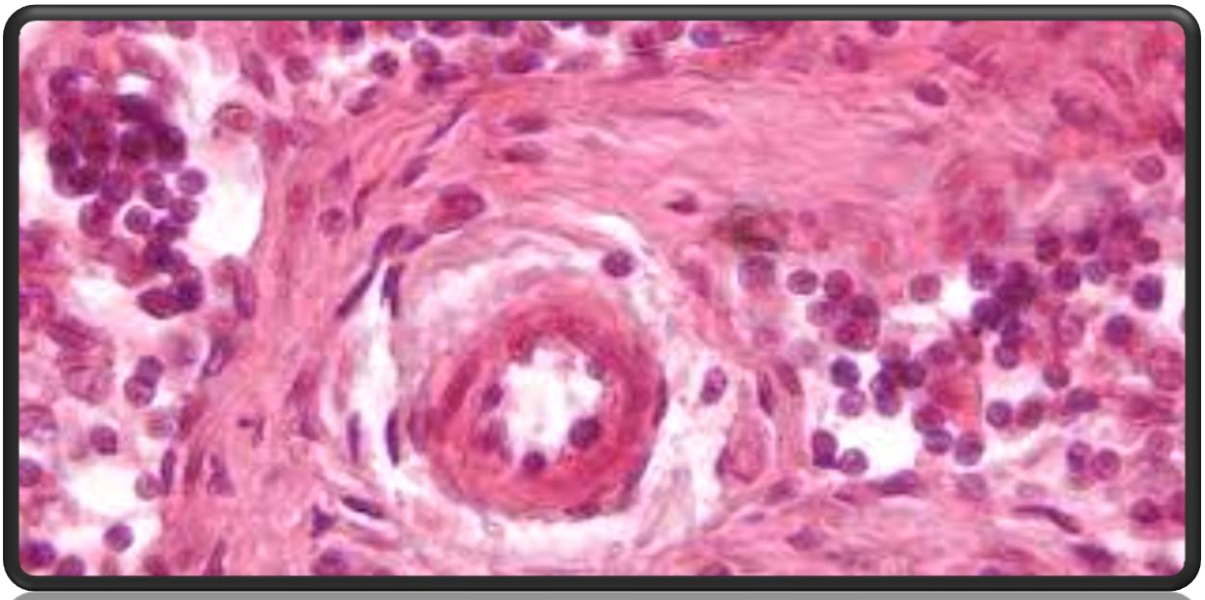

# Introduction to Histology


BY

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# Histology lec.1

Histology is the study of the tissues of the body and how these tissues are arranged t constitute organs . The Greek root histo can be translated as either " tissue " or " web " and both translations are appropriate because most tissues are webs of interwoven filaments and fibers , both cellular and noncellular , with membranous linings . Histology involves all aspects of tissue biology with the focus on how cells ' structure and arrangement optimize functions specific to each organ .

Tissues are made of two interacting components : **cells** and **extracellular matrix** .

**The extracellular matrix** consists of many kinds of molecules , most of which are highly organized and form complex structures , such as collagen fibrils and basement membranes .

The main functions of the matrix are:

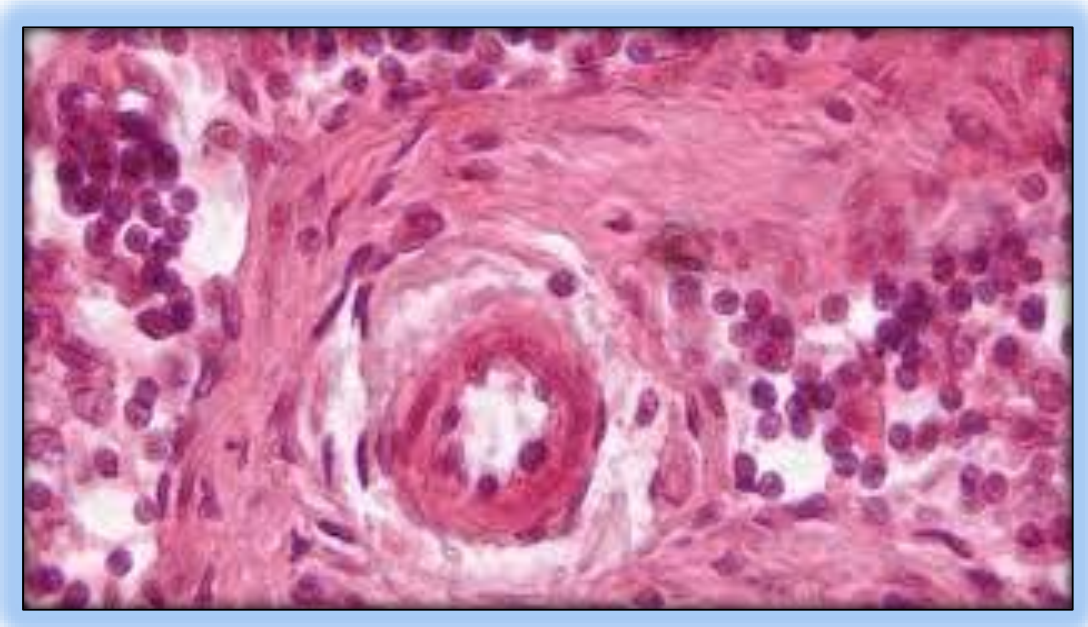
1-to furnish mechanical support for the cells to trans port nutrients to the cells

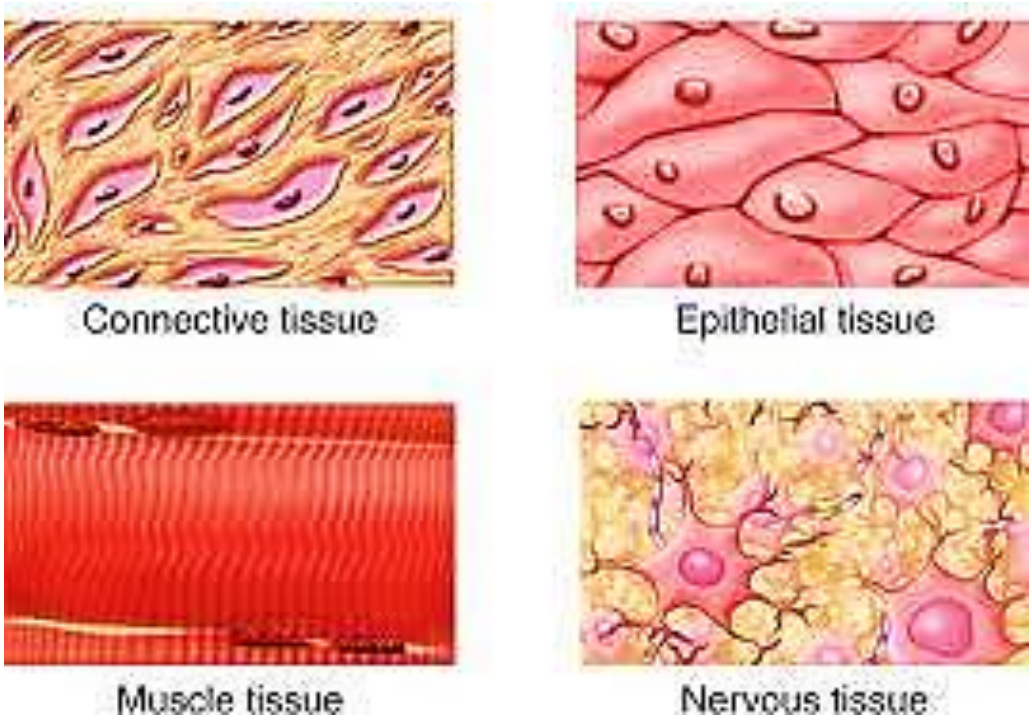
2- to carry away catabolites and secretory products . although the cells produce the extracellular matrix , they are also influenced and sometimes controlled by molecules of the matrix . There is , thus , an intense interaction between cells and matrix .

***Each of the fundamental tissues*** is formed by several types of cells and typically by specific associations of cells and extracellular matrix.

Most organs are formed by an ordered combination of several tissues, except the central nervous system, which is formed almost solely by nervous tissue. The precise combination of these tissues allows the functioning of each organ and of the organism as a whole.

The small size of cells and matrix components makes histology dependent on the use of microscopes. Advances in chemistry, molecular biology, physiology, immunology, and pathology and the interactions among these fields - are essential for a better knowledge of tissue biology. Familiarity with the tools and methods of any branch of science is essential for a proper under standing of the subject.

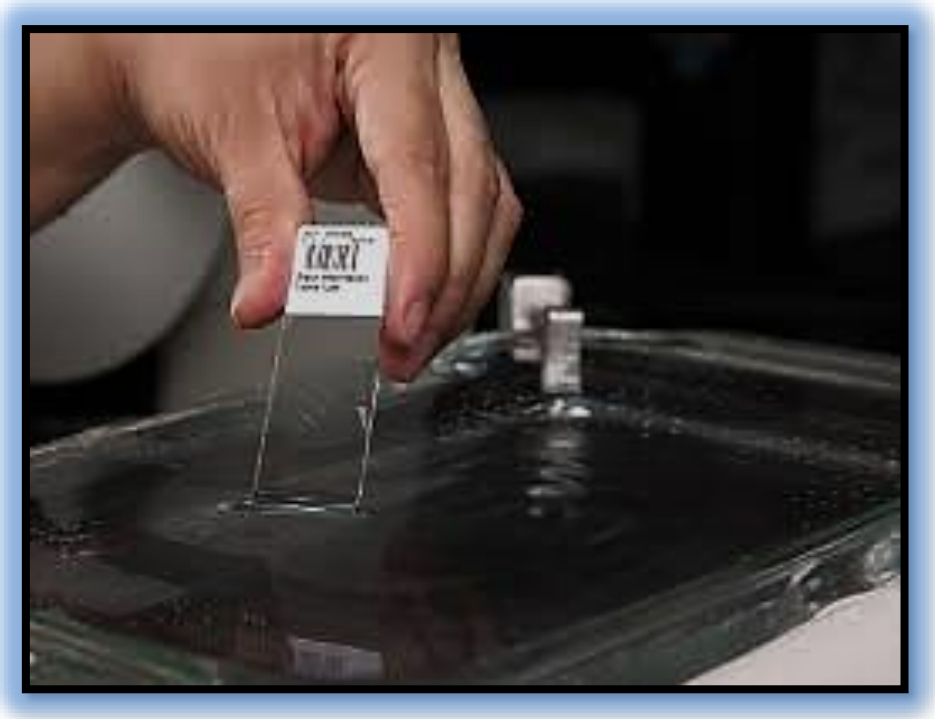





## *Preparation of tissue for study*

The most common procedure used in the study of tissues is the preparation of histological sections or tissue slices that can be studied with the aid of the light microscope. Under the light microscope, tissues are examined via a light beam that is transmitted through the tissue. Because tissues and organs are usually too thick for light to pass through them, they must be sectioned to obtain thin, translucent sections and then attached 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. However, as a practical matter this is seldom feasible, artifacts, distortions, and loss of components due to the preparation process are almost always present.



# *Procedures*

## *Fixation*

Tissues must be immersed in fixation immediately after removal from the body.

10% Neutral Buffered Formation is the routine fixative.

## *Tissue Processing*

Tissue Processing consist of 3 steps:

1- Dehydration

2- Clearing

3- Infiltration (lasting between 8 to 12 hours)

## *Tissue Embedding*

***Embedding*** is enclosing the tissue in the infiltration medium (paraffin) used for processing and then allowing the medium to solidify.

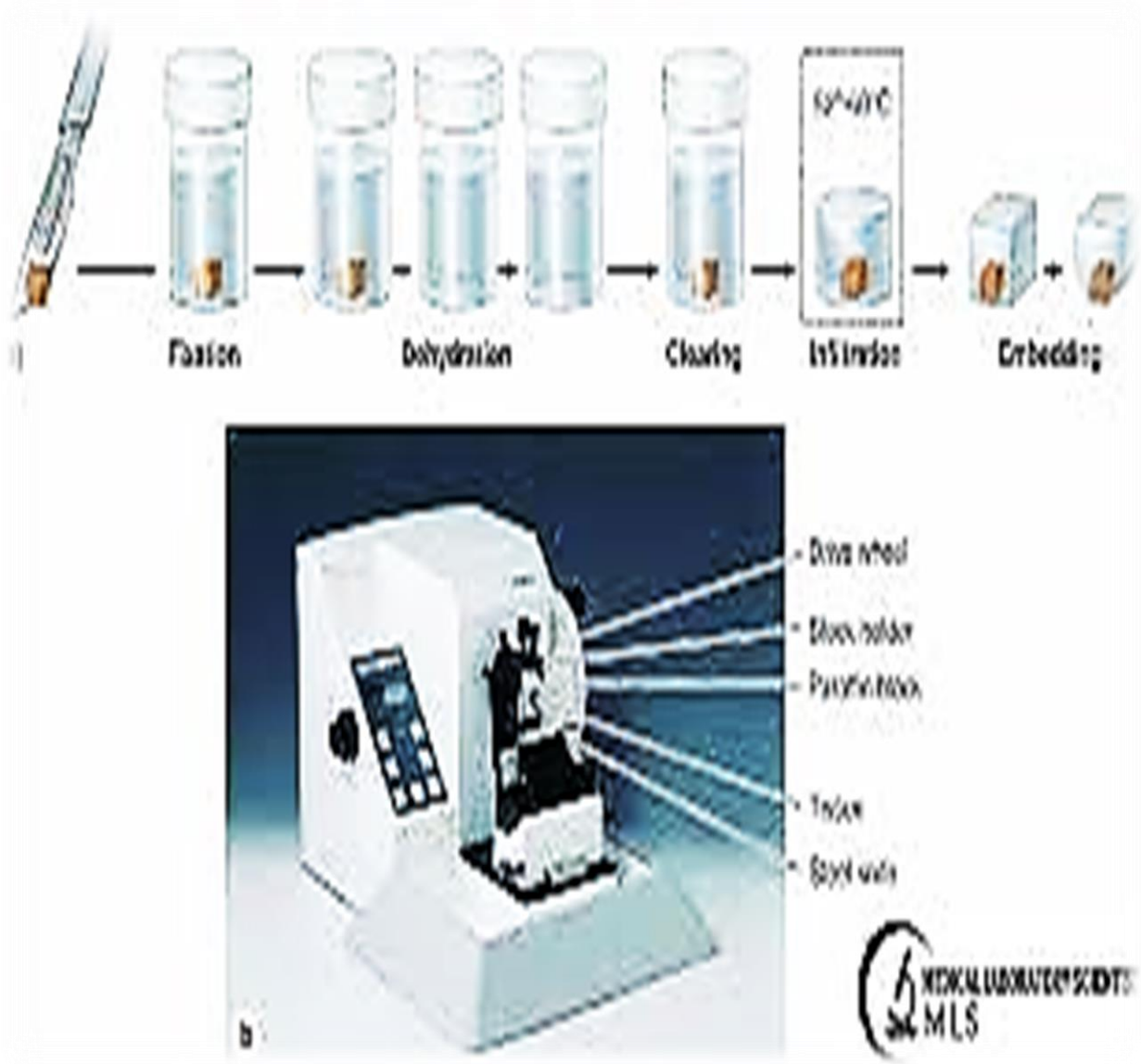
## *Sectioning with a microtome*

The microtome advances the tissue block , toward a sharp knife. The tissue is moved in an up and down motion to cut the tissue

into a presct number of micrometres'. The Paraffin and tissue shavings are shaved off like a ribbon.

## *Tissue Staining*

## *Cover slipping, mounting .*



## *Fixation*

If a permanent section is desired, tissues must be fixed. To :

1-avoid tissue digestion by enzymes present within the cells (autolysis) or by bacteria

2-to preserve the structure and molecular composition, pieces of organs should be treated before, or as soon as possible after, removal from the body .

This treatment fixation can be done by chemical or, less frequently, physical methods. In chemical fixation the tissues are usually immersed in solutions of stabilizing or cross-linking agents called fixatives. Because the fixative needs some time to fully diffuse into the tissues, the tissues are usually cut into small fragments before fixation to facilitate the penetration of the fixative . One of the best fixatives for routine light microscopy is formalin a buffered isotonic solution of 37% formaldehyde

**Embedding & Sectioning:**

Tissues are usually embedded in a solid medium to facilitate separation.

To obtain thin sections with the microtome, tissues mustbe infiltrated after fixation with embedding substances that impart a rigid consistency to the tissue. Embedding materials include **paraffin and plastic resins**.

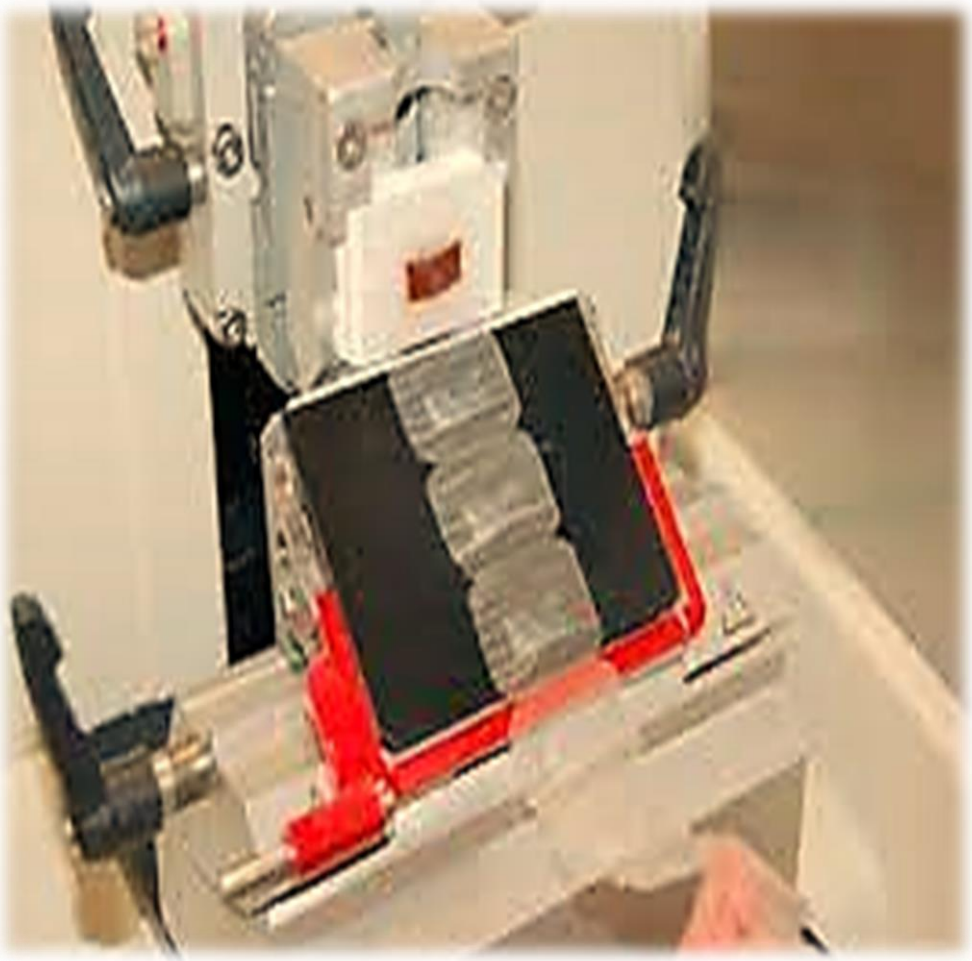
- Paraffin is used routinely for light microscopy; Resins are used for both light and electron microscopy.

- The process of paraffin embedding is ordinarily preceded by ***two main steps: dehydration and clearing.***
- The water is first extracted from the fragments to be embedded by bathing them successfully in a graded series of mixtures of ethanol and water, usually from 70% to 100% ethanol (dehydration). The ethanol is then replaced with a solvent miscible with both alcohol and the embedding medium.

  When the tissues are permeated with the solvent, they normally become translucent (clear), 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.

  After being removed from the oven, the tissue and the impregnated paraffin solidify.

  The hard blocks containing the tissues are then placed in an instrument called a microtome and are sliced by the microtome's steel or glass blade into sections 1 to 10 micrometers thick.



***Problem in the study of tissue section***

- A key point to be remembered in studying and interpreting stained rice sections is that microscope preparations are the end result of a series of processes that began with collecting the tissue and ended with mounting a coverslip on the slide. Several Steps of this procedure may distort the tissues, producing minor structural abnormalities called artifacts. Structures seen microscopically then may differ slightly from the structures before them when they were alive.

***One such distortion is:***

**1-** minor shrinkage of cells or tissue regions produced by the fixative, by the ethanol, or by the heat needed for paraffin embedding. Shrinkage can produce the appearance of artificial spaces between cells and other tissue components. Another source of artificial spaces is the loss of molecules such as lipids, glycogen, or low molecular weight substances that are not kept in the tissues by the fixative or removed by the dehydrating and clearing fluids. Slight cracks in sections also appear as large spaces in the tissues.

**2-**Other artifacts may include wrinkles of the section (which may be confused with linear structures such as blood capillaries) and precipitates of stain (which may be confused with cellular structures such as cytoplasmic granules). Another point to remember in studying histological sections is the impossibility of differentially staining all tissue components on a slide stained by a single procedure. With the light microscope it is necessary to examine several stained preparations with different methods to obtain an idea of the tissue's complete

## *Apoptosis*

is the process of cell suicide or programmed cell death called apoptosis, Apoptosis is a highly regulated cellular activity that occurs rapidly and produces small membrane-enclosed apoptotic bodies, which quickly undergo phagocytosis by neighboring cells or macrophages specialized for debris removal.

Unlike cells under going necrosis as a result of accidental injury, apoptotic cells do not rupture and release none of their contents. This difference is highly significant because release of cellular components causes a rapid series of local reactions and migration of leukocytes in an elaborate reaction called an inflammatory response.

A few examples of apoptosis : In the mature ovary, apoptosis is the mechanism of both the Monthly loss of luteal cells and the removal of excess oocytes and their follicles. Programmed cell death was first discovered in developing embryos, where apoptosis is an essential process for shaping various developing organs or body regions (morpho genesis), such as the tissue between the digits on a developing limb bud. Apoptosis is an important means of eliminating cells whose survival is blocked by lack of nutrients.

## *Necrosis*

The accidental death of cells, a pathologic process, is called necrosis. Necrosis can be caused by microor ganisms, viruses, chemicals, and other harmful agents. Necrotic cells swell; their organelles increase in volume; And finally they burst, releasing their con tents into the extracellular space. Macrophages in the debris of necrotic cells by phagocytosis and then secrete molecules that activate other immune defensive cells to promote inflammation.