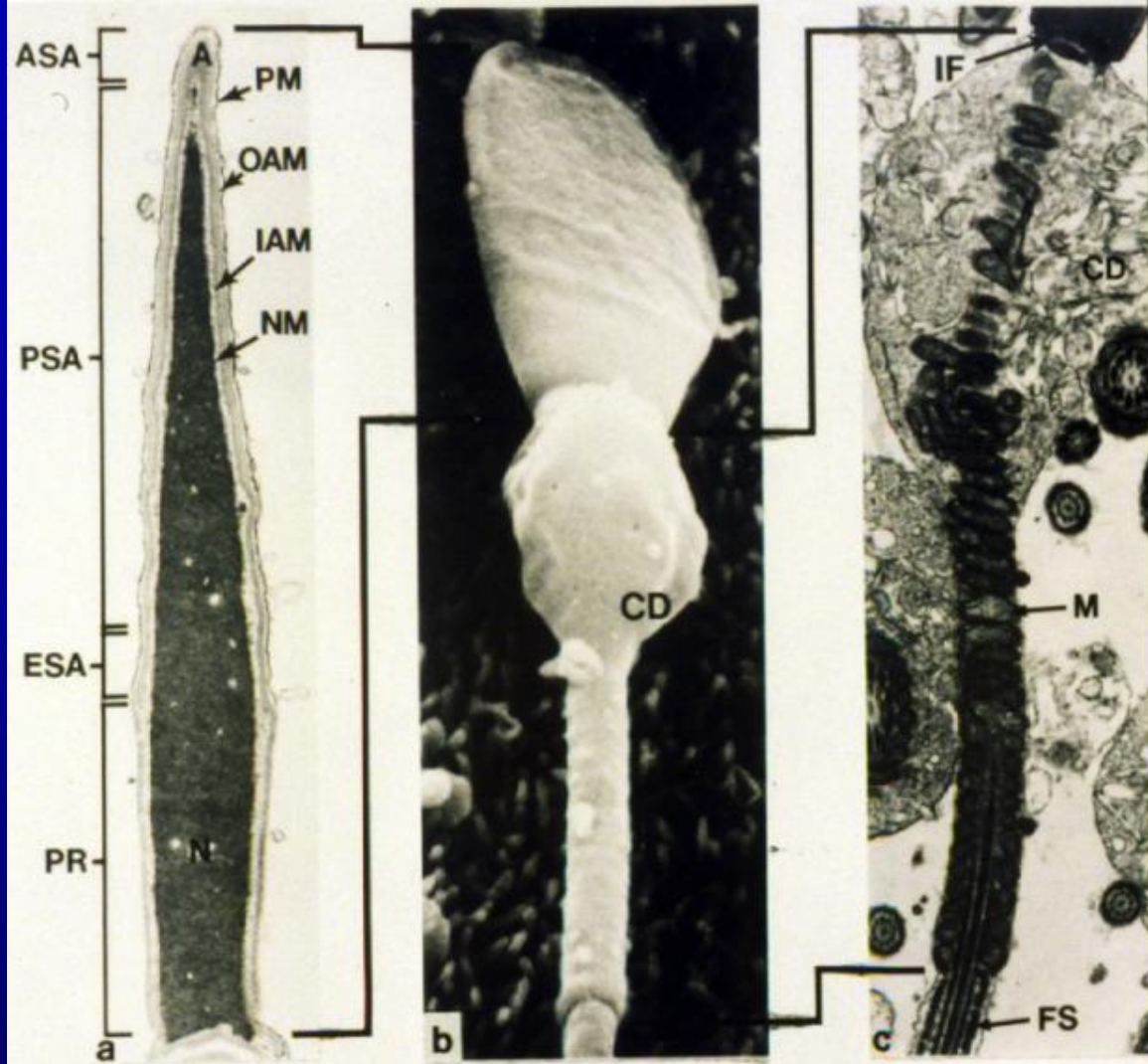
**Figure 1-5.** Photograph of the Zeiss model EM 10 electron microscope. (Courtesy of Carl Zeiss Co.)



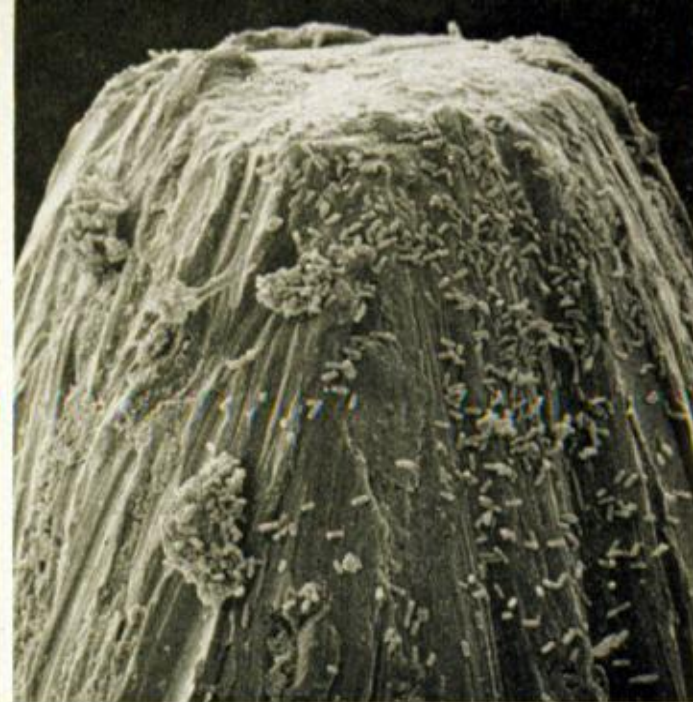
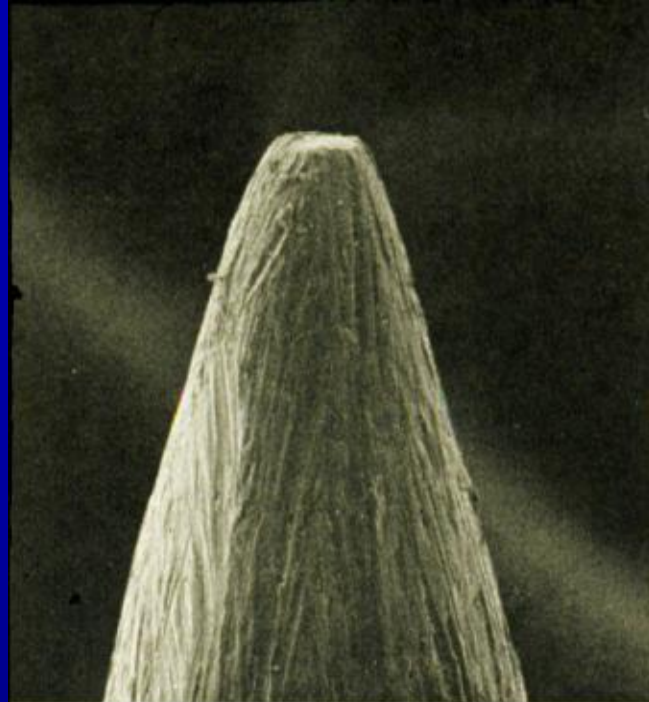




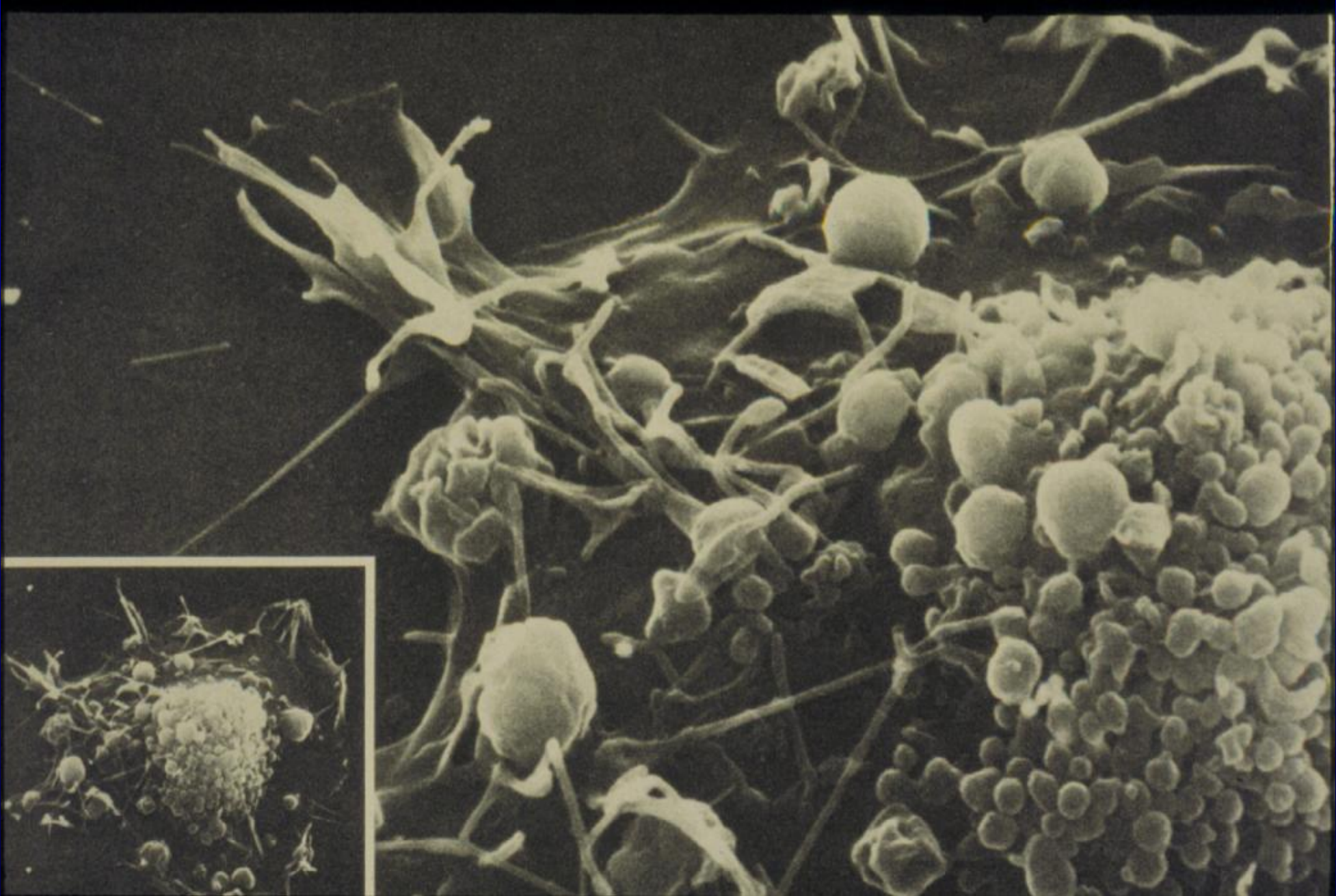




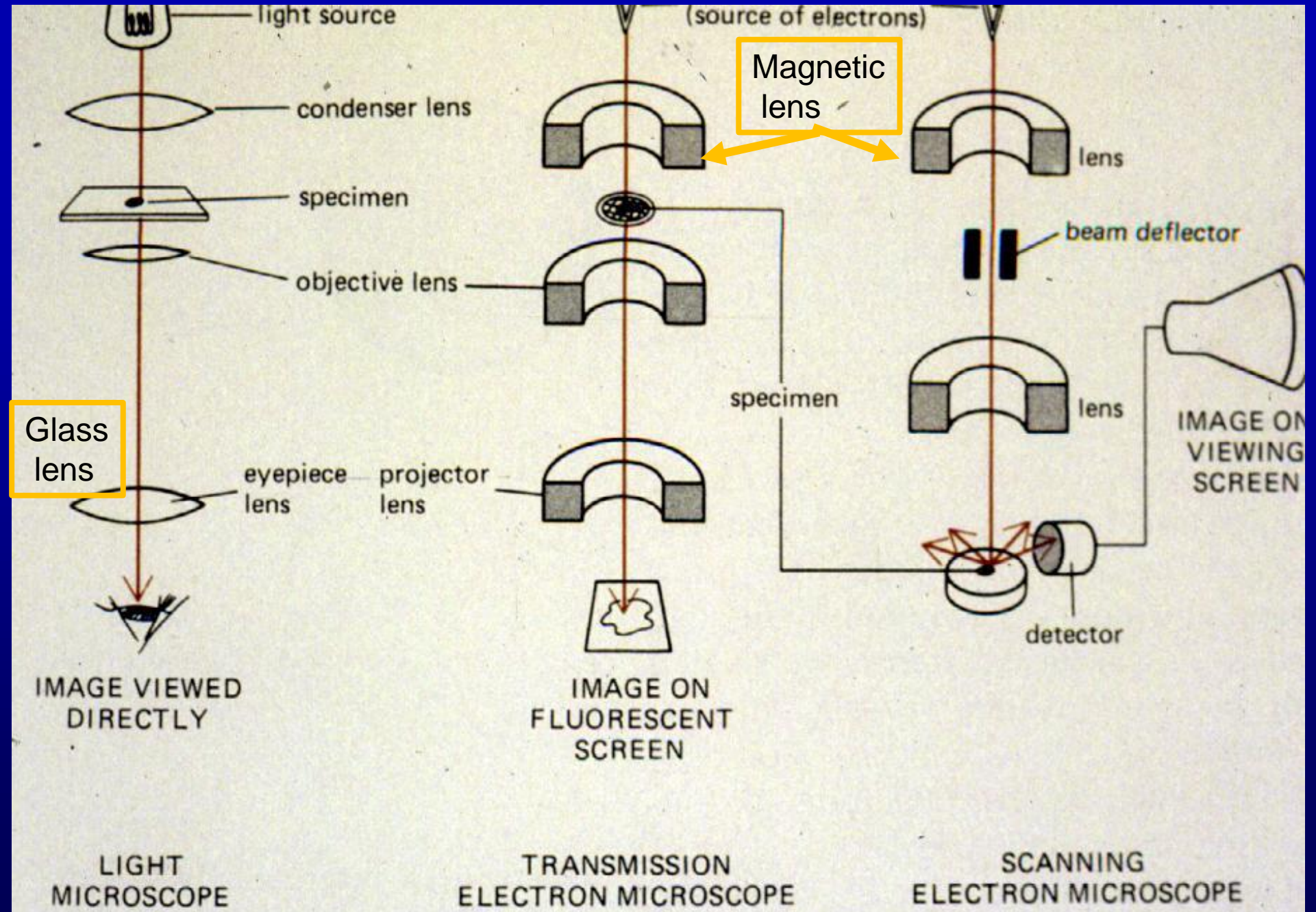






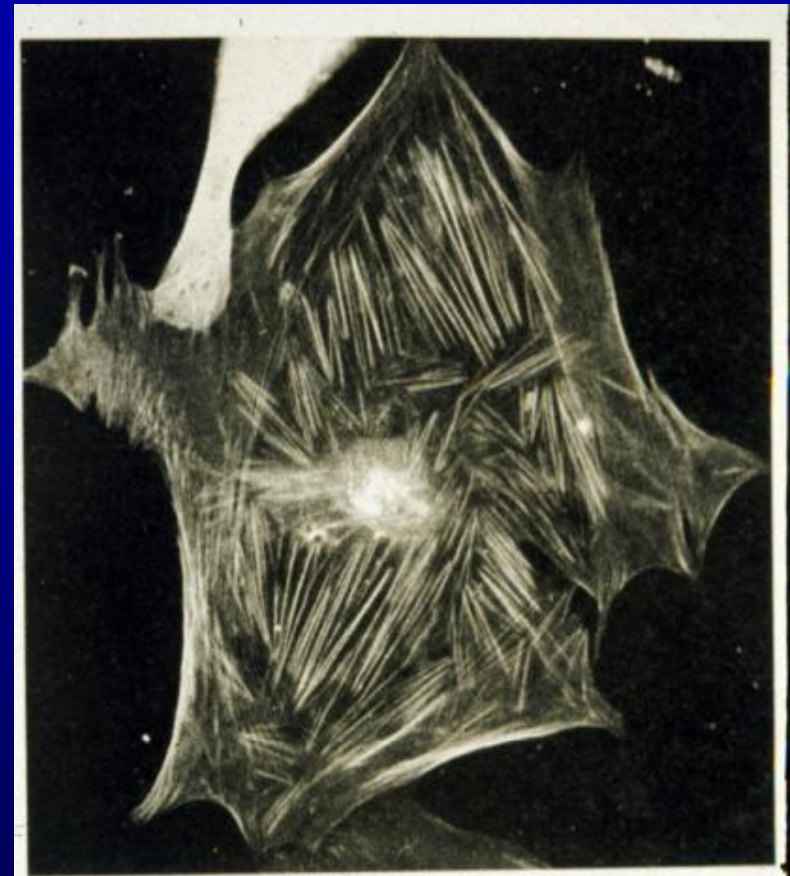






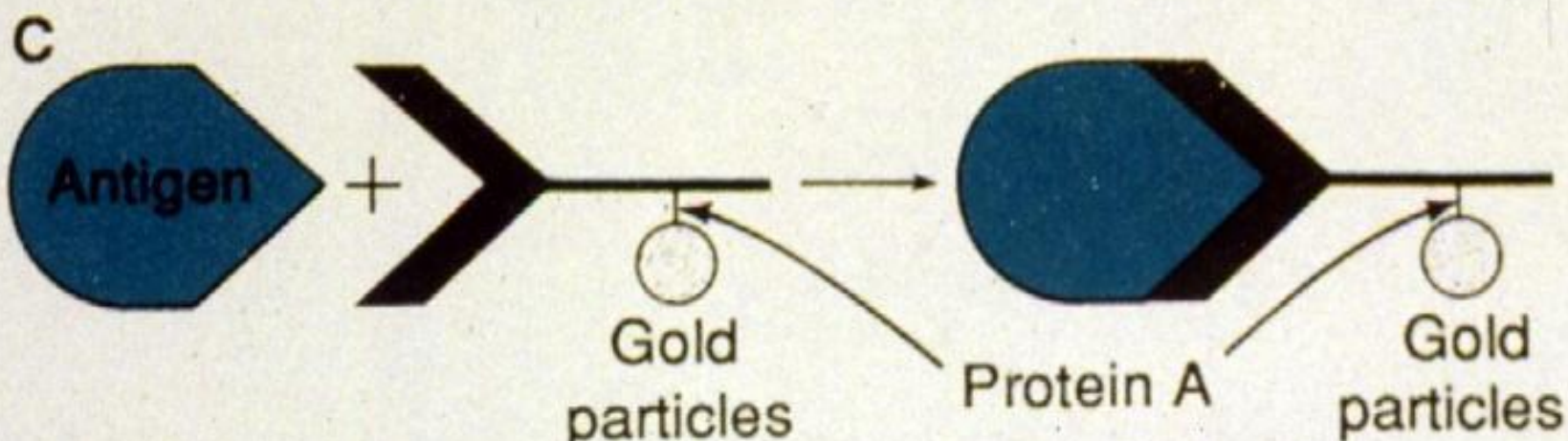
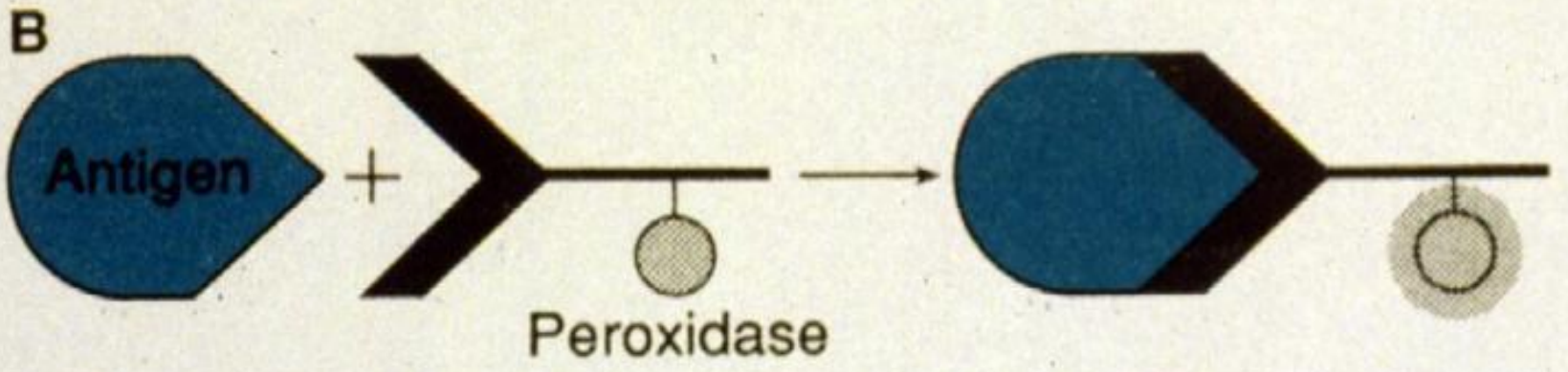
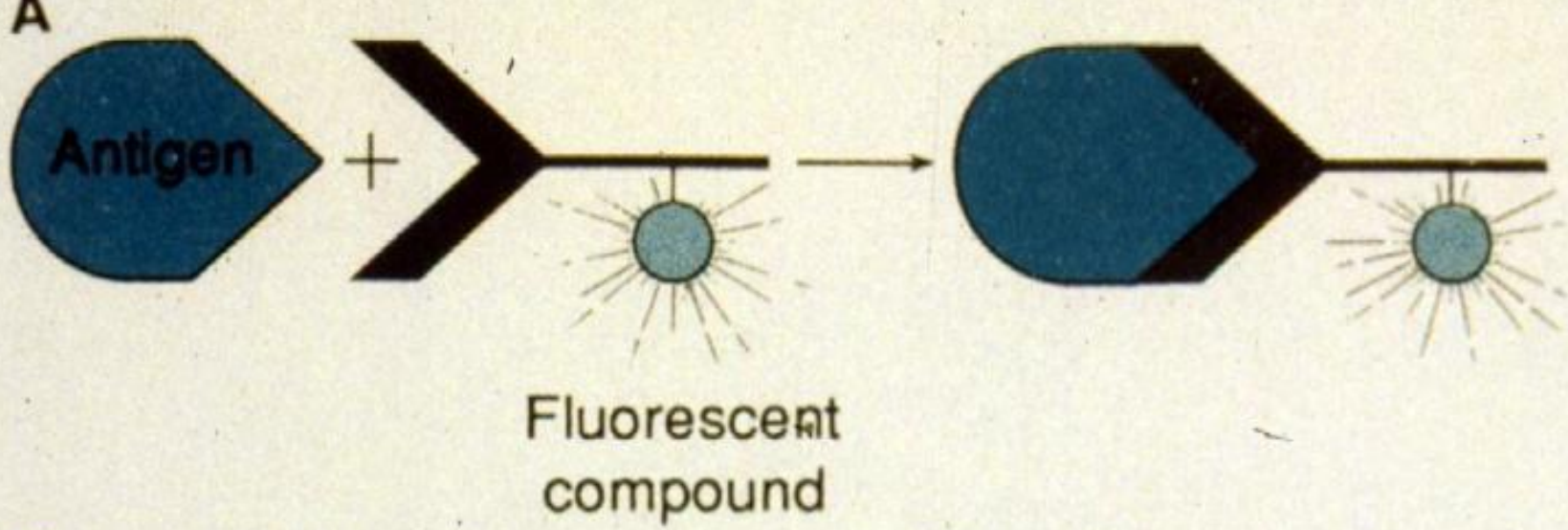
# Other Light and Electron Microscope Procedures

- Immunofluorescence
- Autoradiography
- In Situ Hybridization (ISH)
- Freeze Fracture
  - (Membrane Analysis)

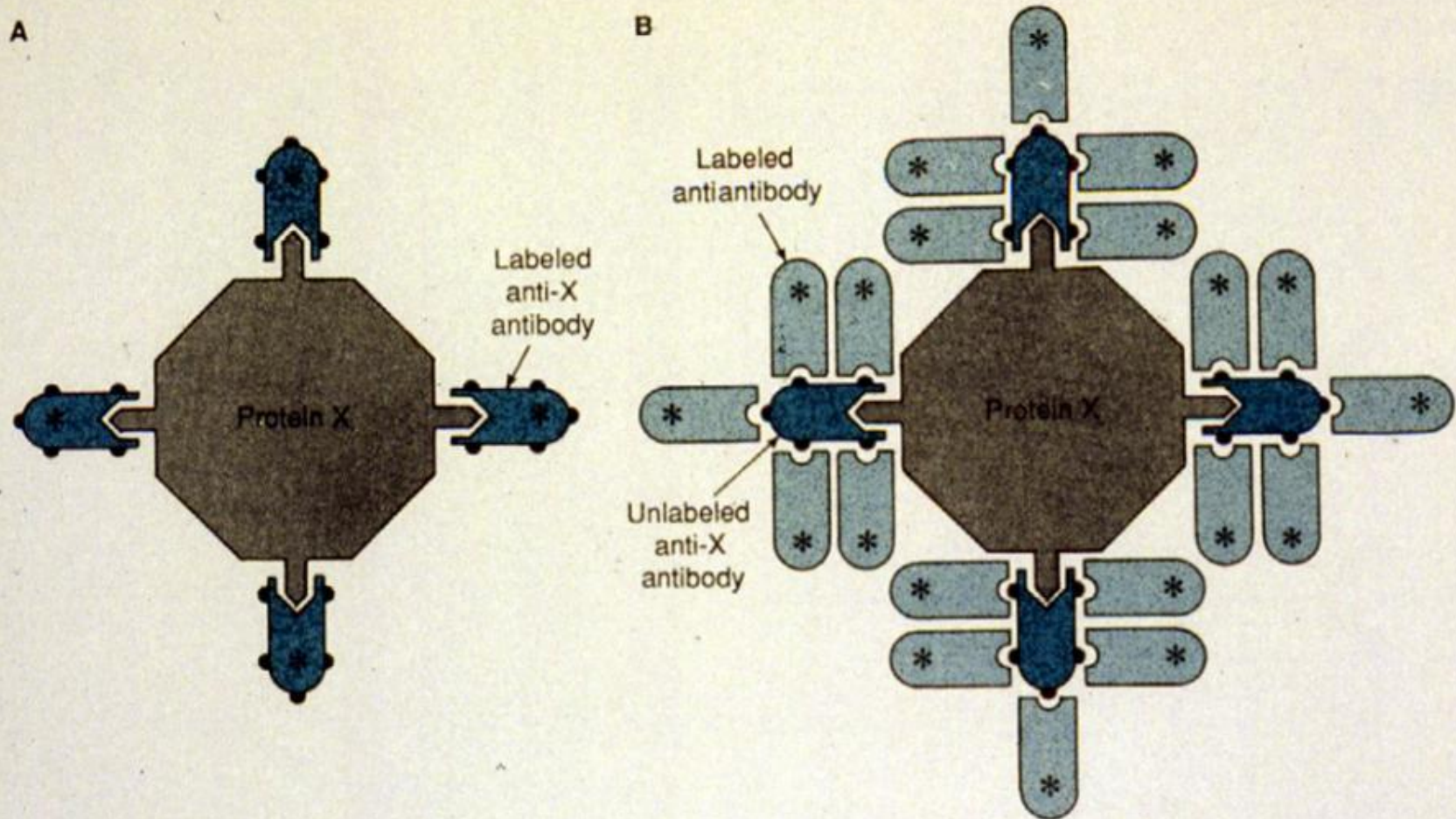


**Figure 2-9.** Actin fibrils composed of aggregates of actin filaments in the cytoplasm of a cultured human fibroblast preincubated in fluorescent actin antibody.  $\times 1767$ . (Reproduced, with permission, from E Lazarides: *J Cell Biol* 1975; **65**:549.)



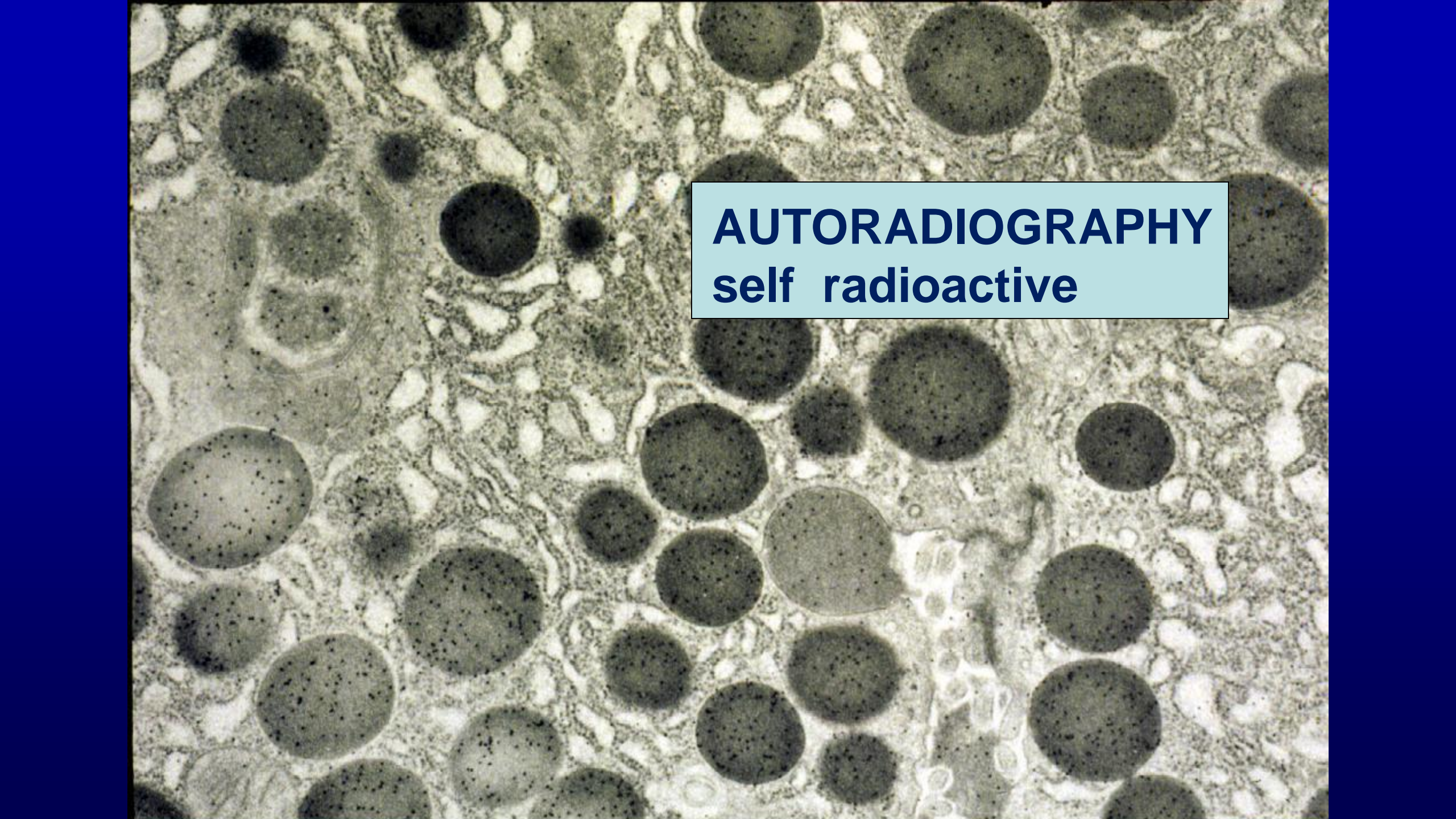






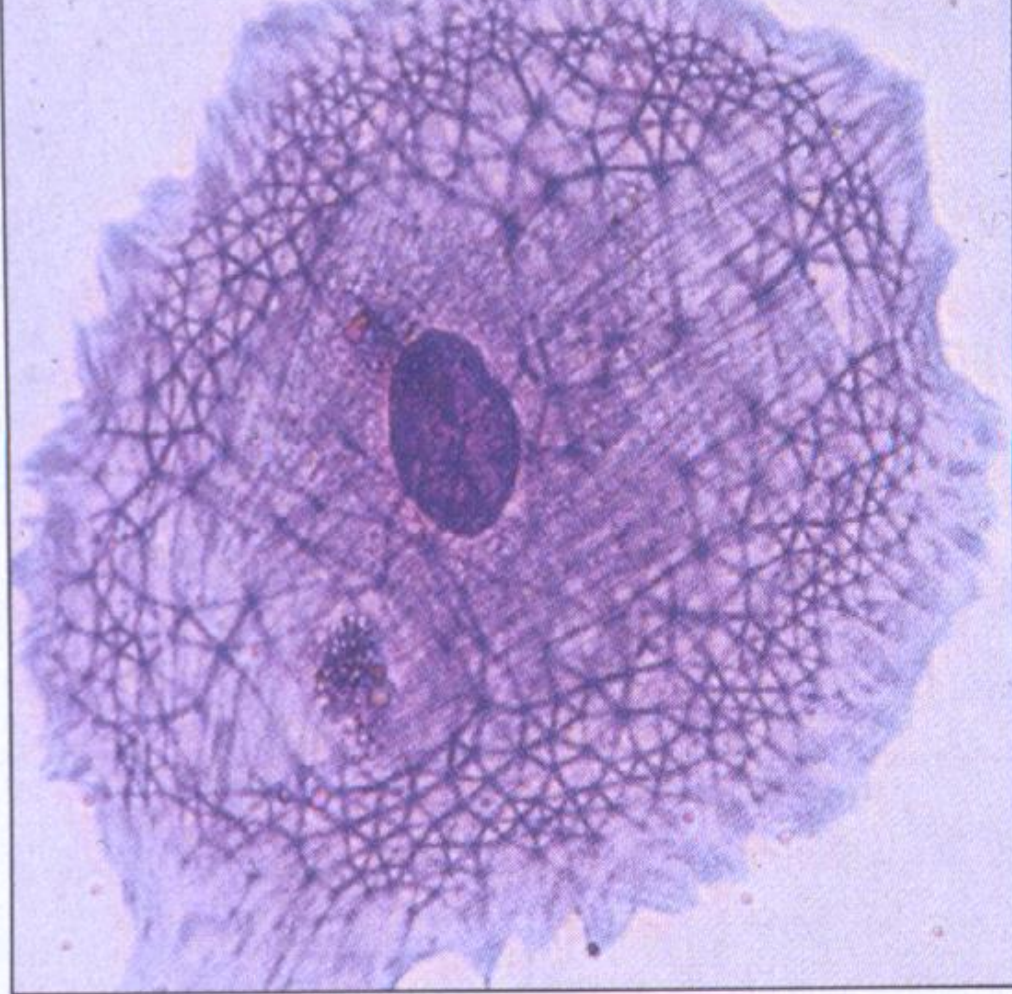
**Figure 2-11.** The direct **(A)** and indirect **(B)** techniques of immunocytochemistry. In the direct technique, a labeled antibody binds to an antigen present in the cells. In this case, each antigen molecule binds a few antibody molecules. In the first step of the indirect technique, nonlabeled anti-x antibody is bound to the antigen; in the second step the labeled anti-antibody then binds to the anti-x antibody. Because each anti-x antibody binds several molecules





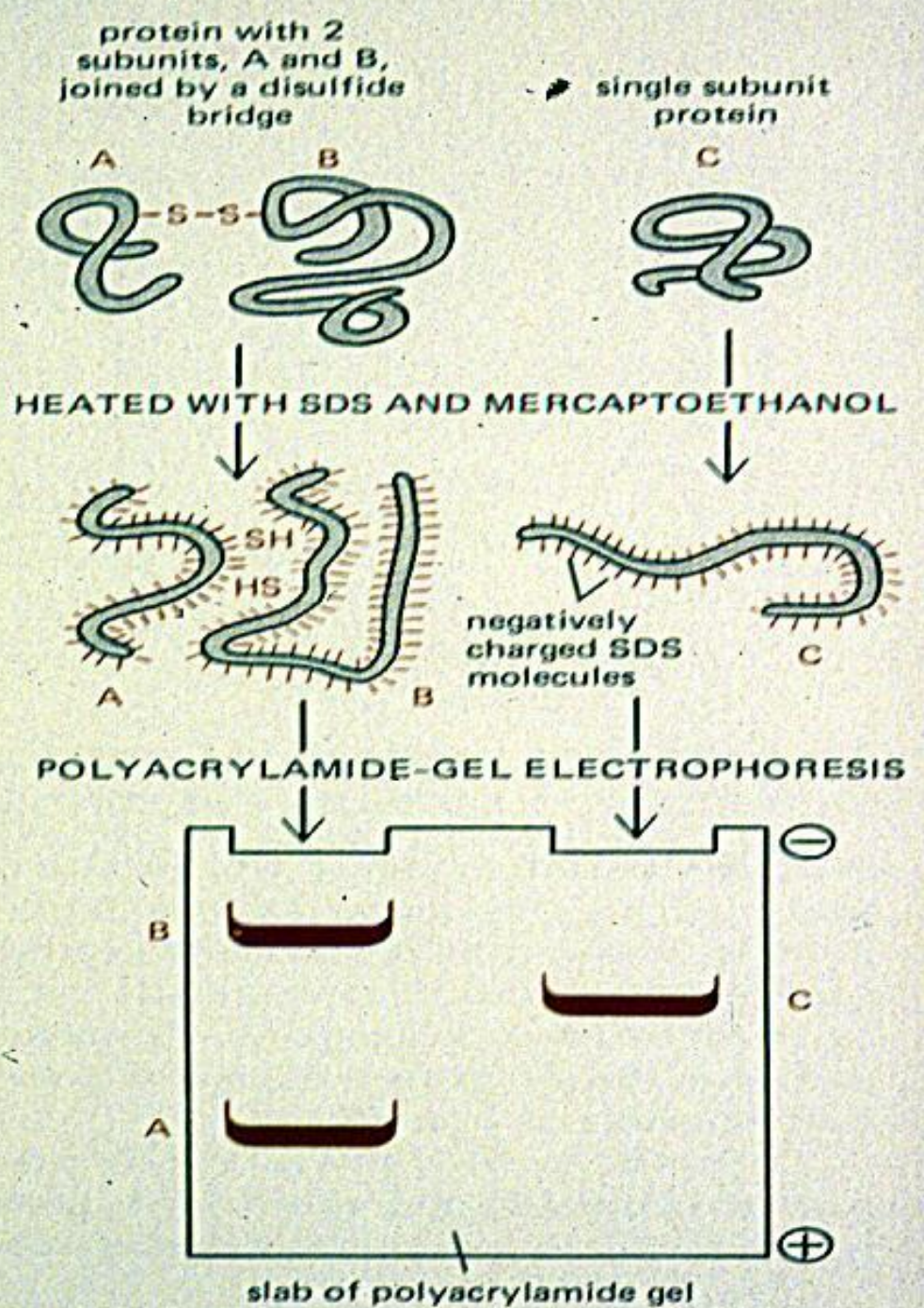
**AUTORADIOGRAPHY**  
self radioactive





10 μm

**Figure 16-1 The cytoskeleton.** A cell in culture has been fixed and stained with **Coomassie blue**, a general stain for proteins. Note the variety of

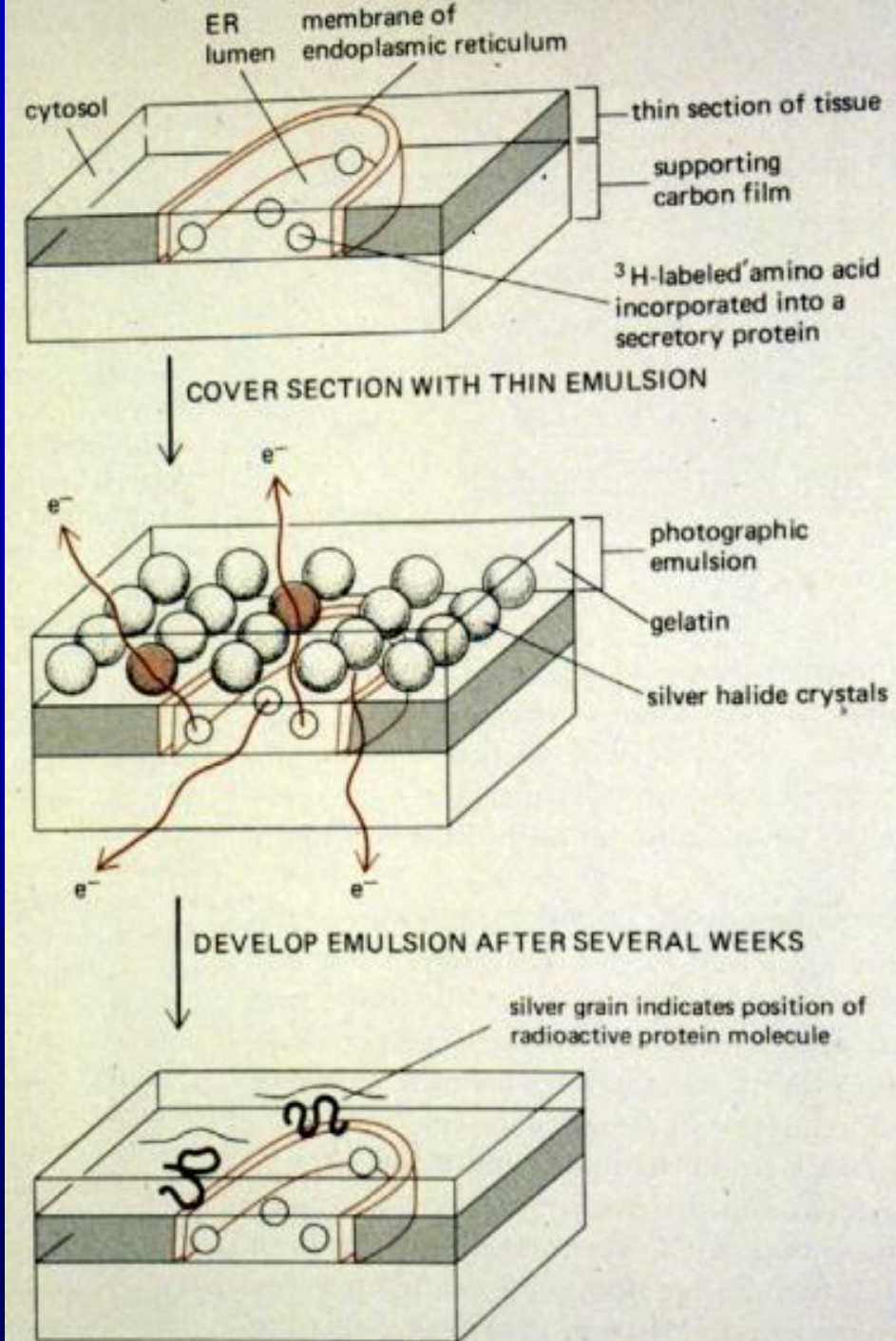




# Evidence for protein pathway

## Autoradiography

Procedure to localize a product (e.g., protein) within a **cell or gel** that is self-radioactive due to the cell's incorporation of radioactive precursors (e.g., radioactive amino acids) into that product that is visualized in a photographic emulsion.



# Autoradiography vs Fluorography Terminology

**Autoradiography** is the direct exposure of film by beta particles or gamma rays.

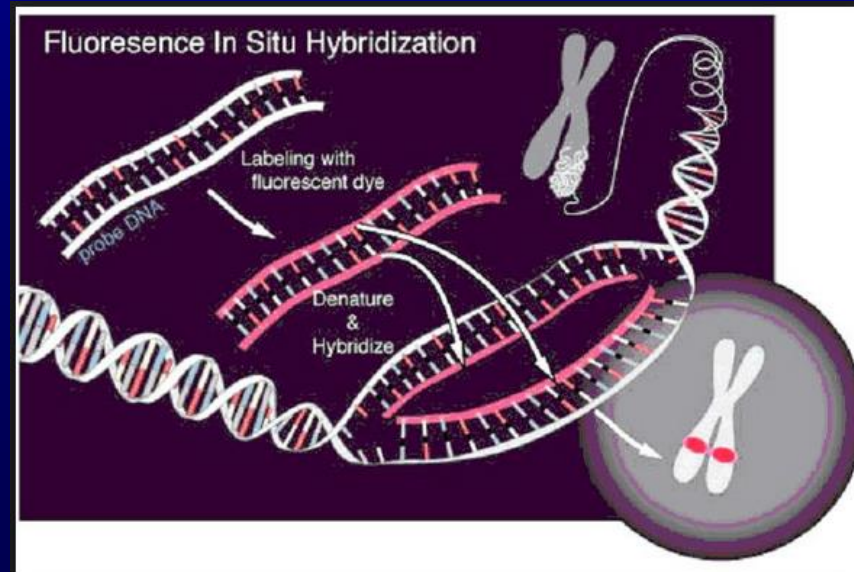
**Fluorography** is the exposure of film by secondary light that was generated by the excitation of a fluor or a screen by a beta particle or a gamma ray.



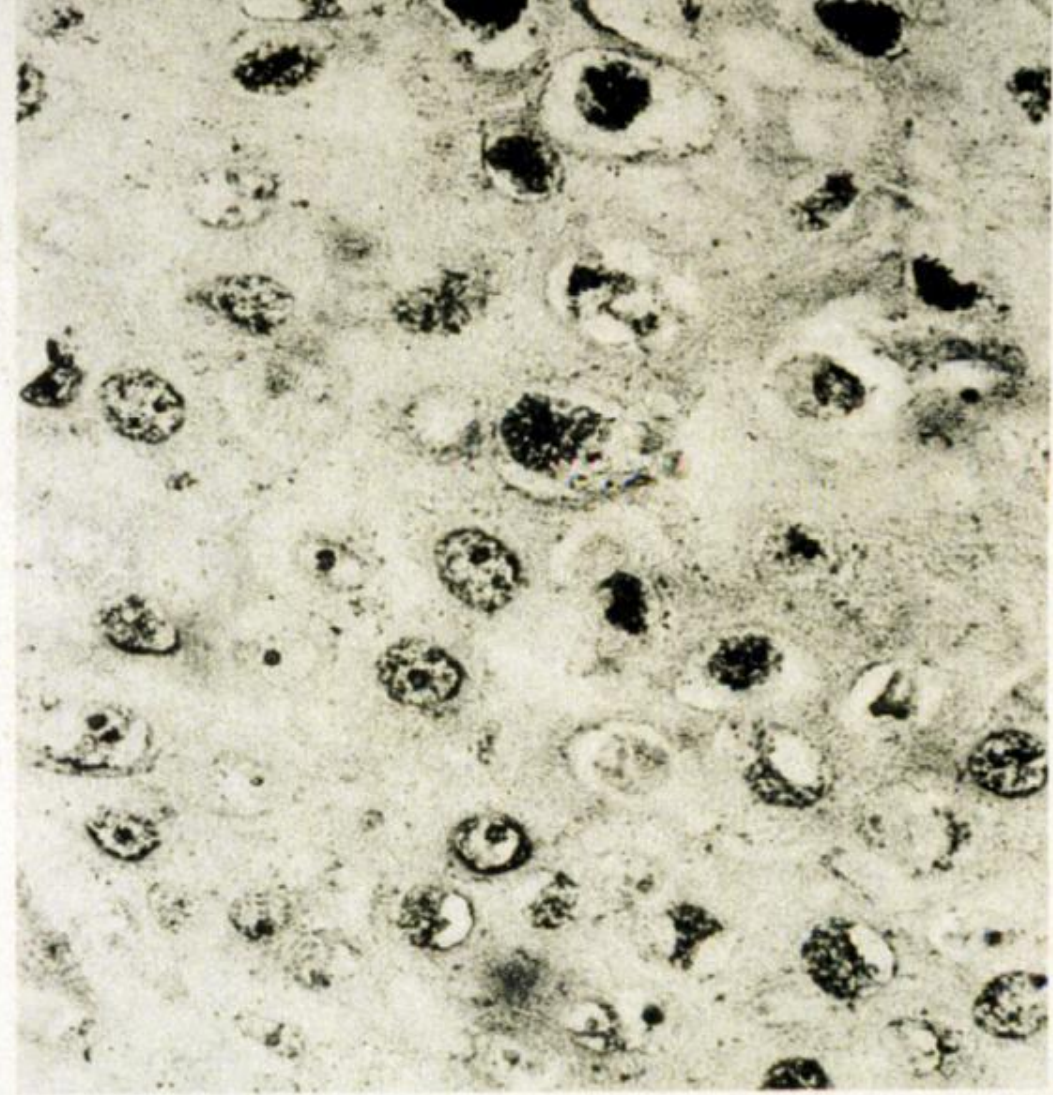
# *In situ* hybridization

- *In situ* hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e., probe) to localize a specific DNA or RNA sequence in a portion or section of tissue (*in situ*).

Source: [http://en.wikipedia.org/wiki/In\\_situ\\_hybridization](http://en.wikipedia.org/wiki/In_situ_hybridization)



**Photomicrograph** of a section of human epithelial tumor (condyloma) in which in situ hybridization with the DNA of the human papilloma virus type II (HPVII) was performed. **Observe dark staining of several nuclei, indicating the presence of the genome of this virus in the tumor, suggesting its possible participation in the genesis of the tumor.**



**Figure 2-13.** Photomicrograph of a section of human epithelial tumor (condyloma) in which in situ hybridization with the DNA of the human papilloma virus type II (HPVII) was performed. Observe dark staining in several nuclei, indicating the presence of the genome of this virus in this tumor, suggesting its possible participation in the genesis of the tumor. (Courtesy of JE Levi )



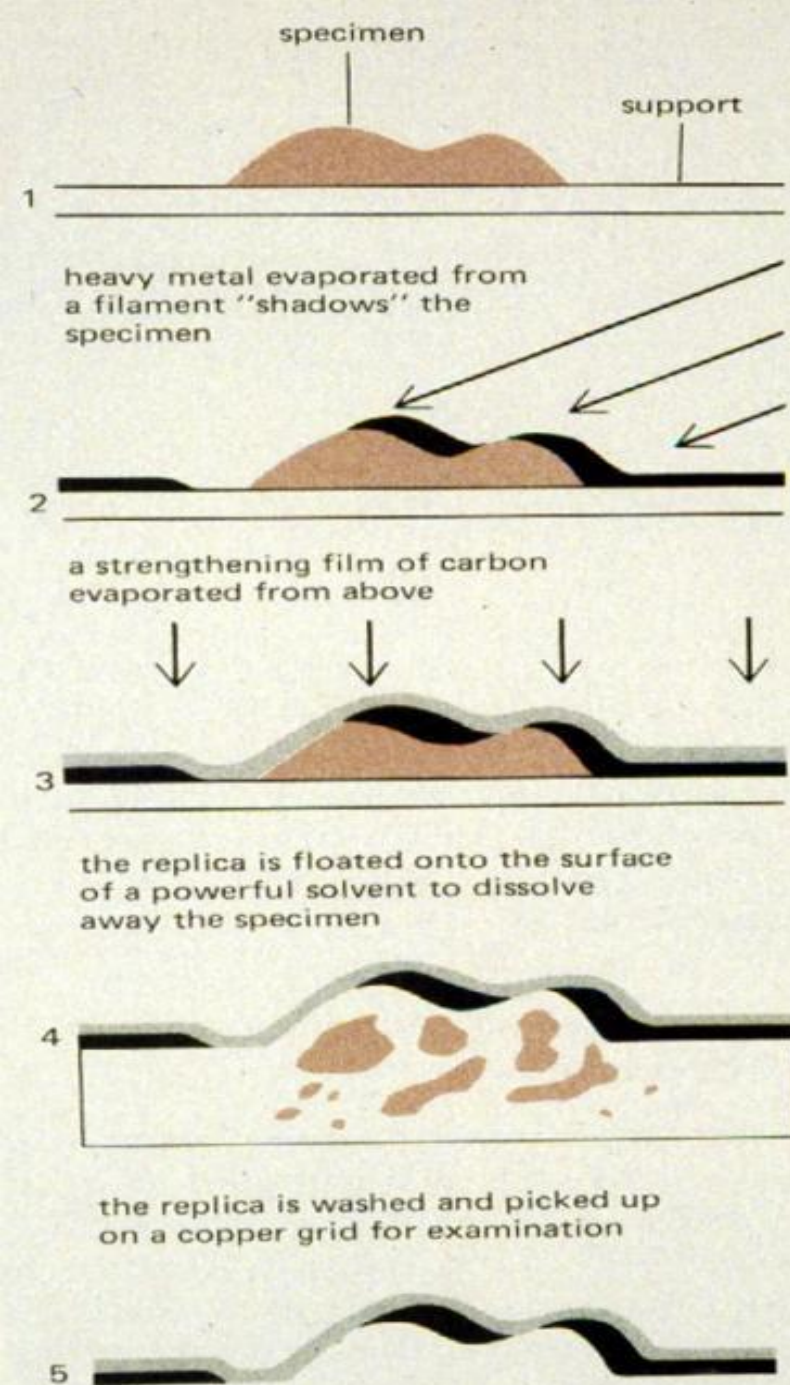
# Carbon Replica

1. Heavy metal evaporated from a filament “shadows” the specimen.

2. A strengthening film of carbon evaporated from above.

3. The replica is floated onto the surface of a powerful solvent to dissolve away the specimen.

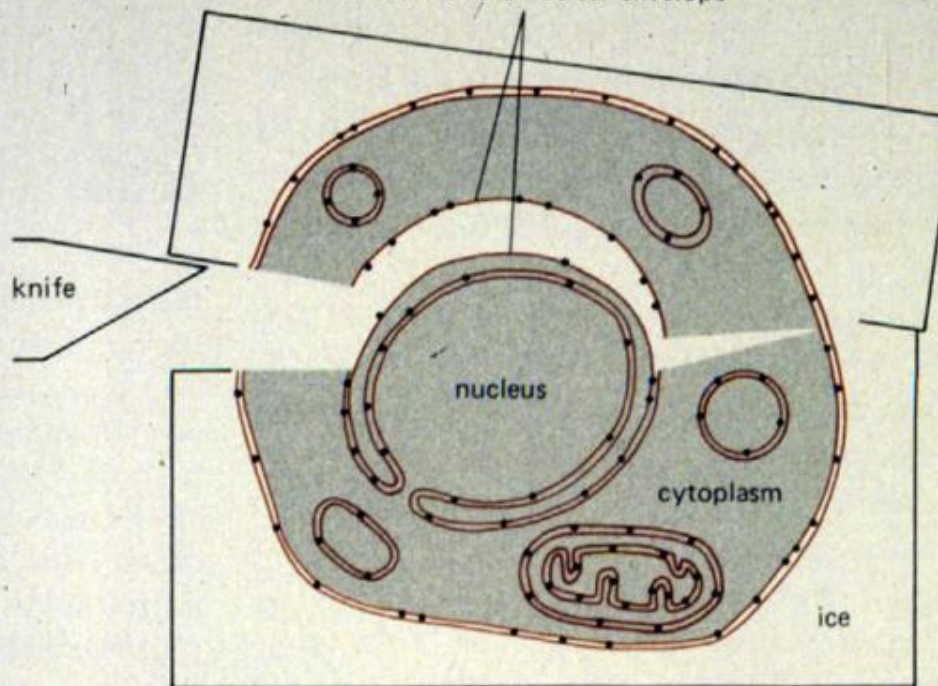
4. The replica is washed and picked up on a copper grid for examination.





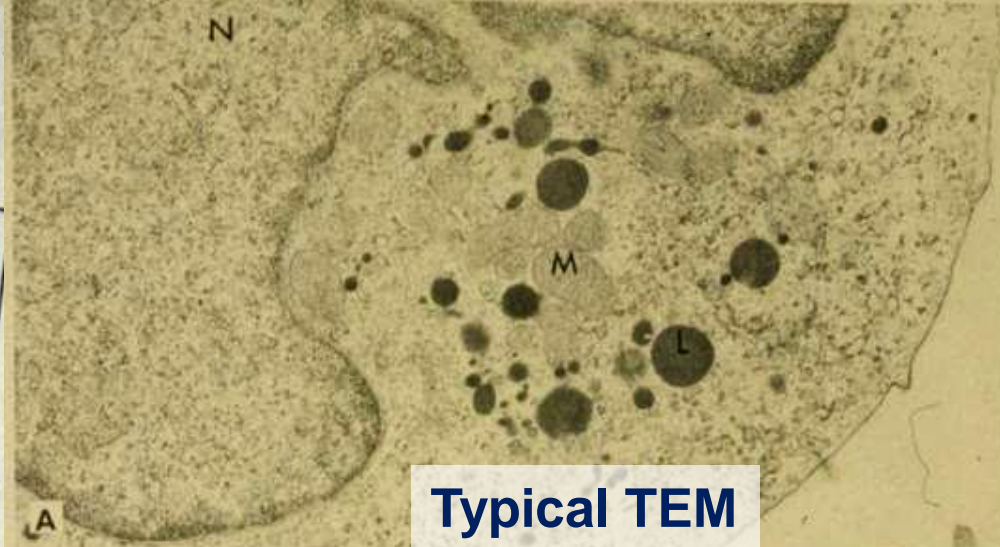
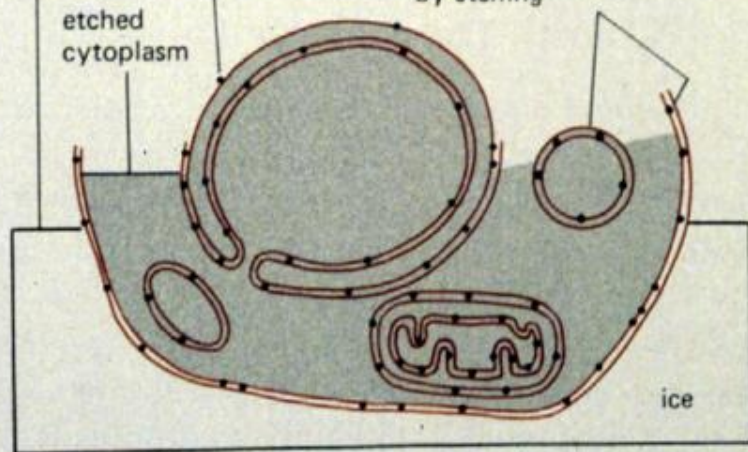
(A) FRACTURE

the 2 fracture faces of the outer membrane of the nuclear envelope

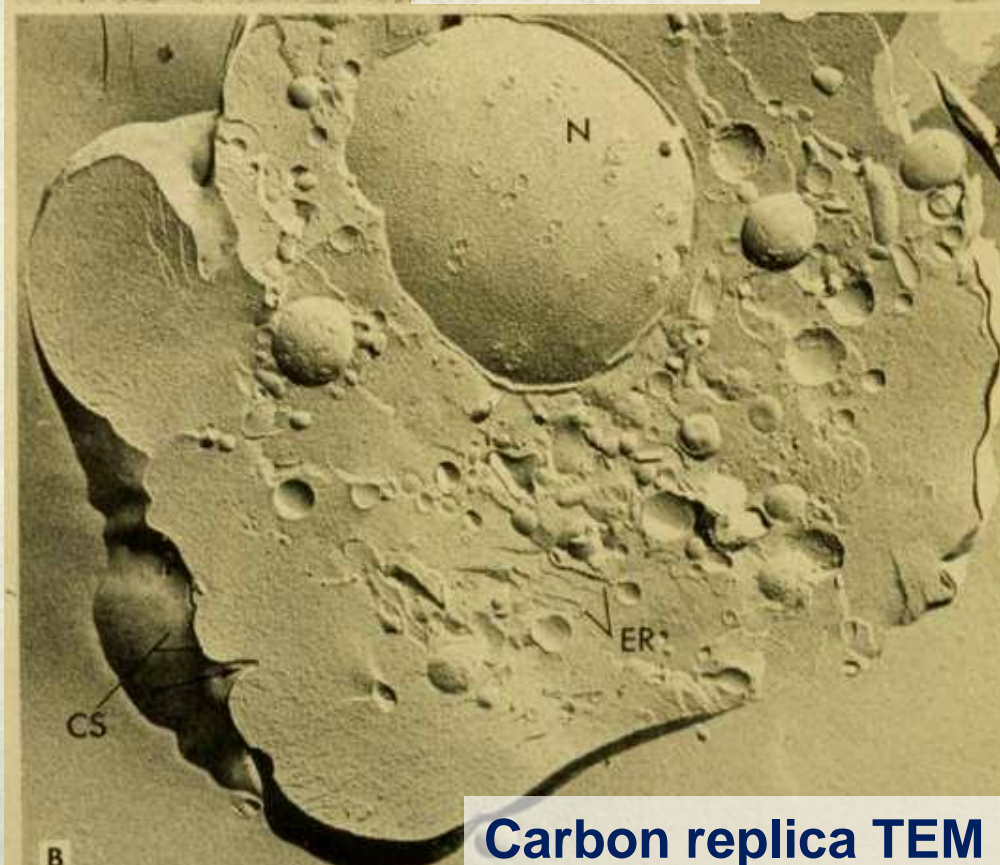


(B) ETCH

etched ice  
etched cytoplasm  
intramembrane particle  
outer surface of plasma membrane and of membrane-bounded organelle revealed by etching



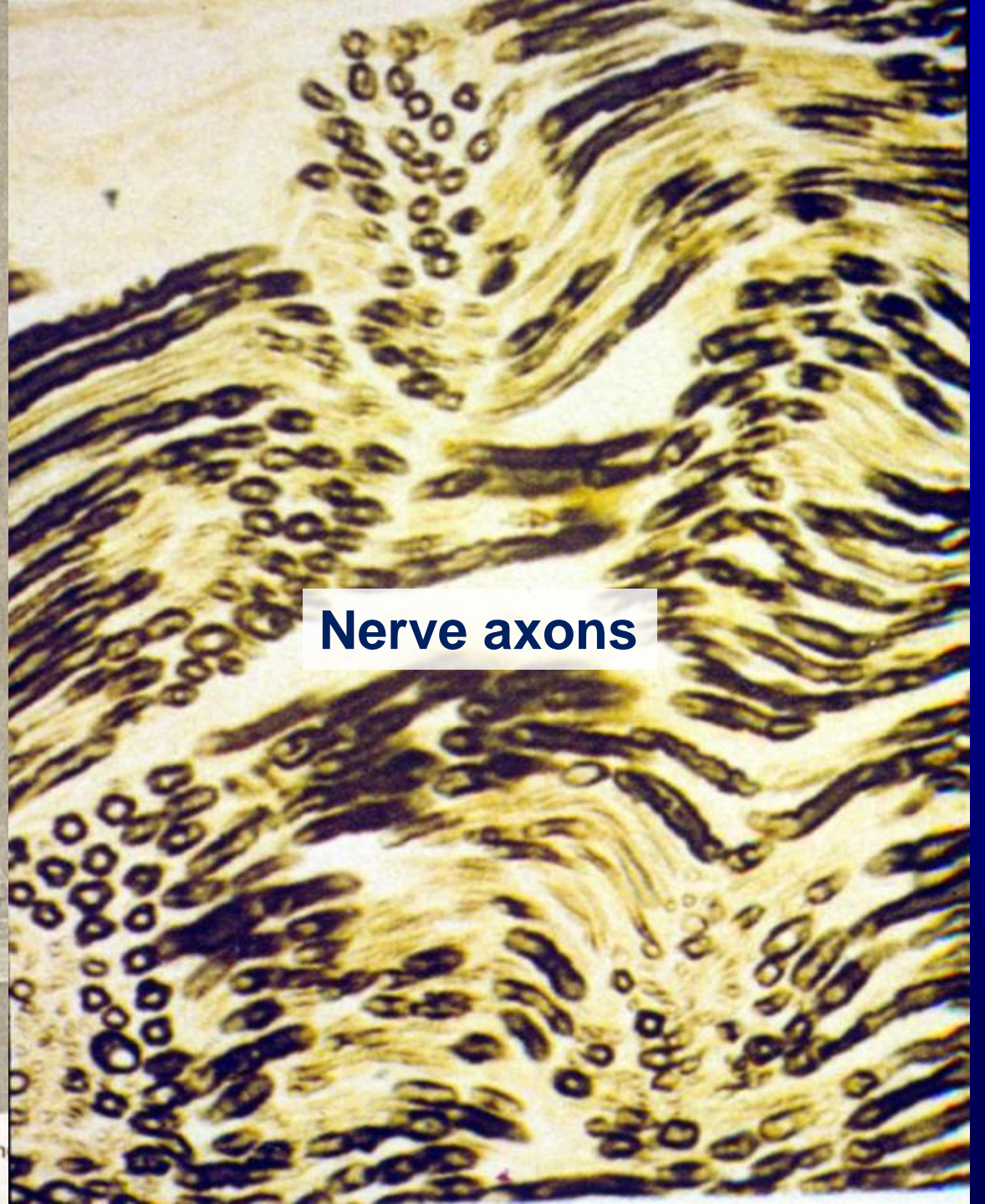
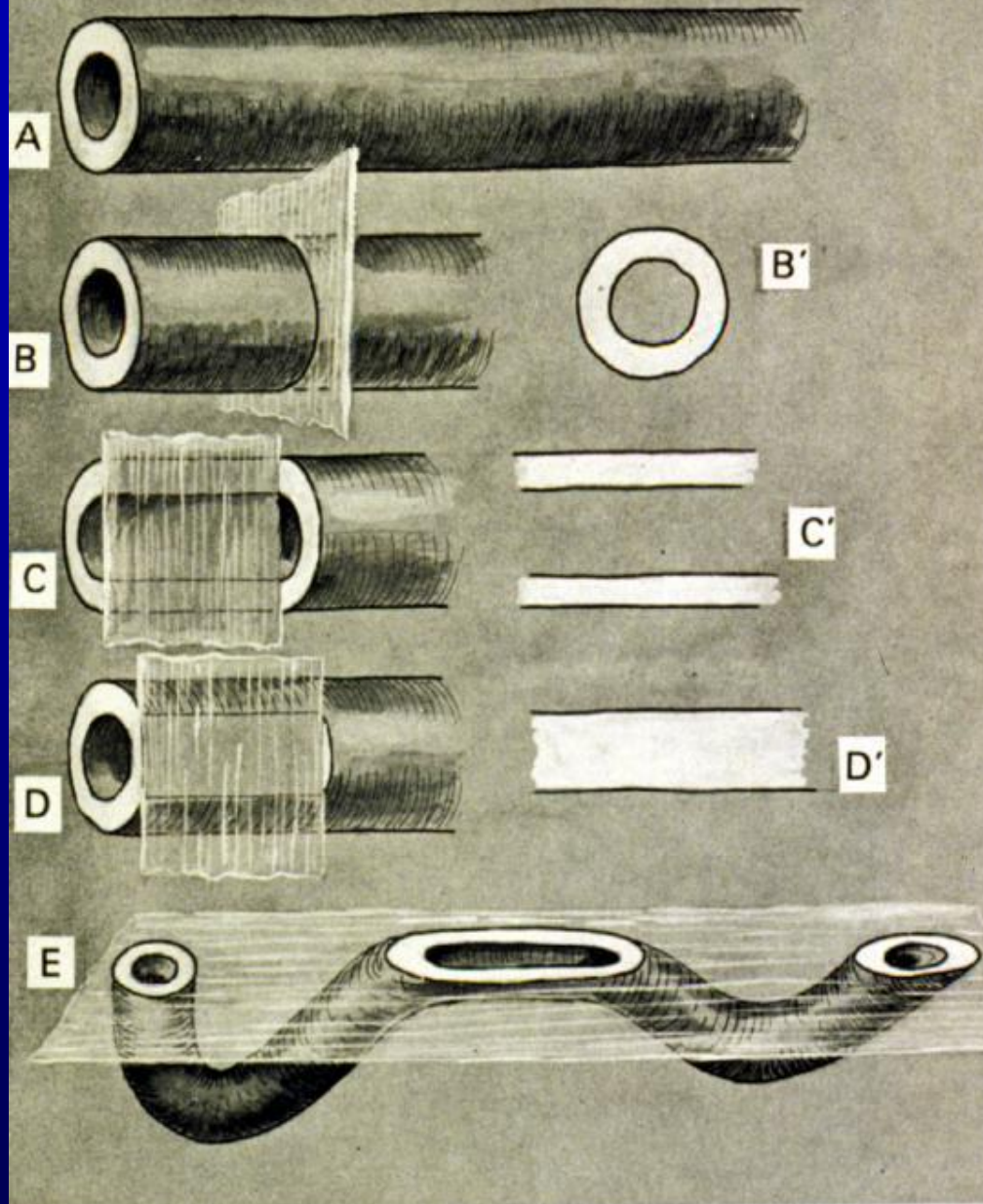
Typical TEM



Carbon replica TEM



# Section orientation



1-3. Various directions of cutting tubes. B, Transverse (cross) section; C, longitudinal section; D, tangential section; E, a curved tube cut.



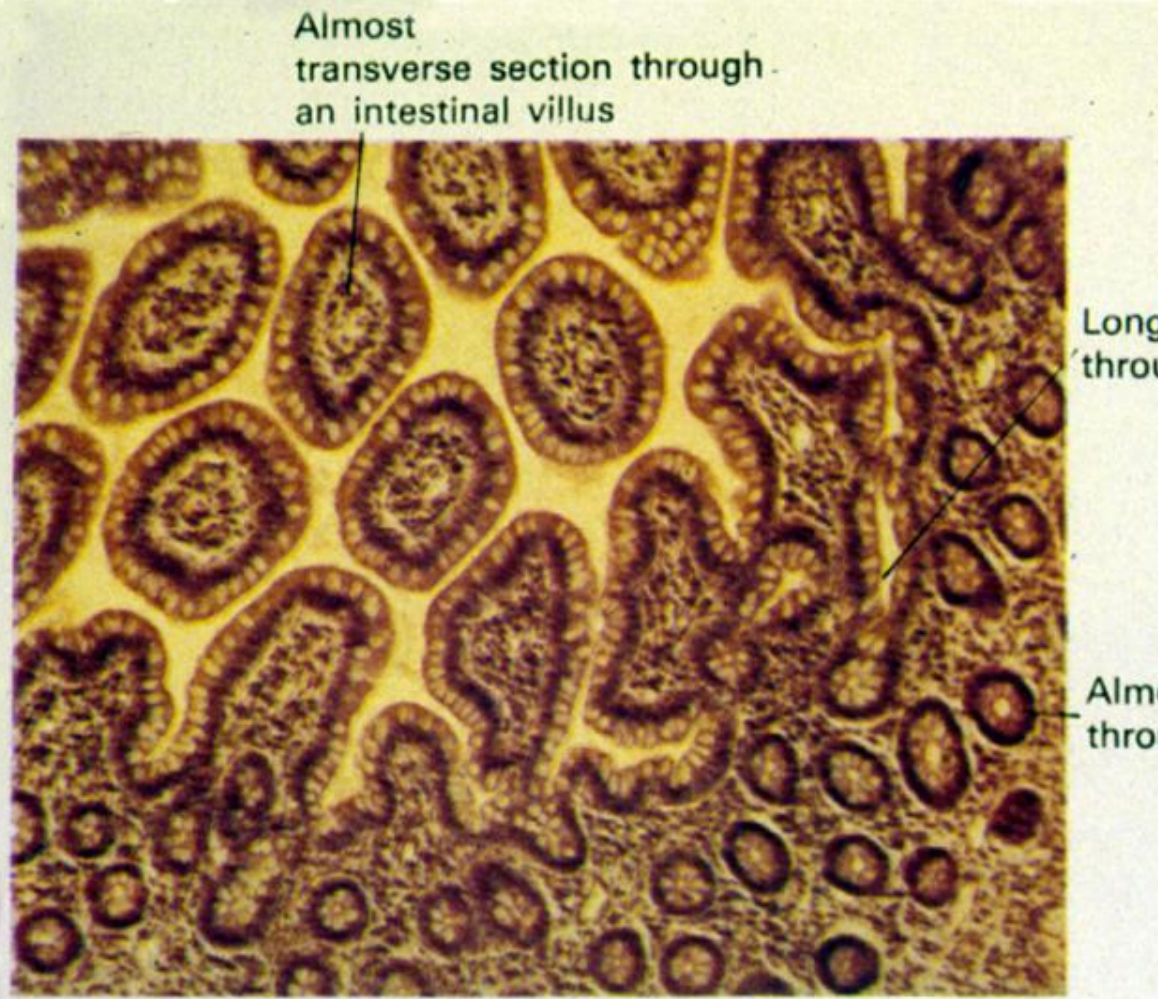
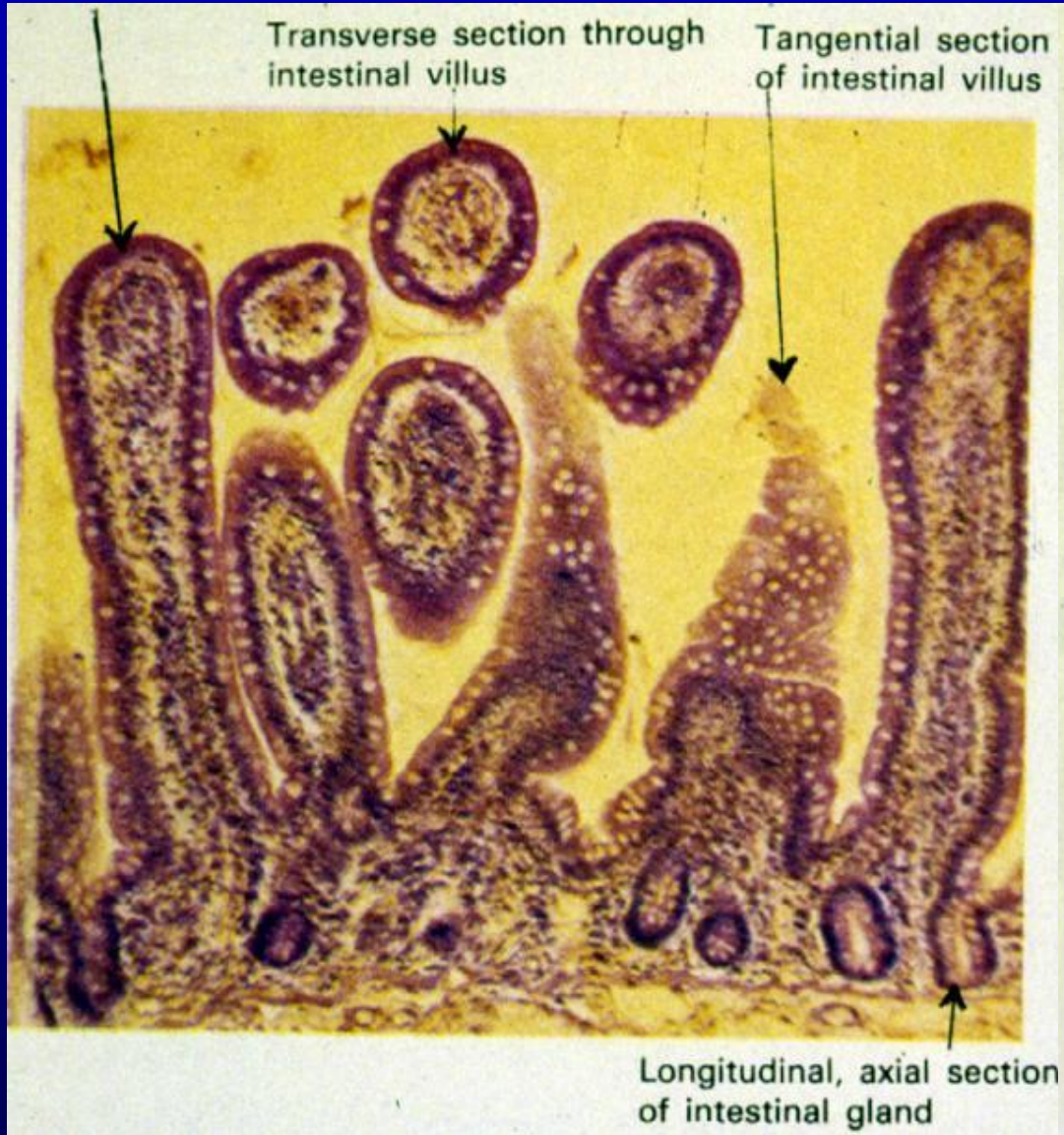


Fig. 1-5. Section almost tangential through the wall of the intestine. Villi and glands are cut nearly transversely. H&E.



# Section Orientation

Plane of section can make it look as if there are more layers of cells than there are, but it can never make it look like there are less than the actual number.

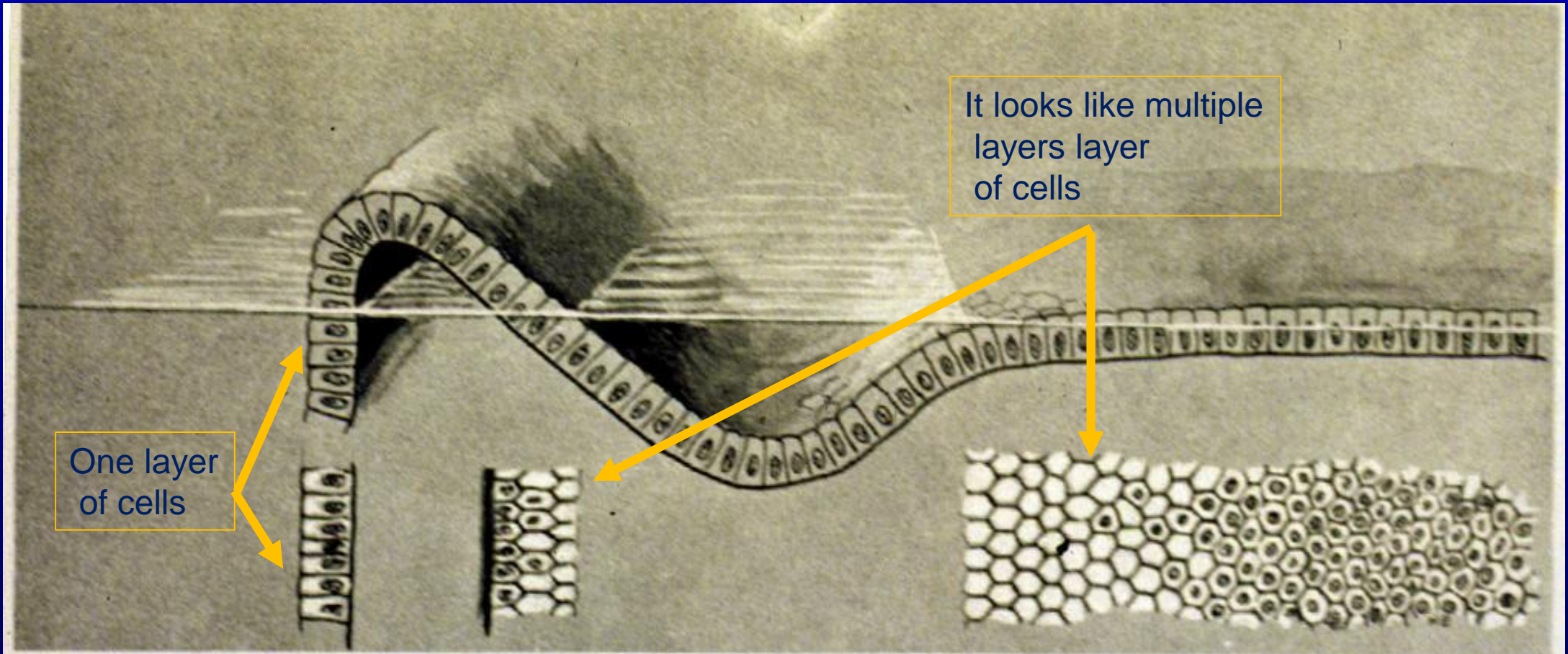
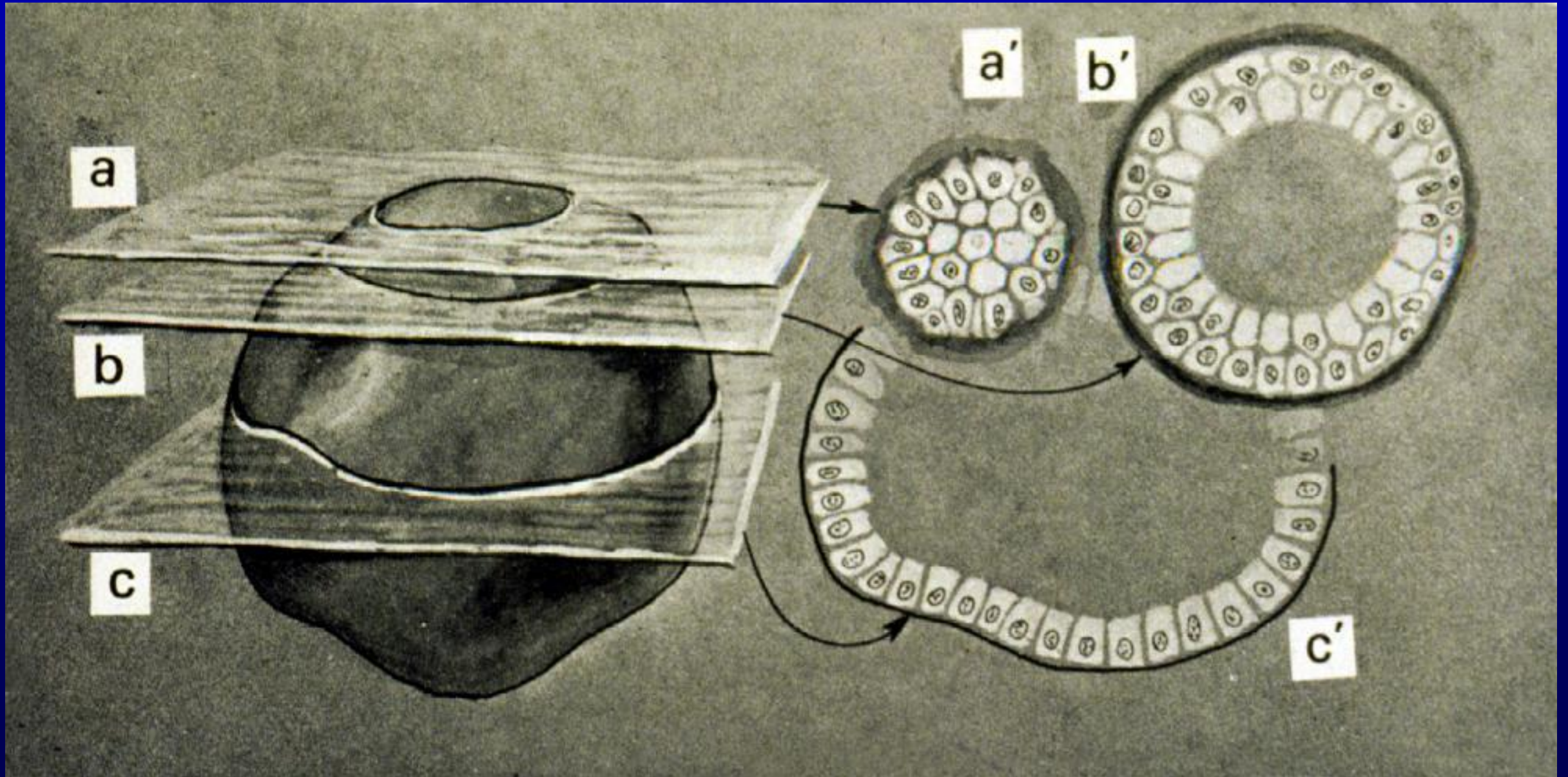


Fig. 1-7. Cutting a sheet, thicker than the slice. The particular sheet shown here is a simple, columnar epithelium. One can observe that the number of layers appears to change with the direction of cutting. At the right, one notices that it will depend on the level of cutting whether cells appear to have a nucleus or not.



# Section Orientation

Plane of section can make it look as if there are more layers of cells than there are, but it can never make it look like there are less than the actual number.





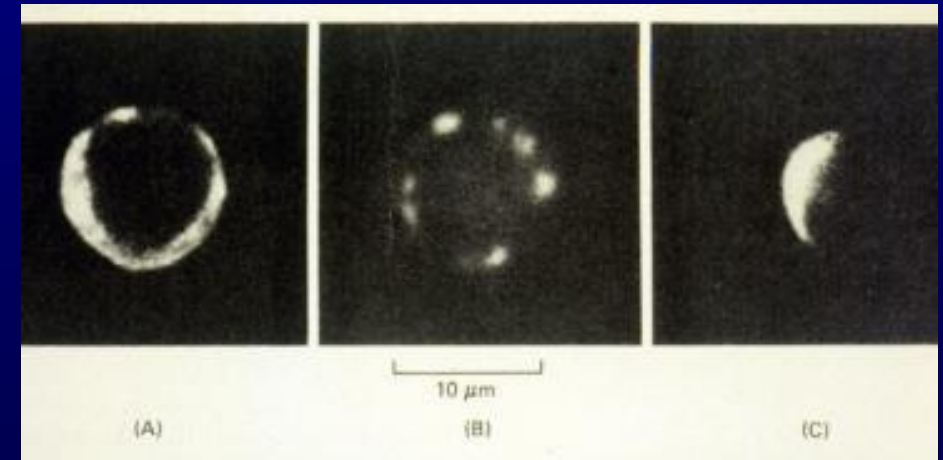
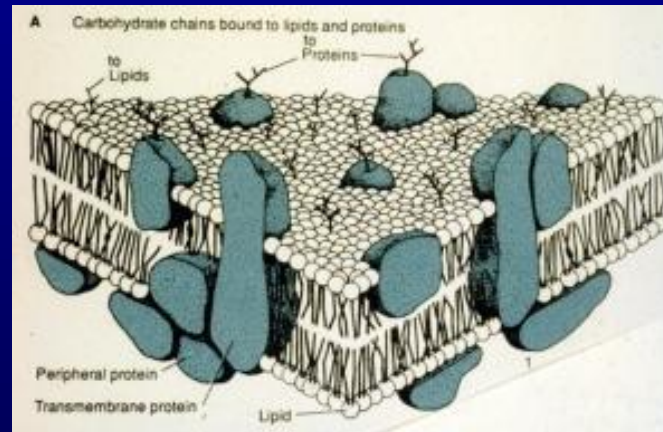
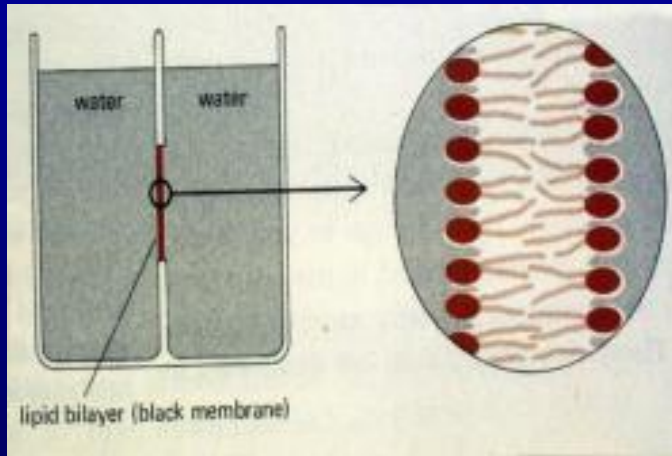






Next time

# Membrane and Receptors





Many illustrations in these VIBS Histology YouTube videos were modified from the following books and sources: Many thanks to original sources!

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