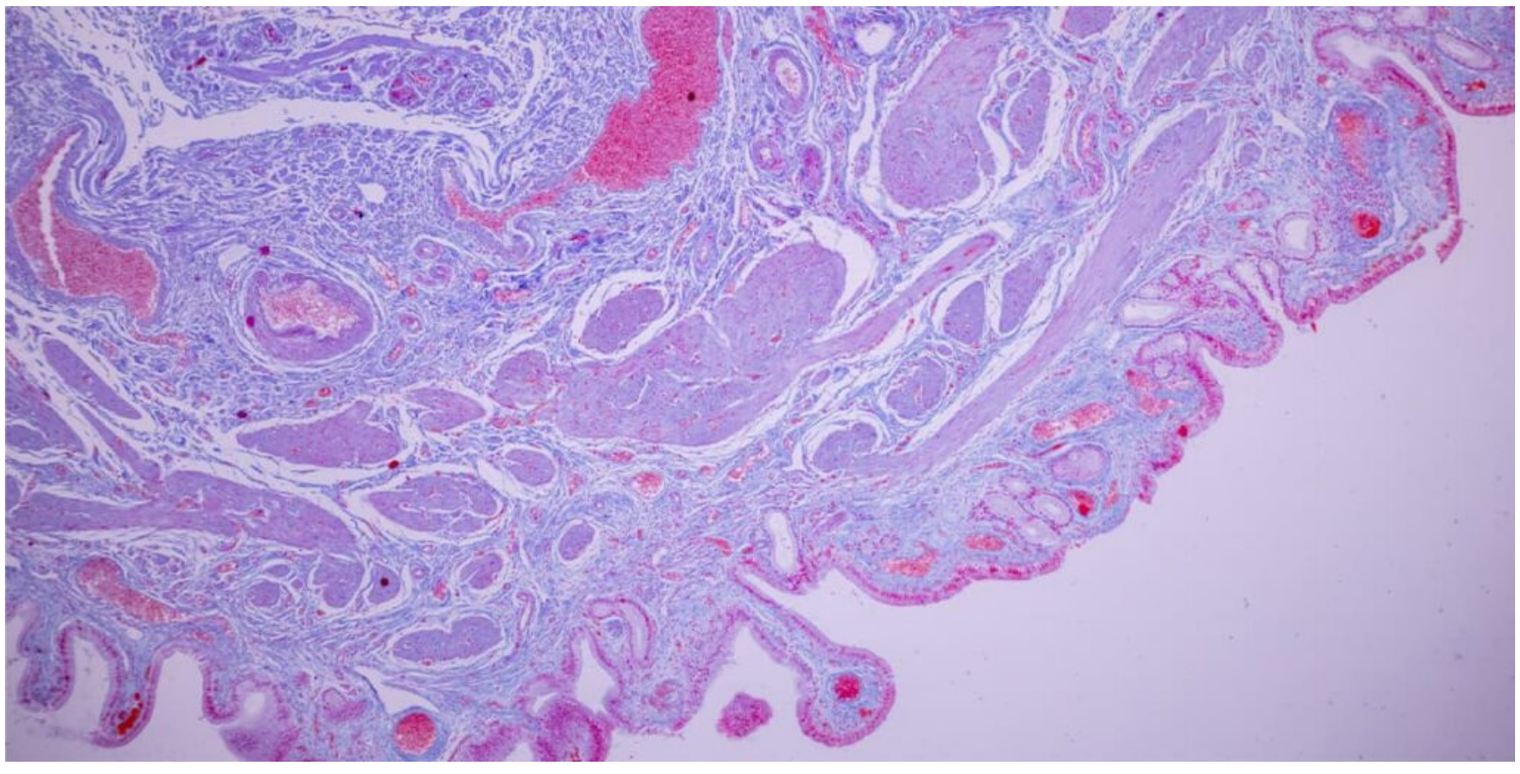
tissue preparation histochemistry &cytochemistry

Assistant Lecturer : fatima tawfik alkhuzaie

جامعة المستقبل/ كلية الطب

college of medicine



Histology: is the study of the tissues of the body and how these tissues are
arranged to constitute organs. Tissues are made of two interacting
components: cells and extracellular matrix (ECM). The main functions of
extracellular matrix are:
1- Provide a mechanical support for the cells,
2- Transport nutrients to the cells,
3- Carry away catabolites and secretory products.

Cells produce the extracellular matrix, but it also influenced and sometimes controlled by molecules of the matrix. Many components of the
matrix recognized by and attaching to receptors present on cell surfaces
which are molecules that cross the cell membranes and connect to structural
components of the intracellular cytoplasm. Each of the fundamental tissues
(except the central nervous system) is formed by several types of cells and
typically by specific associations of cells and extracellular matrix.

Preparation of Tissues for Study

It is the preparation of histological sections or tissue slices that can be studied with the aid of the light microscope. They must be sectioned to obtain thin, transparent sections and then attached to glass slides before they can be examined. The ideal microscope tissue preparation should be preserved so that the tissue on the slide has the same structure and molecular composition as it had in the body.

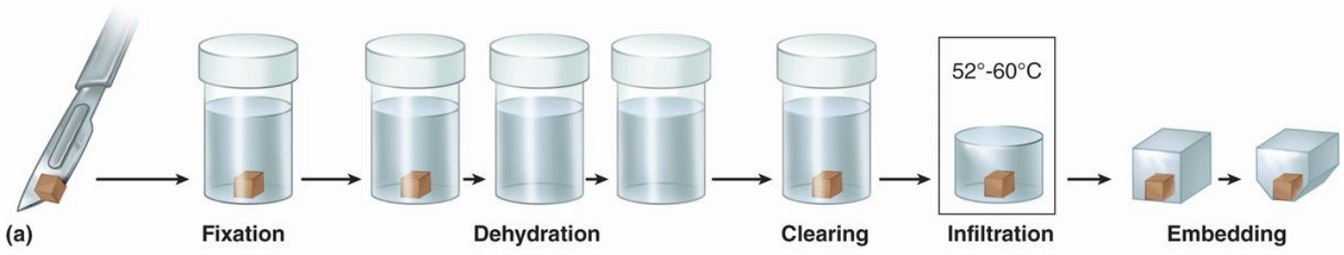
The basic steps used in tissue preparation for histology are:

Figure 1-1: steps of tissue preparation

1- Fixation

If a permanent section is desired, tissues must be fixed. Fixation is used to:

- Terminate cell metabolism,
- Prevent enzymatic degradation of cells and tissues by autolysis (self-digestion).
- Kill pathogenic microorganisms such as bacteria, fungi, and viruses.



• Harden the tissue as a result of either cross-linking or denaturing protein
molecules.
One of the best fixatives for routine light microscopy is formalin, a buffered
isotonic solution of 37% formaldehyde.
Due to high resolution afforded by the electron microscope, a double fixation
procedure, using a buffered glutaraldehyde solution followed by a second fixation in buffered osmium tetroxide, is a standard procedure in preparations
for fine structural studies. The effect of osmium tetroxide is to preserve and
stain lipids and proteins.

Fixation by freezing

It involves the submission of the tissues to rapid freezing. A freezing microtome (cryostat) is then used to section the frozen block with tissue. This
method allows the rapid preparation of sections and it is also effective in the
histochemical study of very sensitive enzymes or small molecules and useful
when structures containing lipids are to be studied (no xylene).

2- Embedding & Sectioning

Tissues are usually embedded in a solid medium to facilitate sectioning. Embedding substances gives a rigid consistency to the tissue. Embedding materials include paraffin and plastic resins. Paraffin is used routinely for light microscopy; resins are used for both light and electron microscopy. It involves two main steps: dehydration and clearing. The water is first extracted by bathing tissue successively in a graded series of mixtures of ethanol and water, usually from 70% to 100% ethanol (dehydration). The ethanol is then replaced with other solvent, xylene. As the tissues are infiltrated with this solvent, they generally become transparent (clearing). Once the tissue absorbs the solvent, it is placed in melted paraffin in an oven, typically at 52–60°C. The heat causes the solvent to evaporate, and the spaces within the tissues become filled with paraffin. The tissue together with its impregnating paraffin hardens after removal from the oven. The hard blocks containing the tissues are then placed in the microtome and are sliced by the microtome's steel or glass blade into sections 1 to10 micrometers thick, (micrometer (1um) = 1/1,000 of a millimeter (mm) = 10_{-6} m). The sections are floated on water and then transferred to glass slides to be stained .

3- Staining

Sections must typically be stained or dyed because most tissues are colorless. Most of these dyes behave like acidic or basic compounds and have a tendency to form electrostatic (salt) linkages with ionizable radicals of the tissues. Tissue components with a net negative charge (anionic) stain more readily with basic dyes and are termed basophilic; cationic components, such as proteins with many ionized amino groups, have affinity for acidic dyes and are termed acidophilic. Basic dyes like toluidine blue, alcian blue, and methylene blue and Hematoxylin stain the nucleic acids, glycosaminoglycans, and acid glycoproteins. Acid dyes (eg, orange G, eosin, acid fuchsin) stain the acidophilic components of tissues such as mitochondria, secretory granules, and collagen. Combination of hematoxylin and eosin (H&E) is used most commonly. Hematoxylin stains DNA of the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of cartilage) blue. In contrast, eosin stains other cytoplasmic components

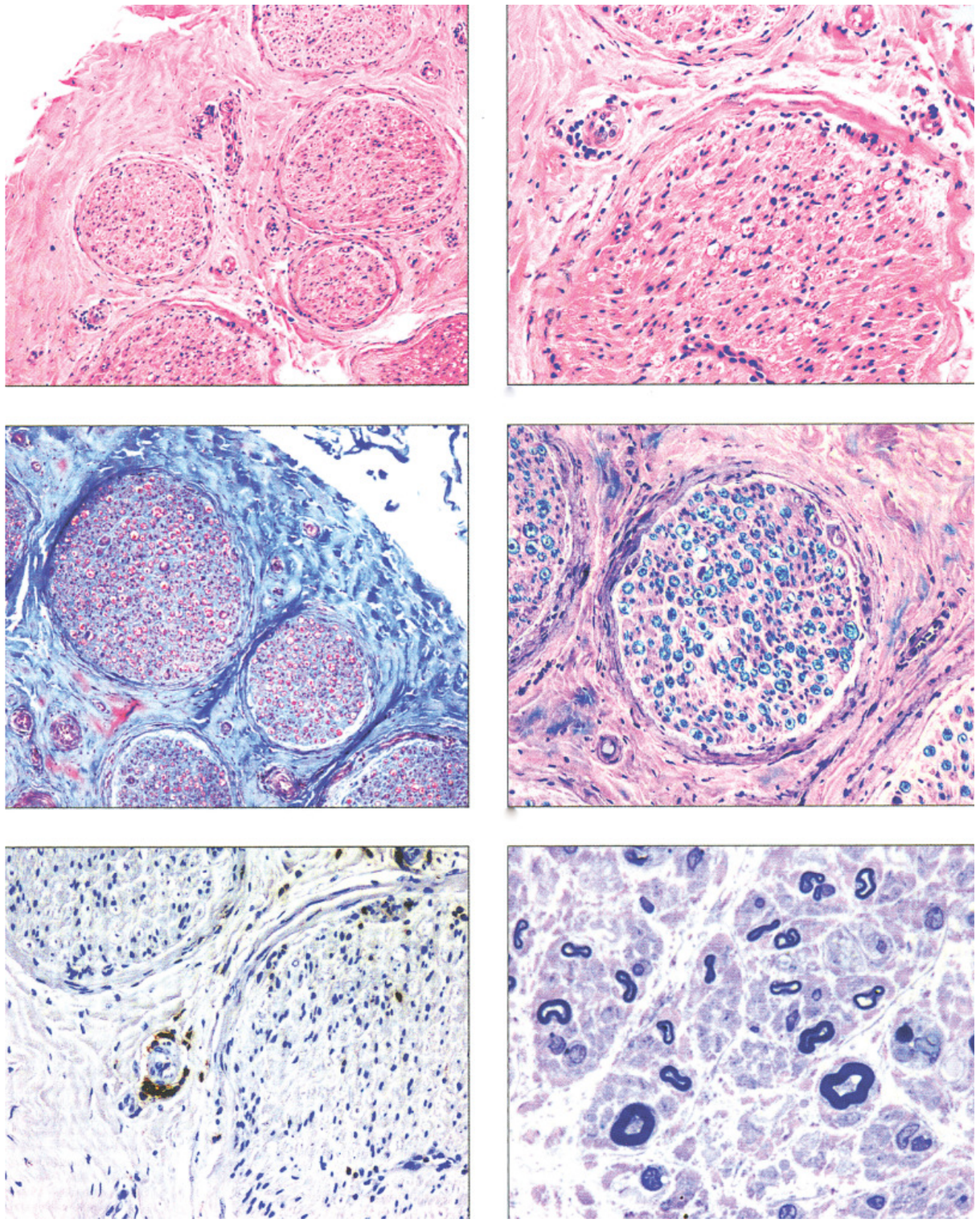
Medical applications

Biopsies are tissue samples removed during surgery or routine medical
procedures. In the operating room or medical center, biopsies are fixed in vials
of formalin for later processing and microscopic analysis in a pathology
laboratory. If results of such analyses are required before the medical
procedure is completed, for example to know whether a growth is malignant
before the patient is closed, a much more rapid processing method is used.

The biopsy is rapidly frozen in liquid nitrogen, preserving cell structures and
at the same time making the tissue hard and ready for sectioning. The frozen
sections are placed on slides for rapid staining and microscopic examination
by a pathologist

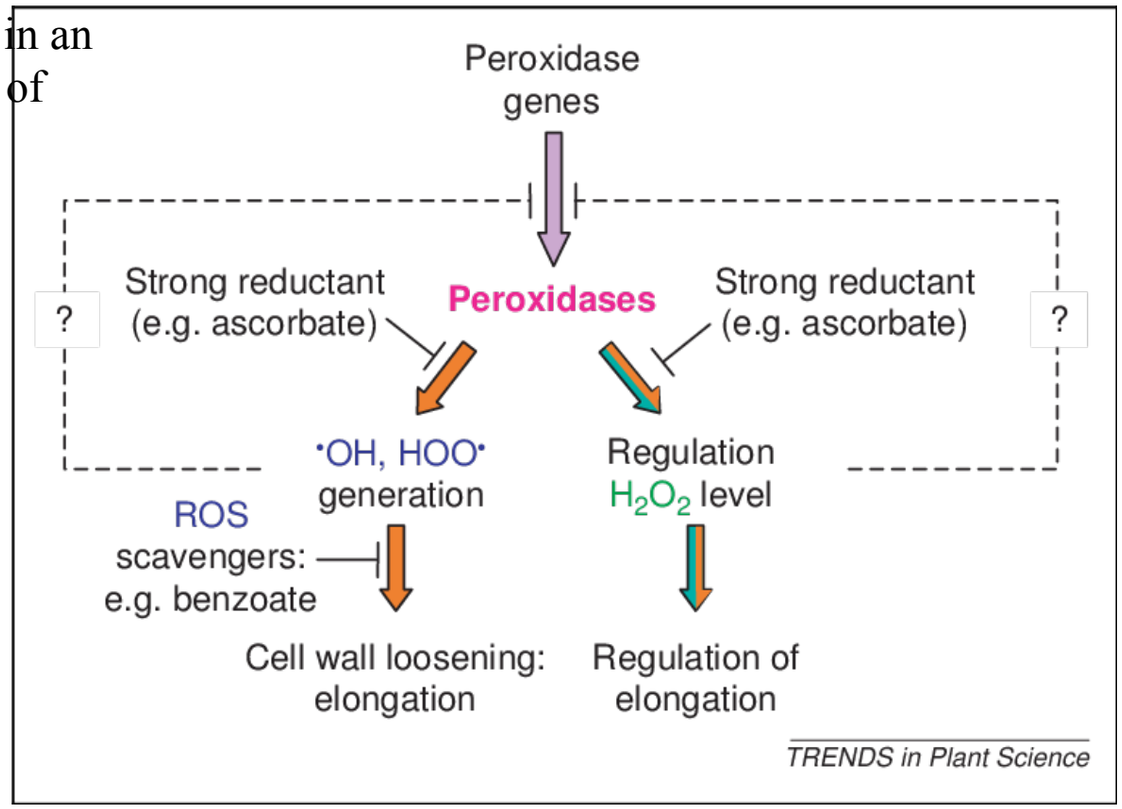
Histochemistry & Cytochemistry
.The terms **histochemistry** and **cytochemistry** indicate
methods for localizing cellular structures in tissue sections
using unique enzymatic activity present in those
structures

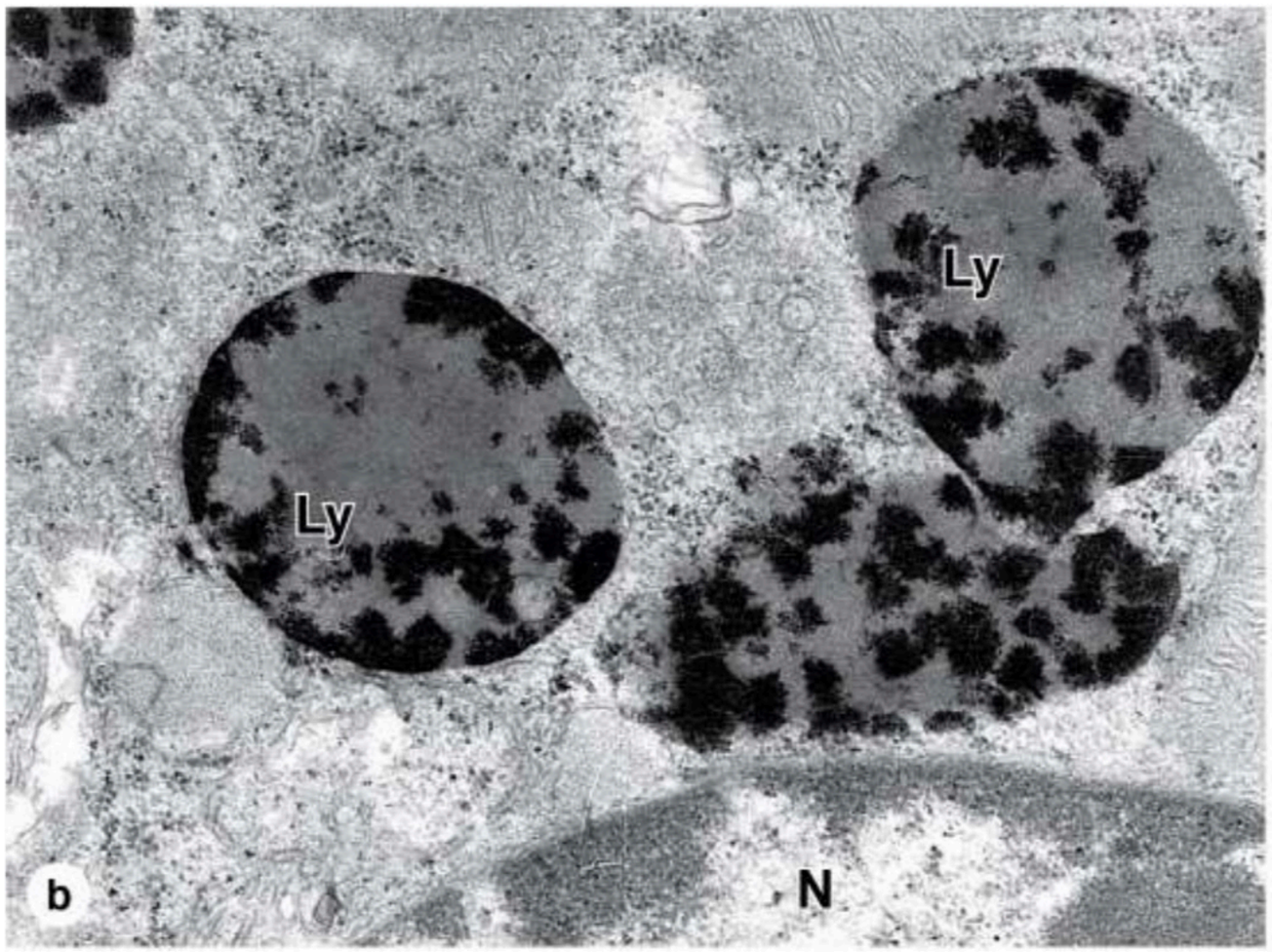
.To preserve these enzymes histochemical procedures are
usually applied to unfixed or mildly fixed tissue, often
sectioned on a cryostat to avoid adverse effects of heat and
paraffin on enzymatic activit



Enzyme histochemistry steps:
(1) Tissue sections are immersed in a solution that contains the substrate of the enzyme to be localized;
(2) The enzyme is allowed to act on its substrate;
(3) At this stage or later, the section is put in contact with a marker compound;
(4) This compound reacts with a molecule produced by enzymatic action on the substrate (the product)
(5) The final reaction product, which must be insoluble and which is visible by light or electron microscopy only if it is colored or electron-dense, precipitates over the site that contains the enzyme.
• When examining such a section in the microscope, one can see the cell regions (or organelles) covered with colored or electron-dense material.

Example:
Detection of Peroxidase enzyme: sections of adequately fixed tissue are incubated in a solution containing **hydrogen peroxide** and **3,3'-diamino-azobenzidine (DAB).**
The latter compound is oxidized in the presence of peroxidase, resulting in an insoluble, brown, electron-dense precipitate that permits the localization of peroxidase activity by light and electron microscopy.





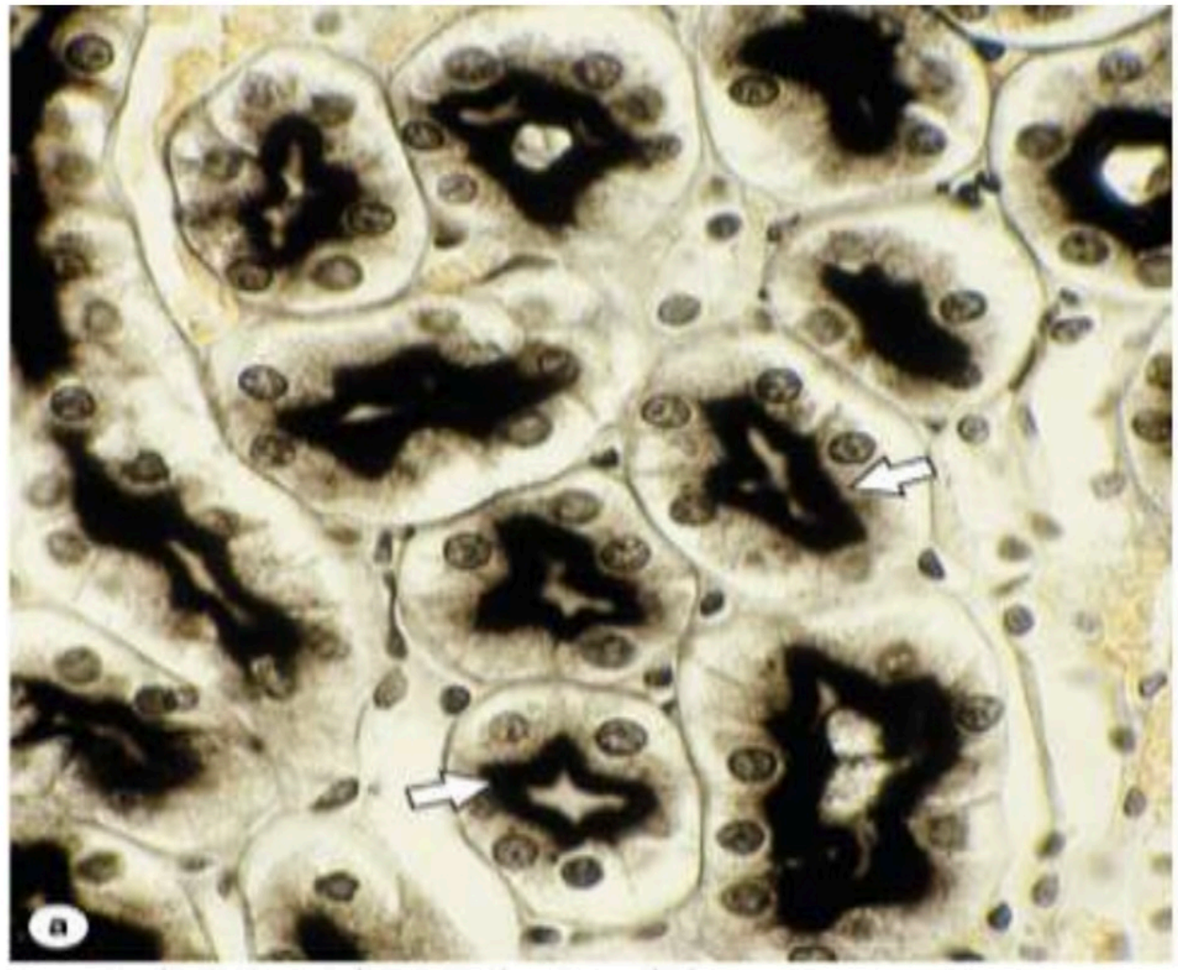


Figure : Enzyme histochemistry, (a): Micrograph of cross sections of kidney tubules treated histochemically by the Gomori method for alkaline phosphatases show strong activity of this enzyme at the apical surfaces of the cells at the lumen of the tubules (arrows). (b): TEM image of a kidney cell in which acid phosphatase has been localized histochemically in three lysosomes (Ly) near the nucleus (N). The dark material within these structures is lead phosphate that precipitated in places with acid phosphatase activity. X25,000.


Thank
you!