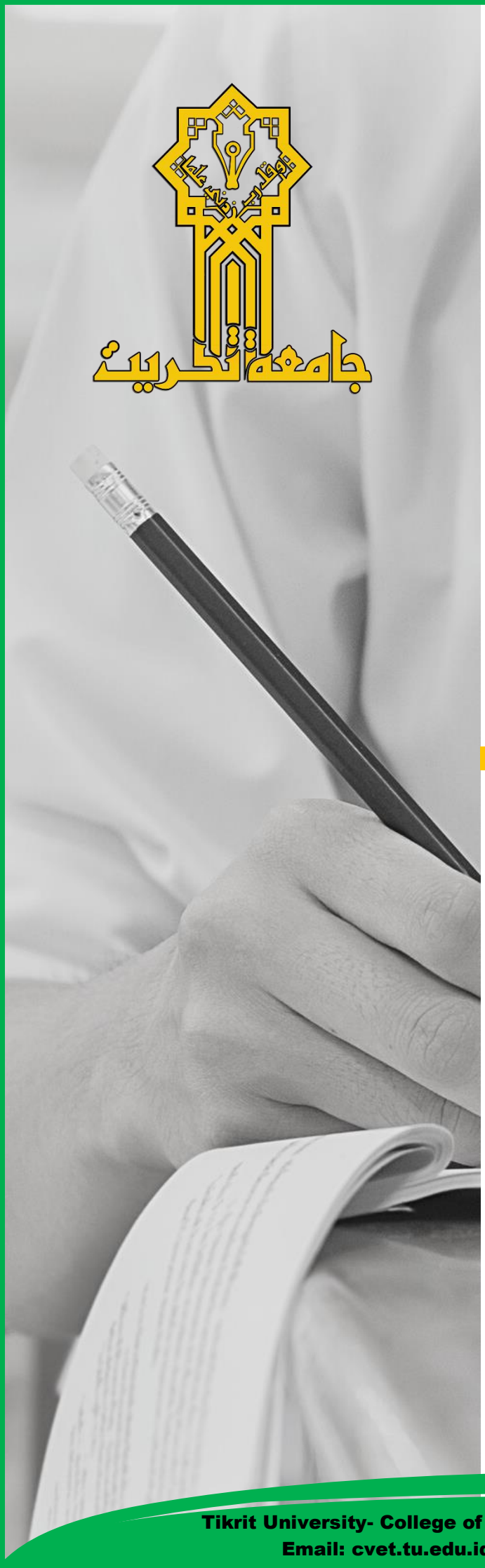


Tikrit University
College of Veterinary Medicine

Preparation of tissue slides

Subject name: Practical Pathology

Subject year: 2024-2025

Lecturer name: Thamer J. Shihab

Academic Email:

dr.thamer.vet@tu.edu.iq

SCAN ME
Lecturers link

Preparation of tissue slides

First practice lesson

Third class-First course

Histology

- **Histology**: Greek: '*histo*'= tissue; '*logy*'= science
- The science that studies different tissues composing different body organs, and the normal structure and cells composing these tissues under light microscope
- Organs usually composed of more than one type of tissues
- Tissues studied under light microscope after being fixed on glass slides and processed

Histopathology

•**DEFINITION OF PATHOLOGY:**

•The word ‘Pathology’ is derived from two **Greek words—pathos** meaning **suffering**, and **logos** meaning **study**.

•**Pathology is, thus, scientific study of structure and function of the body in disease**; or in other words,

•**pathology consists of the abnormalities that occur in normal anatomy (including histology) and physiology owing to disease**.

Tissues Processing

- Tissues taken from organs, before or after death, are processed on microscope slides in multiple steps like cutting, fixation, dehydration, staining, etc.
- After processing, slides are examined under light microscope

Biopsy: It's a histological sample taken from the living animal (through an operation)in order to diagnose the disease.

1-it should be contains both the affected and intact parts of tissue.

2- should be have appropriate size for fixation (about 1 one cm^3).

Autopsy (necropsy):

It's a histological sample taken from the after death or after postmortem examination.

3-fixed immediadiately without washing.

4- Treming for it with a sharp scalp and avoid any destruction to the piece of tissue.

Light Microscope

How to Prepare a Slide

In order to view the cellular components of a tissue, it has to be processed in a step wise manner to produce a "slide" which can be viewed under a microscope.

Fixation

↓

Dehydration

↓

Embedding

↓

Sectioning

↓

Mounting → Staining → Observation

(Junqueira's Basic Histology)

Fixation

Aims of fixation:

1- prevent putrefaction of the tissue by the saprophytic bacteria.

2- prevent autolysis of the tissue architecture by the effecting lysozymes.

3- keep the tissue component as possible as it is in the living body.

Types of Fixatives

1- Formalin fixative 10% (common).
2- Bouin 's solution.
3- Alcohol fixatives e.g.: Methanol, Ethanol, Carnyou's (freezing).
4- Glutral aldehyde (Electron microscope).

The aims of fixation:
1- prevent enzyme action.
2- optimal preservation of tissue outline details.

Processing of the tissue sample

This process can be done Automatically with Histokinett

OR

Manually.

Its include the following steps:

A- **Dehydration:**

by gradual concentrations of alcohol(ethanol 100%):

Distill water	2 hours (washing)
70 % alcohol	2hours
80%	2hours
90%	2 hours
95%	2 hours
100%	2 hours
100%	2 hours

B- Clearance

Replacement of alcohol by Xylene or chloroform for 2 hours / tow times as following:

Xylene (1)	2 hours
Xylene (2)	2 hours

Aim of this treatment is the predisposing the tissue for next treatment;

C- Impregnation with paraffin:

Liquid paraffin (56 °C) 2 hours
Liquid paraffin (56 °C) 2 hour

Aim of this treatment in order to penetrate the tissue components with paraffin.

Bolcking

1- Liquid paraffin (56 °C)

2- 2 L-shape metal pieces

3- 15 min in room temperature

4- overnight in refrigerator

Microtomy

By using the microtome instrument:

1- 5-6 μm (thickness of slices)

(μm = 1x10 meter)

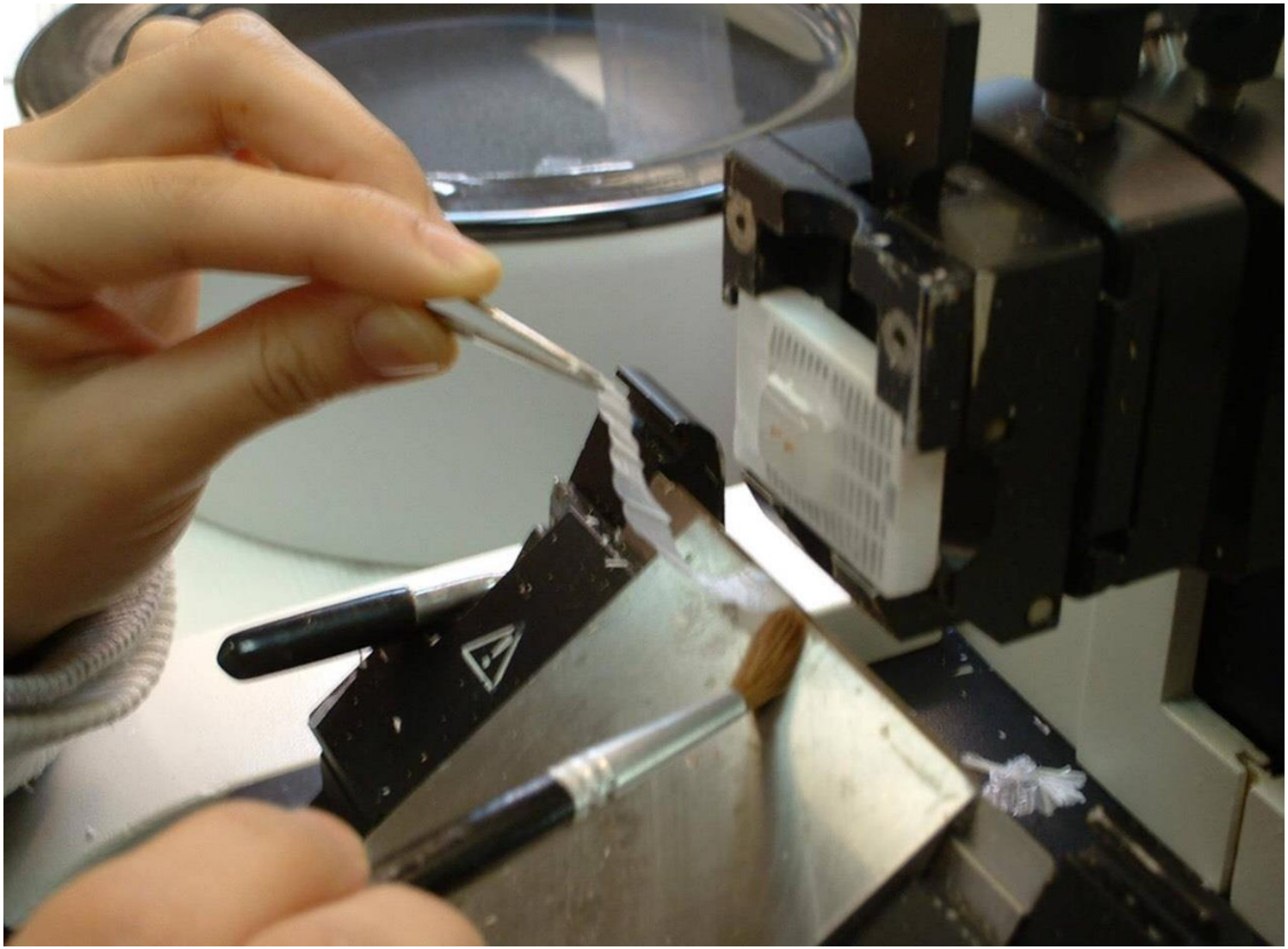
2- in water path 37 °C

3- Clean the slide with a drop of egg albumin or glycerin.

4- Oven 37 °C overnight

60 °C 2 hours.

Sectioning



Staining

- After being fixed on slides, tissue section stained
- Dyes most commonly used in histology are **Hematoxylin and Eosin** (H&E)
  - Nucleus→ **Blue**
  - Cytoplasm→ **Red**

Staining

1- Deparaffinization

Removing of paraffin from tissue skices by:

- Xylene 15-30 min
- Xylene 15-30 min

2- Rehydration:

By using decreased concentrations of ethyl alcohol:

100 %	alcohol	2 min
100 %	alcohol	2 min
90 %	alcohol	2 min
80 %	alcohol	2 min
70 %	alcohol	2 min
	D.W.	2 min

4- DEHYDRATION:

done by the same manner as in step one (rehydration) but with increasing concentrations of ethyl alcohol as following:

70%	2 min
80%	2 min
90%	2 min
100%	2 min
100%	2 min

5- CLEARING:

done by:

Xylene	15-30	min
Xylene	15-30	min

6- Covering with cover slips:

Done by putting a small drop of Canada balsam or DPX, on a clean coverslip (or on clean staining slide) and covered the staining tissue immediately to ovoid drying of tissue, then keep it in the oven for drying.

3- Routine staining: Hematoxyline and Eosin (H&E stain)

1- Hematoxyline 10- 15 min

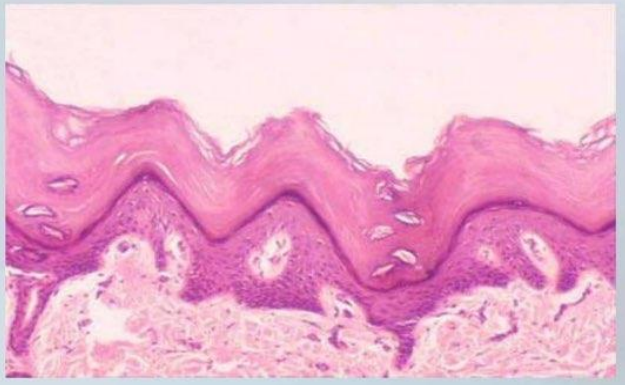
It's a basic stain that give the acidic structures in the tissue like nucleus the **blue color**.

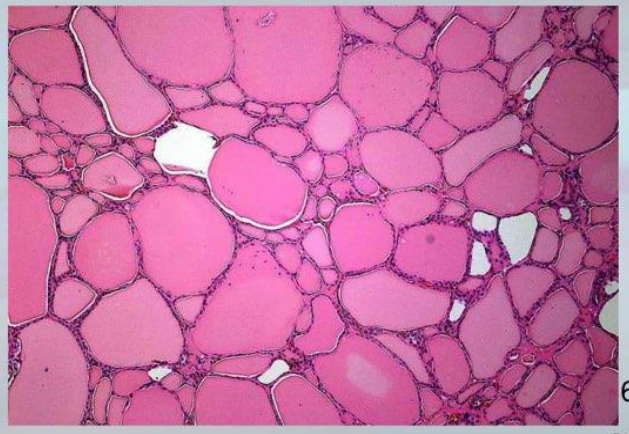
Tap water	10	min	(clear)
Acid alcohol	1-2	dip	

2- Eosin 5 min

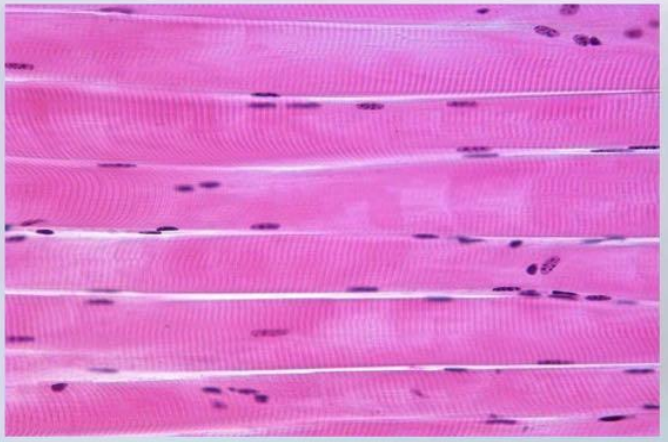
It's acidic stain that gives the basic structure in the tissue like endoplasmic reticulum the red color .

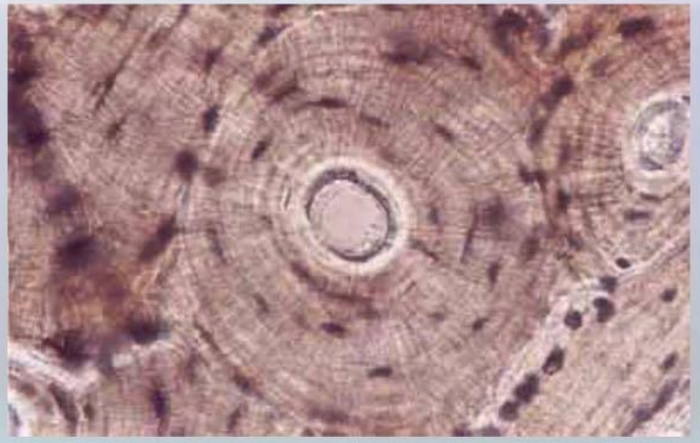
Tissues Under Light Microscope

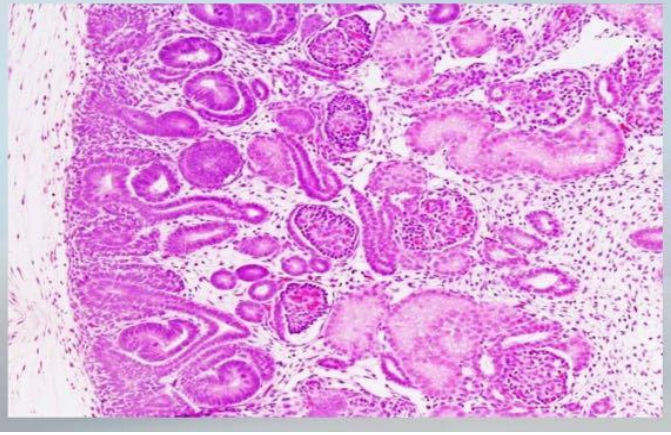




Tissues Under Light Microscope







Sections in Histology

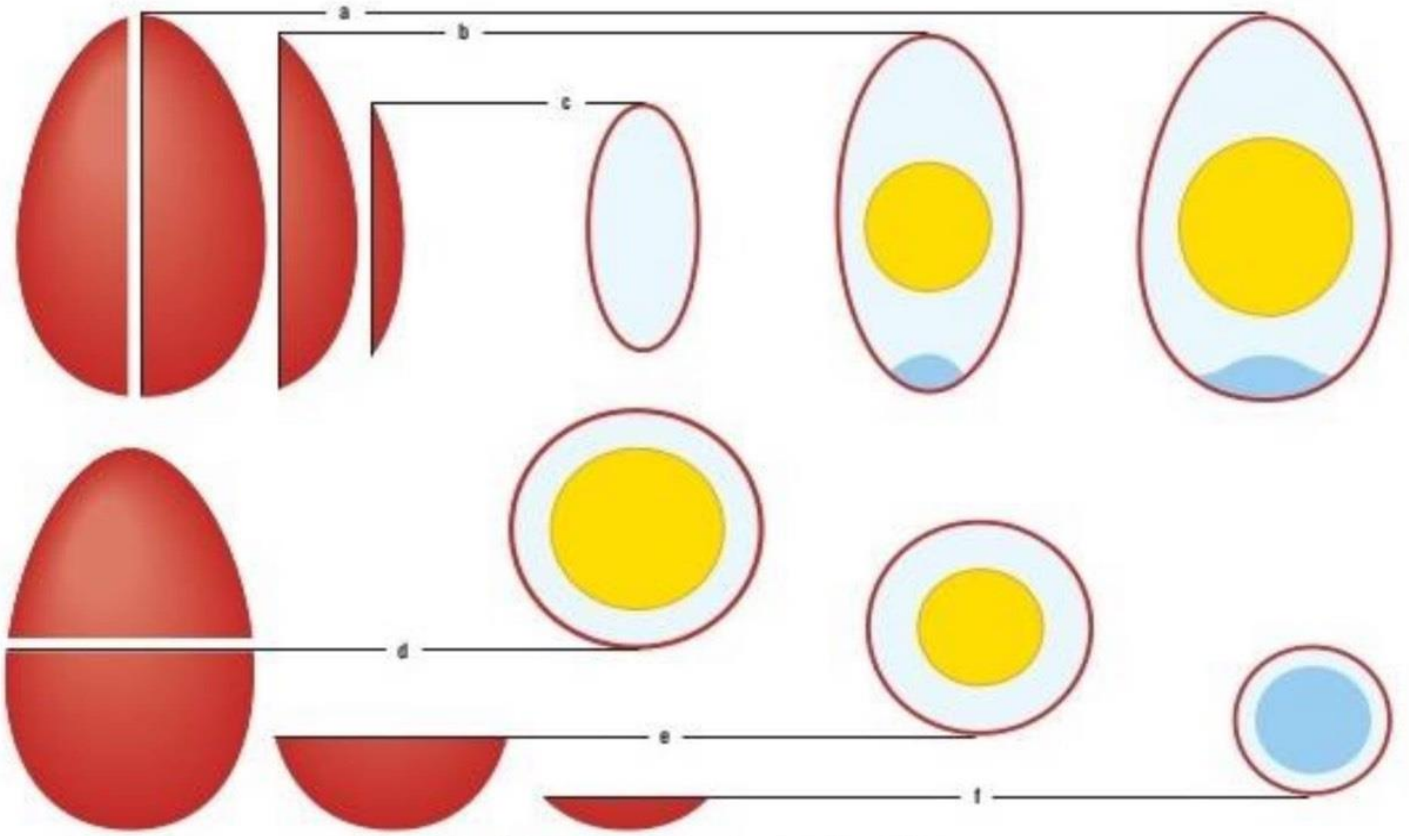


FIGURE 1.1 ■ Planes of section of a round object.

Sections in Histology

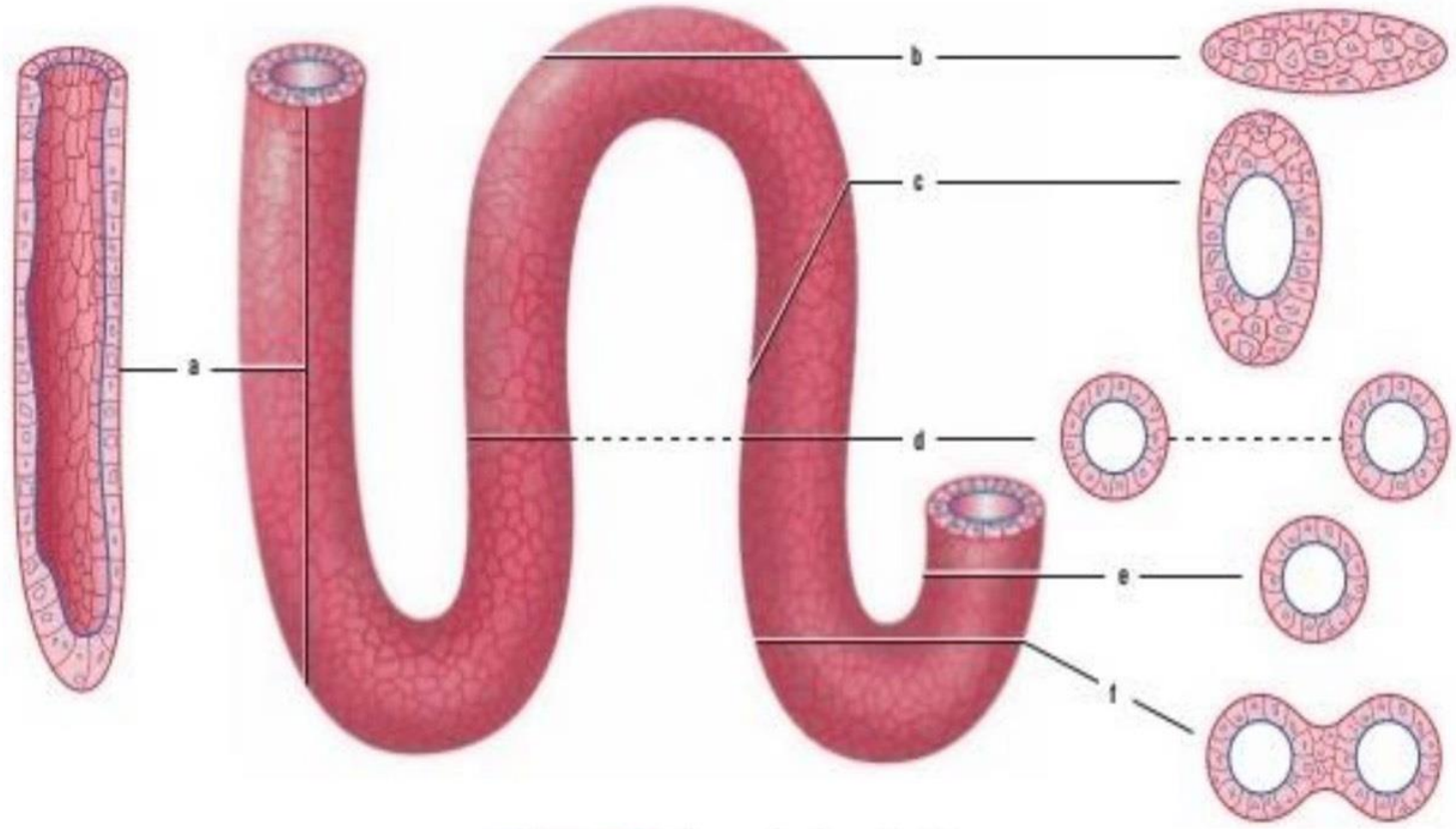


FIGURE I.2 ■ Planes of section of a tube.

Elements of Medical Word

- A medical word consists of some or all of the following elements:
  - **Word root**
  - **Combining form**
  - **Prefix**
  - **Suffix**

Example:

Hist – ***o*** – ***logy***

root comb. form suffix

- How you combine these elements, and whether all or some of them are present in a medical term, determines the meaning of a word

Word Root

- A **word root** is the foundation of a medical term and contains its primary meaning
- All medical terms have at least one word root
- Most word roots are derived from ***Greek*** or ***Latin*** language
- Thus, two different roots may have the same meaning; for example, the Greek word (*dermatos*) and the Latin word (*cutane*) both refer to the skin
- As a general rule, **Greek roots** are used to build words that describe a disease, condition, treatment, or diagnosis. **Latin roots** are used to build words that describe anatomical structures

Table

Examples of Word Roots

This table lists examples of word roots as well as their phonetic pronunciations. Begin learning the pronunciations as you review the information below.

English Term	Greek or Latin Term*	Word Root	Word Analysis
skin	dermatos (Gr)	dermat	**dermat**/itis (dĕr-mă-TĪ-tĭs): inflammation of the skin
			A term that describes a skin disease
	cutis (L)	cutane	**cutane**/ous (sūb-kū-TĀ-nē-ŭs): pertaining to the skin
			A term that describes an anatomical structure
kidney	nephros (Gr)	nephr	**nephr**/oma (nĕ-FRŌ-mă): tumor of the kidney
			A term that describes a kidney disease
	renes (L)	ren	**ren**/al (RĒ-năl): pertains to the kidney
			A term that describes an anatomical structure
mouth	stomatos (Gr)	stomat	**stomat**/itis (stō-mă-TĪ-tĭs): inflammation of the mouth
			A term that describes any inflammatory condition of the mouth
	oris (L)	or	**or**/al (OR-ăl): pertaining to the mouth
			A term that describes an anatomical structure

Combining Forms

- A **combining form** is created when a word root is combined with a **vowel**
- The vowel, known as a *combining vowel,* is usually an ***o***, but sometimes it is an ***i***
- The combining vowel has no meaning of its own, but enables two word elements to be connected
- Like the word root, the combining form is the basic foundation to which other word elements are added to build a complete medical word

Combining Forms

Table **Examples of Combining Forms**

This table illustrates how word roots and vowels create combining forms. Learning combining forms rather than word roots makes pronunciation a little easier because of the terminal vowel. For example, in the table below, the word roots gastr *and* nephr *are difficult to pronounce, whereas their combining forms* gastr/o *and* nephr/o *are easier to pronounce.*

Word Root	+	Vowel	=	Combining Form	Meaning
erythr/	+	**o**	=	erythr/o	red
gastr/	+	**o**	=	gastr/o	stomach
hepat/	+	**o**	=	hepat/o	liver
immun/	+	**o**	=	immun/o	immune, immunity, safe
nephr/	+	**o**	=	nephr/o	kidney
oste/	+	**o**	=	oste/o	bone

Prefix

- A **prefix** is a word element attached to the beginning of a word or word root
- However, not all medical terms have a prefix
- Adding or changing a prefix changes the meaning of the word
- The prefix usually indicates a number, time, position, direction, or negation
- Many of the same prefixes used in medical terminology are also used in the English language

Prefixes

Table **Examples of Prefixes**

This table lists examples of prefixes as well as their phonetic pronunciations. Begin learning the pronunciations as you review the information below.

Prefix	+	Word Root	+	Suffix	=	Medical Word	Meaning
an- (without, not)	+	**esthes** (feeling)	+	**-ia** (condition)	=	**anesthesia** ăn-ĕs-THĒ-zē-ă	condition of not feeling
hyper- (excessive, above normal)	+	**therm** (heat)	+	**-ia** (condition)	=	**hyperthermia** hī-pĕr-THĔR-mē-ă	condition of excessive heat
intra- (in, within)	+	**muscul** (muscle)	+	**-ar** (pertaining to)	=	**intramuscular** ĭn-tră-MŬS-kū-lăr	pertaining to within the muscle
para- (near, beside; beyond)	+	**nas** (nose)	+	**-al** (pertaining to)	=	**paranasal** păr-ă-NĀ-săl	pertaining to (area) near the nose
poly- many, much	+	**ur** (urine)	+	**-ia** (condition)	=	**polyuria** pŏl-ē-Ū-rē-ă	condition of much urine

Suffix

- A **suffix** is a word element placed at the end of a word that changes the meaning of the word
- In the terms tonsill*/itis*, and tonsill*/ectomy*, the suffixes are *–it is* (inflammation) and *–ectomy* (excision, removal)
- Changing the suffix changes the meaning of the word
- In medical terminology, a suffix usually describes a pathology (disease or abnormality), symptom, surgical or diagnostic procedure, or part of speech
- Many suffixes are derived from Greek or Latin words

Suffixes

Table **Examples of Suffixes**

This table lists examples of pathological suffixes as well as their phonetic pronunciations. Begin learning the pronunciations as you review the information below.

Combining Form	+	Suffix	=	Medical Word	Meaning
	+	**-itis** (inflammation)	=	**gastritis** găs-TRĪ-tĭs	inflammation of the stomach
gastr/o (stomach)	+	**-megaly** (enlargement)	=	**gastromegaly** găs-trō-MĔG-ă-lē	enlargement of the stomach
	+	**-oma** (tumor)	=	**gastroma** găs-TRŌ-mă	tumor of the stomach
	+	**-itis** (inflammation)	=	**hepatitis** hĕp-ă-TĪ-tĭs	inflammation of the liver
hepat/o (liver)	+	**-megaly** (enlargement)	=	**hepatomegaly** hĕp-ă-tō-MĔG-ă-lē	enlargement of the liver
	+	**-oma** (tumor)	=	**hepatoma** hĕp-ă-TŌ-mă	tumor of the liver

Examples

Echo-	***cardio-***	***graphy***	(=imaging of the heart by ultrasound)
prefix= sound	**root=** heart	**suffix=** imaging	
Electro-	***cardio-***	***graphy***	(=recording the heart electrical activity)
prefix= electricity	**root=** heart	**suffix=** imaging	
Gastro-	***eter-***	***itis***	(=inflammation of stomach and small intestine)
root 1= stomach	**root 2=** small intestine	**suffix=** inflammation	

Examples

- Root: ***cardium*** = heart
- ***Peri-cardium***: around the heart
- ***Cardio-gram***: recording of heart
- ***Cardio-myo-pathy***: disease of the heart muscle

Common Roots

- Nephro – kidney
- Hepato – liver
- Pulmo – lung
- Cardio – heart
- Cyto – cell
- Rhino – nose
- Naso – nose

Special Stains

- **1- Van gieson's stain (collagen fibers: Pink).**
- **2- Sudan III (Fat: orange).**
- **3- Van kossa (calcium: dark blue).**
- 4- Congo red (Amyloid: red).
- 5- Periodic acid shift stain (PAS) (Fungi: red).
- 6- Muci-carmin (Fungi: red; Glycogen:).
- 7- Acid Fast stain (Ziehl-Nelson stain) (*Mycobacterium*: red).
- 8- Giemsa stain (cytoplasmic granules: red or purple).

Thank you