HISTOLOGY PRACTICAL MANUAL

FOR

Second Year Health Science Students

PART 1: Primary Tissues

SCHOOL OF ANATOMICAL SCIENCES
UNIVERSITY OF THE WITWATERSRAND

Anatomical Sciences

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

PRACTICAL HISTOLOGY FOR SECOND YEAR HEALTH SCIENCE STUDENTS

By

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SCHOOL OF ANATOMICAL SCIENCES

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF THE WITWATERSRAND

JOHANNESBURG, SOUTH AFRICA

CONTENTS

Glossary

- Compiled by TN Augustine -

The information in this section serves to stress the importance of understanding histological terminology and integrating that knowledge with other aspects of study of the human body. Most histological terms are derived from Greek or Latin. This glossary will aid you in understanding the roots of these terms by illustrating their meaning and usage.

Please note: this glossary does not serve as a comprehensive guide to all the terms you may encounter in the duration of this course, nor does it serve as a comprehensive dictionary that would be used to elicit precise definitions of terms. Students should populate this glossary with more terms, their derivations and definitions to ensure their own learning and understanding.

Terms

Tissue sections are stained with different dyes in order to view limited cellular detail using light microscopy. The suffix -**PHILIA** or -**PHILIC** is derived from *Greek: philia* – to love. In combination with different prefixes, in the context of histological stains, you will encounter the following terms:

Basophilic: *Greek: base-loving*
A structure that stains with a basic dye e.g. haematoxylin (used in conjunction with a mordant) is referred to as being basophilic i.e. the structure has an *affinity for basic dyes*. Basic dyes react with *anionic* components of cells. Consider the staining reaction of nuclei with haematoxylin.

Acidophilic: *Greek: acid-loving*
A structure that stains with an acidic dye e.g. eosin is referred to as being acidophilic. This structure is thus *cationic* in nature e.g. cytoplasm commonly stains acidophilically. A cell could exhibit acidophilia in the *subnuclear* (beneath the nucleus) compartment of the cytoplasm indicating that a number of *cationic* cellular components are present in the region.

Eosinophilic: As per acidophilic, except in this instance the term refers to the dye eosin.

The prefix **EPI** is derived from *Greek: epi* - upon. This prefix is generally used to refer to the outermost lining tissue of a specific structure, the latter indicated by the use of particular suffixes. You will encounter the following examples:

Epithelium: EPI + *Greek: thele* – nipple or teat
Epithelium is a tissue composed of cells that cover body surfaces and line cavities. Recall that cavities are hollow structures, lining epithelium in this instance could be referred to as the innermost layer.

Epidermis: EPI + *Greek: dermis* – skin
Skin is composed of a number of layers. The uppermost layer of skin is the epidermis i.e. the epidermis lies *upon* the dermis.

Epimysium: EPI + *Latin: mysium – mus/musculus* - muscle
A dense connective tissue sheath *surrounding a collection of fascicles* that comprise an *entire muscle*. Consider the gross structure of biceps brachii as seen during dissection or in a wet / plastinated specimen – the tissue referred to as *deep fascia* by gross anatomists is histologically, the epimysium.

Epineurium: EPI + *Latin: nervus; Greek:* - nerve
A dense connective tissue sheath *surrounding a collection of fascicles* that comprise an *entire nerve.* Consider the sciatic nerve that you would see on gross inspection of a dissected gluteal region – the fascial covering of the sciatic nerve that you touch is referred to histologically, as the epineurium.

The prefix **PERI** is derived from *Greek: peri* - around. This prefix is generally used to refer to a lining tissue of a specific structure, the latter indicated by the use of particular suffixes. Pay attention to the suffixes and compare them with other terms in which they are used. You will encounter the following examples:

Perimysium: PERI + *Latin: mysium – mus/musculus* - muscle
A connective tissue sheath that *surrounds a group of muscle fibres* to form a bundle known as a *fascicle*. Compare with **epimysium**.

Perineurium: PERI + *Latin: nervus; Greek:* nerve
A connective tissue sheath that *surrounds a group of nerve fibres* to form a bundle known as a *fascicle*. Compare with **epineurium**.

Periosteum: PERI + *Greek: osteo* – bone
A layer of connective tissue that may cover bone.

Perichondrium: PERI + *Greek: chondro*- cartilage
A connective tissue sheath that may cover certain types of cartilage.

The prefix **ENDO** is derived from *Greek: endo* - within or inner. This prefix is generally used to refer to the inner lining tissue of a specific structure, the latter indicated by the use of particular suffixes. Pay attention to the suffixes and compare them with other terms in which they are used. You will encounter the following examples:

Endomysium: ENDO + *Latin: mysium – mus/musculus* - muscle
A connective tissue sheath that *surrounds an individual muscle fibre.* Compare with **perimysium** and **epimysium**.

Endoneurium: ENDO + *Latin: nervus; Greek:* nerve
A connective tissue sheath that *surrounds an individual nerve fibre.* Compare with **perineurium** and **epineurium**.

Endosteum: ENDO + *Greek: osteo* - bone;
A layer of connective tissue that covers bone facing the marrow cavity, and trabeculae of bone within the marrow cavity.

The prefix **EX(TRA)-** is derived from *Latin: ex* – out. It is used in a combining form to mean outer or exterior. The prefix **INTRA-** is derived from *Latin: intra* - within or inner. In combination with different suffixes, this prefix is generally used as a combining form meaning within or inside. However, this prefix is commonly confused with **INTER-,** which is also derived from *Latin* but rather means among or amid. You will encounter the following examples:

Extracellular matrix: EX + cellular + *Latin: mater* – mother tissue refers to matrix

Extracellular matrix refers to those components secreted by cells that reside in the intervening spaces between cells. Consider the different types of connective tissue proper and specialised connective tissues (bone, cartilage, blood).

Intramembranous ossification: INTRA + membranous + *Greek: osteo* – bone

Intramembranous ossification is a form of osteogenesis where bone is formed within mesenchyme i.e. bone is formed without the use of a cartilage model.

Interstitial: INTER + *Latin: sistere* – to place or stand between

You will encounter the term interstitial in the phrase *interstitial growth*, which refers to growth within a matrix e.g. chondrocytes within a cartilage matrix may divide, thus producing new cells and matrix to allow for growth; or *interstitial lamellae*, which refers to remnants of concentric lamellae formed during bone remodelling and found between osteons.

Cells and their surrounding extracellular matrix are commonly arranged in layers. There are a number of different terms employed to describe this arrangement. Consider the following terms:

Stratified: *Latin: stratum,* plural *strata* – layer(s).

A term used when classifying or describing *epithelia* composed of many layers of cells e.g. stratified squamous keratinized epithelium. Consider the function of a stratified epithelium.

Pseudostratified: *Greek: pseudo* – false; *Latin: stratum,* plural *strata* – layer(s)

A term used when classifying or describing an *epithelium* that appears to be composed of many layers but is in fact, not e.g. pseudostratified ciliated columnar epithelium

Lamina: *Latin: lamina,* plural *laminae* – thin plate(s)

This term is used for a variety of structures that exhibit a plate-like appearance, or commonly refer to a layer. You will encounter this term as part of *lamina propria*, the connective tissue layer underlying epithelia and forming part of the mucosa; or as part of *lamina rara externa*, which is a component of the glomerular basement membrane.

Lamella: *Latin:* diminutive of *lamina,* plural *lamellae* – thin plate(s)

Lamella is as a diminutive of lamina and is also used to refer to a layer. You will encounter this term used in mature bone, to describe the arrangement of the collagen fibres and other constituents of bone; or in *lamellar bodies*, membrane- bound organelles found in keratinocytes, and so-named due to their structure as viewed using electron microscopy.

Stratum: *Latin stratum,* plural *strata* – layer(s)

The term *stratum* refers to layers and is commonly used in histology where several layers are in apposition to each other. You will encounter *stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum* as the layers of the epidermis.

Tunica: *Latin: tunica* – sheath
The term tunica refers to a layer of tissue ensheathing a structure. You will encounter this term when studying the structure of blood vessels i.e. *tunica intima, tunica media and tunica adventitia*; as well the *tunica albuginea* and the *tunica vasculosa* forming the capsule of the testis.

Zona: *Greek: zona* – girdle or belt
Histologically the term zona refers to a zone or belt. You will encounter the term in the suprarenal gland, with the cortex being subdivided into three zones – *zona glomerulosa, zona fasciculate and zona reticulari*s – based on the arrangement of the cells.

There are many cell types that you will encounter. Considering the prefix and suffix employed in these terms will allow you to recall the location, function and the state of differentiation of the cells in question. The suffix –**BLAST** is derived from *Greek: blast* – bud, germ, sprout and is generally used with reference to cells that retain a differentiation capacity, while the suffix **– CYTE** is derived from *Greek: cyto* – cell. Consider these examples:

Fibroblast: *Latin: fibra* – fibre + BLAST
The principal cell of connective tissue, responsible for the synthesis of collagen, reticular and elastic fibres as well as other components of ground substance. Inactive fibroblasts are in some texts, referred to as *fibrocytes*.

Leukocyte: *Greek: leuko* – white + CYTE
White blood cells.

See osteoblast; osteocyte; osteoclast; chondroblast; chondrocyte; chondroclast

The following terms are associated with bone. The prefix **OS-** is derived from *Greek:* osteo – bone.

Osteoblast: OSTEO + *Greek: blast* – bud, germ, sprout
Osteoprogenitor cells differentiate into osteoblasts. These cells retain the ability to divide and are able to secrete osteoid.

Osteocyte: OSTEO + *Greek: cyto* – cell
Osteocytes are the principle cell type of bone involved in maintenance of the bone matrix.

Osteoclast: OSTEO + *Greek:* κλαστός – broken
Osteoclasts are not part of the osteogenic lineage but are rather derived from cells in the mononuclear phagocytic system. These cells are however, responsible for remodelling bone.

Osteogenesis: OSTEO + *Greek:* genesis
The suffix –GENESIS refers to the formation of structures. As such osteogenesis is the process by which bone forms.

The following terms are associated with cartilage. The prefix **CHONDRO-** is derived from *Greek: chondro* – bone.

Chondroblast: CHONDRO +*Greek: blast* – bud, germ, sprout
The chondroblast is responsible for laying down cartilage during chondrogenesis.

Chondrocyte: CHONDRO +*Greek: cyto* – cell
Chondrocytes are the principle cell type of bone involved in maintenance of the cartilage matrix.

Chondroclast: CHONDRO +*Greek:* κλαστός – broken
Chondroclasts are not part of the chondrogenic lineage but are rather derived from cells in the mononuclear phagocytic system. These cells are however, responsible for remodelling cartilage.

Chondrogenesis: CHONDRO + *Greek:* genesis
The suffix –GENESIS refers to the formation of structures. As such chondrogenesis is the process by which cartilage forms.

The following terms are often misunderstood.

Spicule: *Latin: spica. Plural spiculae.* A small sharp process.
The word spicule is also often encountered in descriptions of spongy bone and in the process of osteogenesis where osteoid is laid down in spicules, which then coalesce to form larger structures known as trabeculae.

Trabeculum: *Latin: trabs – beam. Plural trabeculae.* Resembling a small bar or beam.
The word trabeculum is a descriptive word encountered in describing the histological structure of spongy bone, and down growths of connective tissue capsules.

Mucous: *Latin/Greek: muco* - mucous
Mucous is a viscous substance containing water, mucin / mucinogen granules and inorganic salts. Mucous cells are thus mucin-secreting cells, commonly found in salivary glands and the respiratory and gastrointestinal tracts.

Mucosa: *Latin/Greek: muco* – mucous.
Mucosa refers to a mucous membrane and should neither be confused with mucous secretions nor with cell membranes. Mucosa refers collectively to a surface epithelium, the underlying connective tissue and at times, a layer of smooth muscle – muscularis mucosae. Mucosa lines internal cavities that link to the exterior. i.e. the gastrointestinal tract, the respiratory tract and the urogenital tract.

Lacuna: *Latin: lacus* – lake; *Plural: lacunae*.
Lacuna is a diminutive of lacus and refers to a pit or hollow. You will encounter the term in cartilage, where chondrocytes are located within lacunae; or in bone, where osteocytes are found in lacunae.

Fenestra: *Latin, Plural: fenestrae* – windows.

Fenestrated thus refers to a structure with 'windows' or openings that allow substances to diffuse readily through the layer on which the opening is found. You will encounter this term in association with the internal elastic membrane of large blood vessels or in fenestrated capillaries where the openings may be covered by a diaphragm.

Striation: *Latin: stria* – stripe or column; *Plural: striae*
Striations in histology are commonly used with reference to structures that exhibit stripes due to the arrangement of certain cellular components. You will encounter this term as striated border, where it describes the appearance of short, numerous microvilli on the apical domain of epithelial cells; or as striated skeletal muscle, where the striations refer to the ordered arrangement of myofilaments; or as the striated duct of the salivary glands, where the striations are due to infoldings of the basal plasma membrane of the epithelial cells lining the duct.

Some histological terms often refer to diminutives of larger structures – these terms cannot be used interchangeably as they refer to essentially different structures with their own corresponding specialised functions. Consider the following terms and their ensuing diminutives.

BRONCHUS – bronchiole
ARTERY – arteriole
VEIN – venule
FIBRE – fibril – filament
NODE – nodule
CANAL – canaliculus

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Introduction

Histology is the study of the structure and function of tissues, providing a link between gross anatomy, physiology, cell biology and biochemistry. This set of notes will direct the student to the main features of the virtual slides and electron micrographs to be studied in the practical sessions. Your textbooks, atlases and lecture notes, provide further information.

✎ ATTENDANCE AT ALL PRACTICAL CLASSES

Attendance at practical classes is **compulsory**. You are expected to remain in the laboratory for the duration of the practical class.

✎ CERTIFICATE OF DUE PERFORMANCE

Students may be refused a due performance (DP) certificate if they **fail to attend and actively participate** in practicals and tutorials in Histology. A DP certificate is required before a student is permitted to write the final examination at the end of the year. Please consult the School Notice Board.

✎ PREPARATION

The timetable and practical notes indicate what is to be covered in each practical class. Students are required **to prepare in advance** for practical classes by revising their lecture notes, reading the relevant pages in their textbooks and preparing their own notes. **If you have not prepared adequately, the demonstrators need not assist you and you may be asked to go to the Library and prepare.**

The majority of practicals are preceded by a **pre-practical** tutorial. These tutorials provide an **outline** of the subject to be studied and pinpoint problem areas. They do not substitute for the preparation you are required to do.

✎ DEMONSTRATORS

Demonstrators are available in practicals **to assist** students where necessary. **Attempt to solve a problem yourself before seeking aid from a demonstrator**. Bear in mind that demonstrators will expect you to have done the necessary preparation from your textbook and will not assist you unless this is the case.

✎ PRACTICAL MANUAL

The practical manual has pages provided for drawings and notes. Your practical manual provides a record of the virtual slides studied during the course and should be kept to complement your notes. The preparation of accurate drawings assists greatly in the understanding of a particular organ or tissue and also forms the basis for revision at the year-end. Make macroscopic, low power (L.P.) and high power (H.P.) fully labelled drawings of all virtual slides studied unless otherwise stated.

Certain sections in this practical manual will not be studied by all courses.
If the following letters appear in bold and in brackets next to a slide or section it will only be studied by that particular course/s:

M	**:**	**ANAT 2020 (MBBCh II, BHSc II & BSc (BME))**
PT/OT	**:**	**ANAT 2033 (BScPT II and BScOT II)**
P	**:**	**ANAT 2031 (BPharm II)**
D	**:**	**ANAT 2030 (BDS II)**
N	**:**	**ANAT 2005 (BNurs II)**

✎ STRUCTURE AND FUNCTION

Variations in structure are related to differences in function. Wherever possible, try to appreciate the function of an organ in relation to the structure that you can see.

✎ TABLES OF COMPARISON AND SUMMARIES

Tables of comparison and summaries are invaluable when correlating information and they are useful for revision too. Each section of this book will finish off with a revision section. Answer all the questions under this heading and elsewhere in the practical instruction notes and make your answers brief but to the point.

✎ OlyVIA

OlyVIA is an image viewer software which allows you to view and examine virtually scanned slides. It will allow you to examine and navigate through each of the scanned histological slides, which appear on the Anatomical Sciences database, at a variety of different magnifications, similar to a traditional microscope.

The computer you are assigned to at the start of the year is your responsibility. Please take good care of it. If you encounter any technical problems please report them to **Mr L du Plessis or Mr T Meadows**, you can find them in room **2P14**. The student may not use the computer for any other purpose but the study of histology. **USB storage devises as well as taking "screen shots" with your cell phone or any other device is strictly prohibited. Any infringement of these rules will result in disciplinary action.**

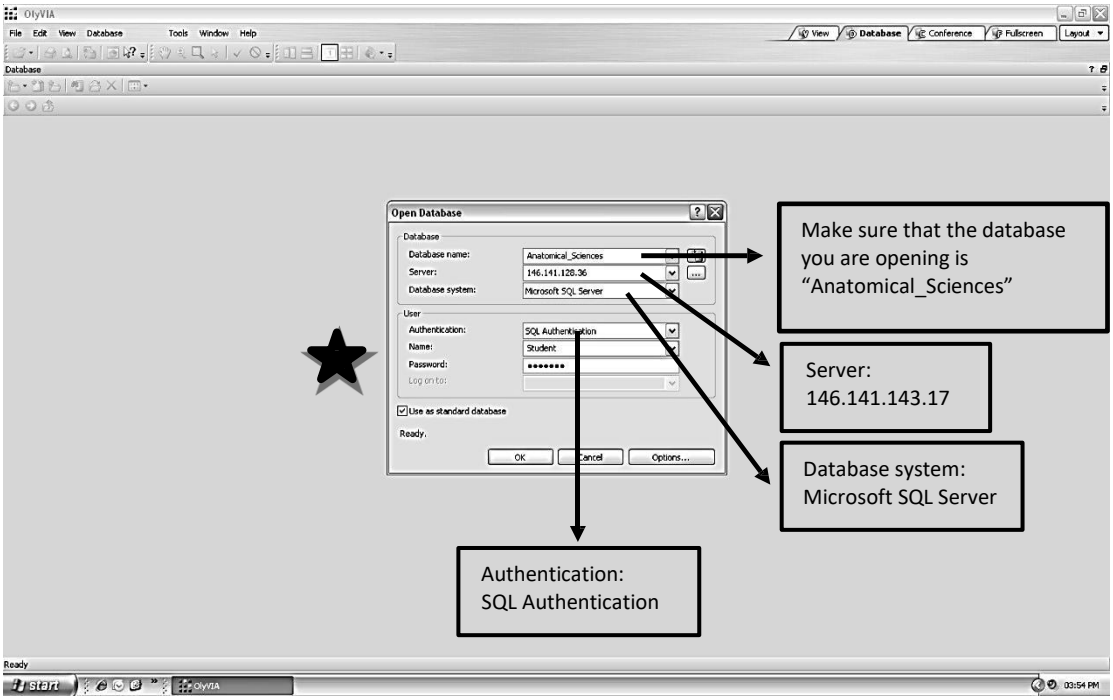
❖ LABORATORY RULES

No eating
No drinking
No cell phones
No bags
No hats or caps will be allowed during the histology practical classes.

How to use OlyVIA

On your desktop double click on the **OlyVIA** icon 

The following screen will appear:



The program will prompt you for a username and password. To login to the database use the following login details and click "Ok":

Name: Student
Password: Student

Note: *Make sure you enter the username and password as it appears above, as it is case sensitive!*

The **Anatomical_Sciences** Database should open automatically

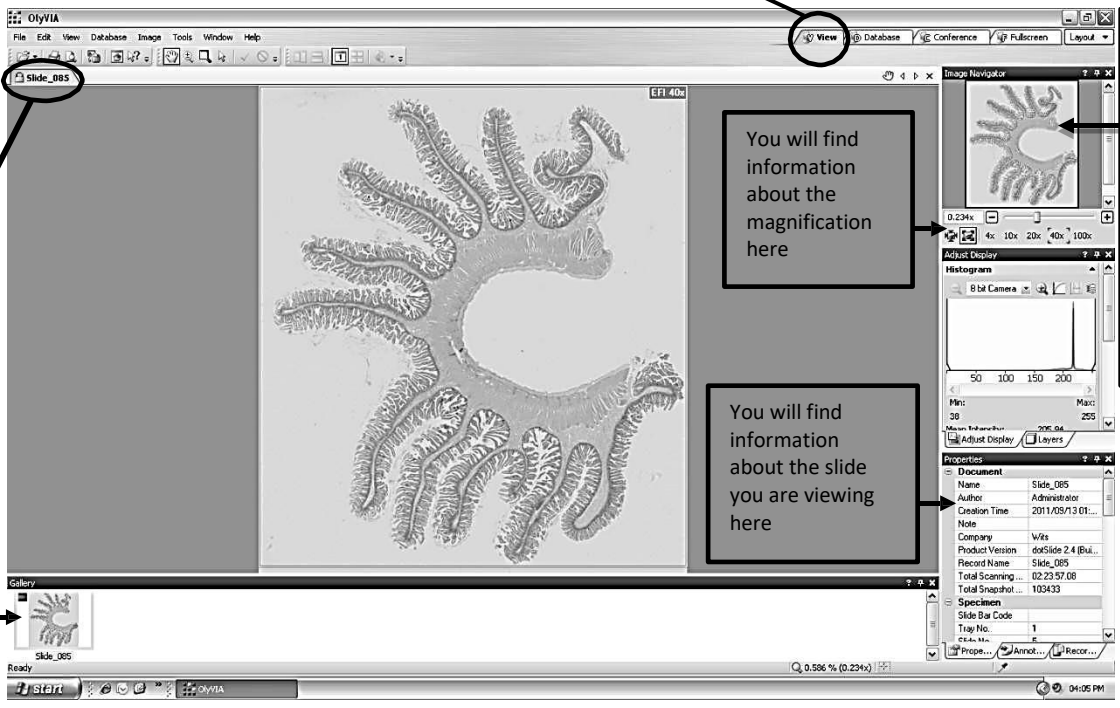
Click on the folder labelled **"Slides"**

The entire data base should now appear before you:

Expansion (clicking on the "+") will list all the slides for you in the database in numerical order.

Double click on the slide you wish to examine

The slide will open automatically under the "**View**" tab



You will find information about the magnification here

You will find information about the slide you are viewing here

You will see a red block in the Image Navigator window as soon as you increase the magnification. It will indicate your position in the slide you are viewing.

Multiple slides can be opened at the same time from the database. All opened slides will appear on the bottom of your screen. Click on the slide to view it, or click on the tab above the image.

In this view you can now examine your slide. Use your mouse wheel for zooming in and out (magnification) of the section. The section will increase or decrease in magnification in region where your mouse is pointing. To move around the section simply click and drag your mouse to the desired location.

Cytology

OBJECTIVES

This practical is designed to familiarize the student with the basics of cytology. Some of this work should be revision from first year.

After studying the cell, you should be able to:

Identify and describe the macroscopic (including the ultrastructure) of a typical cell
Identify and describe the nuclear and cytoplasmic contents and organelles related to specific functions of a typical cell (e.g. protein synthesis)
Identify cell inclusions (e.g. lipid)
Identify cell products at the ultrastructural level (e.g. collagen, bone matrix, elastin, basal lamina)

NOTES

Cytology is the specialized study of cell structure and function.

Cells are small, defined units comprising a nucleus surrounded by membrane-limited cytoplasm. The nucleus, separated from the cytoplasm by a membranous envelope, contains genetic information in DNA (deoxyribonucleic acid). The watery cytoplasm contains a variety of small organelles vital for the viability and proper functioning of the cell.

Cells make up tissues, which in turn together constitute organs and as such perform a variety of functions including forming a cellular barrier over all the body surfaces. Cells secrete all kinds of substances including mucous, enzymes, antibodies and hormones to name but a few; cells also absorb, contract, transmit (nerves) and store (e.g. lipid). These are just a few examples of the functions cells perform. Cells can divide and they also die. Normal cells generally have a defined lifespan.

The existence of cells as the basic unit of tissue structure has long been known.

PRACTICAL WORK

To complete this practical work use your textbook

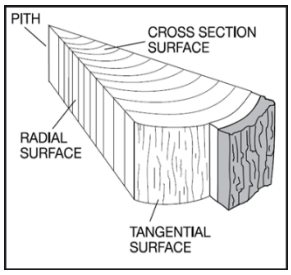
1. **"Histology: A Text and Atlas" by Ross M. and Paulina W. Sixth Edition, 2011 (M),**

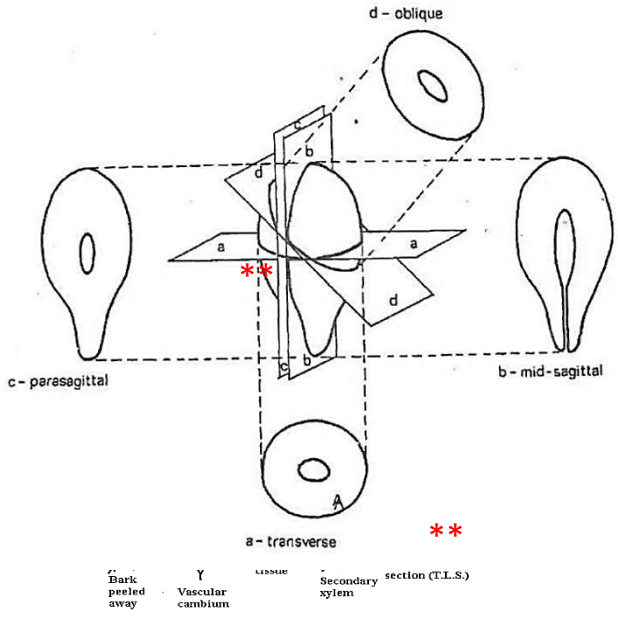
2. **"Junqueira's Basic Histology Text and Atlas" by Mescher Al. Thirteen Edition, 2013 (PT/OT/P/D/N).**

I.PLANES OF SECTION

The structures you see in histological slides appear two-dimensional, but are slices of tissue taken from three-dimensional organs or structures. An object, when cut in different ways, can produce quite different appearances in section. Illustrate your answer by means of simple diagrams.

The diagram below illustrates how sections may be cut in three planes through an organ such as the uterus, viz. cross or transverse (a), longitudinal (b) and (c) and oblique (d). In the longitudinal plane, sections may be cut midsagittally (b) i.e. through the midline cutting the organ into two symmetrical halves, or parasagitally (c). Parasagittal sections may not pass through the lumen of a hollow organ. Note that in each case, the outline of the section is different; also the outlines of cells and fibres and other structures within the organ will be different.





What profiles or outlines might you expect if a long tubular (hollow cylindrical) thick-walled structure was sectioned or cut in a number of different planes (transverse, oblique, peripheral longitudinal, radial longitudinal)?

> "Radial longitudinal sections are prepared parallel to radii of the organ, whereas tangential longitudinal sections are made at an angle to radii of the organ"

What would similar sections through a hollow ovoid and a hollow vesicular (or spherical) structure look like? Illustrate your answer by means of simple diagrams.

II.MAGNIFICATION

- Measure the size of a few cells and their nuclei shown
- See Figs 5.1b Page 106 and Fig. 7.6 Page 202 (M); **Fig 4.14 page 85 (PT/OT/P/D/N)**

i. Measure both the major and minor diameters (longer and shorter axes) and give the final answer in micrometers (μm)
ii. Estimate the ratio of the nucleus to the cytoplasm.

Example: If the cell measures 20mm and the magnification = 320x then:

20mm	= 20,000 μm
20,000/320	= ~62.5 μm

What does this exercise tell you about cells in general?

What do you think would occur if the tissue were not sectioned through the centre of the cell?

Is the proportion of nucleus to cytoplasm shown in any (single) given section of a cell necessarily representative of the whole cell? Give reasons for your answer.

III.THE PLASMA MEMBRANE

Note the plasma membrane seen in Fig 2.2 Page 27 (M); **Fig 2.1 page 20 (PT/OT/P/D/N)**.

The carbohydrate-containing cell coat of the plasma membrane of epithelial cells may also be called the glycocalyx: no other cellular membrane has a glycocalyx.

- Measure the width of the plasma membrane.
- Suggest a general function for the glycocalyx.

IV.CELL ORGANELLES

(a) The Endoplasmic Reticulum

Draw diagrams of the endoplasmic reticulum (ER) shown in Fig 2.25 on Page 46 and Fig 2.30 on Page 49 (M); **Fig 2.10 Page 30 (PT/OT/P/D/N)**.

Does the ER in the photomicrographs have the same form i.e. is the ER rough or smooth?

Give the main functions of both rough ER and smooth ER.

(b) The Golgi Apparatus

Note Fig 2.33 and Fig. 2.34 on Page 51 (M); Fig 2,13 page 34 **(PT/OT/P/D/N)** showing the Golgi apparatus.

Draw a diagram showing how proteins for secretion pass from the ER through the Golgi and into secretory granules (see, for instance, Fig. 2.35 Page 52 (M), **Fig 2.14 Page 35 (PT/OT/P/D/N).**

(c) Lysosomes

Intracellular digestion occurs by means of lysosomes. Lysosomes are vesicles containing digestive enzymes, which typically function at acid pH, e.g. acid phosphatase. Two types of intracellular digestion are recognised - *heterophagy* and *autophagy*. Define these terms.

Draw and label a diagram to illustrate the sequence of events, which occurs during the digestion of unwanted material in a cell (see Fig. 2.21 on Page 41 (M); **Fig 2.15 Page 36 (PT/OT/P/D/N).**

Lysosomes are very small and as such are not often seen in sections viewed with light microscope unless specifically labelled or stained. However when they are particularly large or abundant, lysosomes are recognisable.

Name one cell type where lysosomes may be seen in light microscope sections.

What is a residual body?

Clinical Correlation

Tay Sachs Disease. This is a type of lysosomal storage disease, where the absence of the enzyme lysosomal galactosidase prevents the degradation of a specific type of ganglioside in neurons. The accumulation of this ganglioside leads to a disruption of cell function and eventual cell death. Presentation of this disease is gradual, with children appearing normal at birth but thereafter exhibiting slow growth, alterations of facial features and bone and joint deformities.

Ross & Pawlina, (2011). Histology A Text and Atlas 6th Edition.

(d) Mitochondria

Note Fig. 2.37 Page 54 (M); **Fig 2.20 Page 42 (PT/OT/P/D/N).**

Draw and label a diagram of a mitochondrion. Make sure you label the different "spaces" formed by the two membranes. Note that the mitochondria in most cells exhibit cristae of the flat, shelf-like form (lamelliform), but the mitochondria of steroid-secreting cells exhibit tubular or vesicular cristae.

What is the main function of mitochondria?

Look at the mitochondria in Fig. 20.18 Page 715 (M); **Fig 19.10 Page 396 (PT/OT/P/D/N)**. Does there appear to be a preferential distribution of these mitochondria? What might this mean in functional terms?

V. THE NUCLEUS

Look at the nuclei in Fig. 3.1 Page 77 (M); **Fig 3.3 Page 58 (PT/OT/P/D/N)**. Observe the areas of heterochromatin and euchromatin.

Comment on the relative "activity" or "inactivity" of these nuclei and relate this concept to the heterochromatin distribution (i.e. the relative proportions of heterochromatin and euchromatin). Explain your answer.

Note the nuclear membrane in Fig. 3.5. Page 82 (M); **Fig 3.5 Page59, (PT/OT/P/D/N)**.

- What is the pore diameter?
- What is the function of nuclear pores?

Draw a fully labeled diagram of the structure of the nuclear envelope, including a nuclear pore, at the level of the high powered fully resolved TEM (Transmission Electron Microscope) (See Fig 3.7, Page 84 Note the nuclear membrane in Fig. 3.5. Page 82 (M); Fig 3.5 Page59 **(PT/OT/P/D/N).**

VI. THE NUCLEOLUS

Describe the appearance of the nucleolus (refer to Fig. 3.1 Page 77 and Fig. 3.4, Page 79 Note the nuclear membrane in Fig. 3.5. Page 82 (M); **Fig 3.3 Page 58 (PT/OT/P/D/N).**

What is/are the function(s) of the nucleolus?

RECAPITULATION
CYTOLOGY

1. Use a table to describe the functions of the membranous and non-membranous organelles contained with cell cytoplasm.

2. Consider the staining reaction of cell cytoplasm, which contains large numbers of a) mitochondria and b) rough endoplasmic reticulum as seen using H&E under light microscopy.

3. What are inclusions?

4. Describe the appearance of glycogen as seen under TEM and LM (refer to Fig. 18.12 (TEM) on Page 640 and Plate 66 (H&E) on Page 659 Note the nuclear membrane in Fig. 3.5. Page 82 (M); **Fig 16.13 Page 334 and Fig 16.14 Page 335 (PT/OT/P/D/N),** giving reasons for your answer.

PART 1: The Primary Tissues

Consist of:

1. Epithelium
2. Connective Tissue
3. Muscle
4. Nervous Tissue

These primary tissues are found in different combinations in all the organs of the body.

These four tissues are the basis of all histology. They should be studied diligently. The organ systems should not be attempted before the basic tissues are well understood.

A NOTE ON STAINING TECHNIQUES

1. The most common dyes used for staining tissue sections are haematoxylin (blue) and eosin (pink). Haematoxylin acts as a basic dye and reacts with cell components that are acidic (e.g. DNA, thus staining the chromatin blue); structures that stain with basic dyes are said to be basophilic. Eosin is an acidic dye and is therefore bound by basic structures (e.g. cytoplasm of many cells), which stain pink and are said to be acidophilic or eosinophilic. Please do not refer to structures as staining "blue" or "pink" with H & E.

2. In order to demonstrate specific structures other stains may be used for the demonstration slides e.g. the Periodic Acid-Schiff technique (PAS) is used to demonstrate acid mucopolysaccharides. Structures that stain magenta with this stain are referred to as Pas +ve. Osmium Tetroxide, which is a fixative, will stain lipid black and is often used to demonstrate myelin. It is also used in electron microscopy.

Epithelium

OBJECTIVES

To understand and recognize three main epithelia in various sections obtained from both light and electron microscopes.

To be able to see the differences in the morphology of these cells (width vs. height) as well as their location and function (always try to relate structure of the cell/tissue/organ vs. function).

NOTES

An epithelium is a sheet of epithelial cells, which covers the body surfaces, lines body cavities and hollow organs or constitutes glands (see later: epithelial derivatives - glands).

Study epithelia according to the following method:

General Topography

The number of layers of cells

- Simple - one layer
- Stratified - more than one layer

Variations in ***size and shape*** of the cell in the uppermost layer

- Squamous (flat)
- Cuboidal
- Columnar

The shape, size and position of the nucleus in relation to the cell shape
The staining reaction of the cytoplasm - presence or absence of granules
Cell borders - whether distinguishable or not
Surface specialisations - whether present or absent

- Microvilli (striated or brush border)
- Cilia
- Stereocilia

Keratinisation - whether present or absent

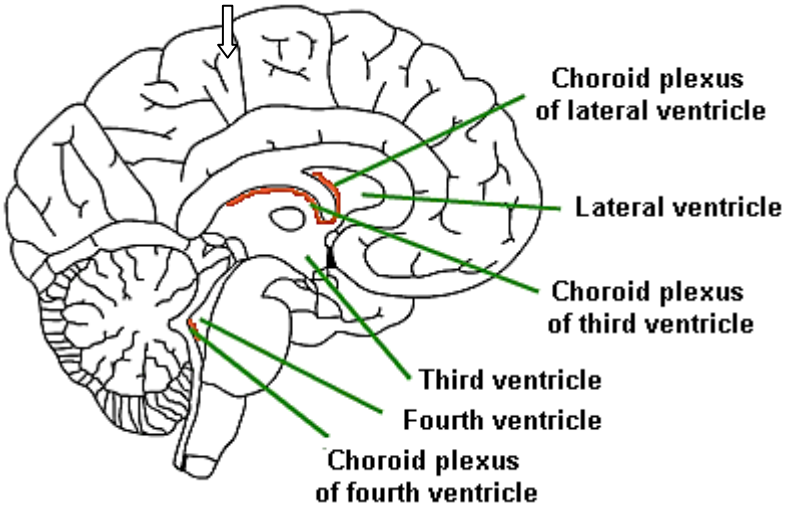
The classification of the epithelium is descriptive and based on the number of cell layers (simple or stratified) and the shape of the surface/uppermost cells (squamous, cuboidal or columnar). In some cases, a third feature – ***apical specializations*** of the cell surface domain, may be added to provide the full classification of the epithelia.

PRACTICAL WORK

I. SIMPLE EPITHELIA

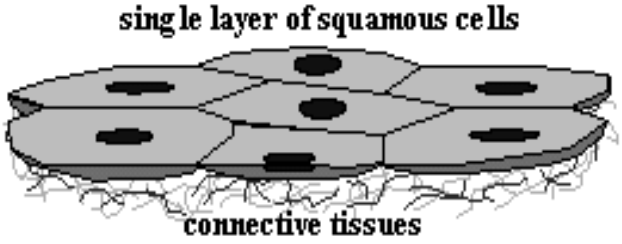
(a) Simple squamous epithelium

Choroid Plexus
Slide: 66
Stain: H&E

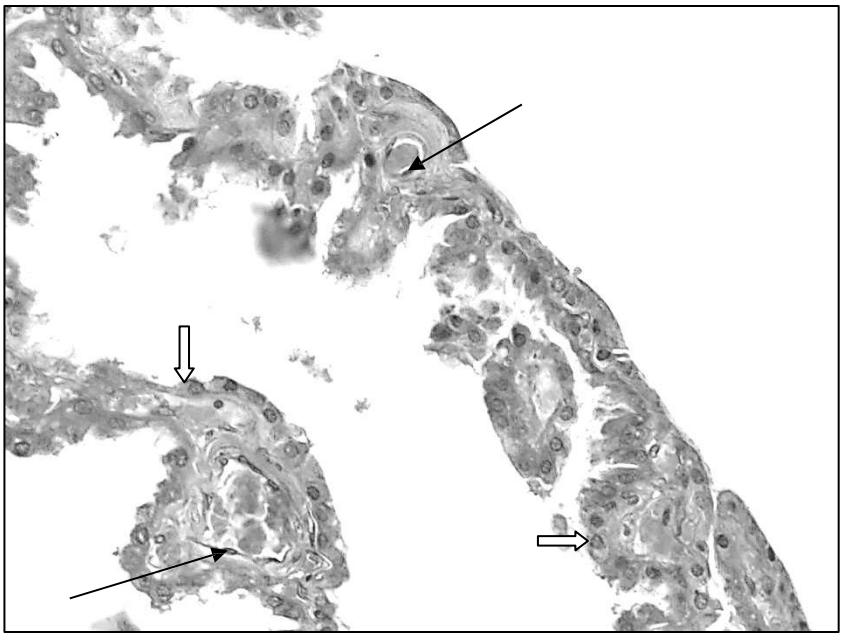


http://library.thinkquest.org/28457/csf.shtml

Study the choroid plexus under L.P. and note the solid mass of the brain tissue and adjacent to it the delicate vascular choroid plexus. The choroid plexus is composed of a lining epithelium - simple cuboidal epithelium. Below this is a core of connective tissue with numerous blood vessels of varying size. The blood vessels contain eosinophilically stained red blood cells and its lumina are lined by simple squamous epithelium. Thus you will be observing two types of simple epithelia both simple squamous and simple cuboidal epithelia within the choroid plexus.



Find a few blood vessels (recognized by the presence of eosinophilically stained red blood cells. These are labelled with a thin black arrow in the photomicrograph below) and study them under H.P.



Draw a few squamous – flat cells lining the lumen of the blood vessel and note how nuclei in some of these cells bulge into the lumen.

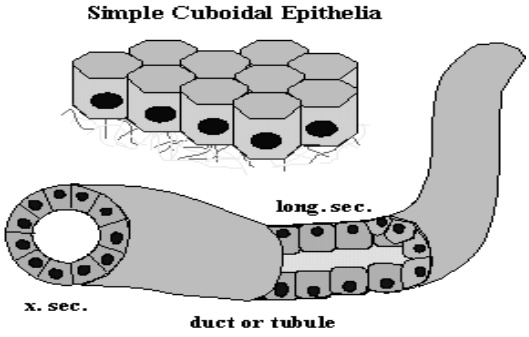
This type of epithelium lines the lumina of all blood vessels.

Note: *The light microscope does not resolve the basal lamina of a simple squamous epithelium. The basal lamina is a material secreted by the epithelium. The basement membrane consist of the basal lamina and reticular fibres contributed by the underlying connective tissue.*

What is the term used to describe the simple squamous epithelium lining a blood vessel?

(b) Simple cuboidal epithelium

Choroid Plexus
Slide: 66
Stain: H&E



A simple cuboidal epithelium lines the vascular choroid plexus. These cells are known as ependymal cells. Apical surface specialisations include microvilli and cilia.This epithelium is exposed to the lumen of the ventricle filled with cerebrospinal fluid, which is produced by these cells. This type of epithelium is also found, for example, lining the ducts of some glands (see the above diagram).

Study and draw a few cuboidal cells under 20x and 40x magnification and show their relationship to the basement membrane (these cells are labelled with a white arrow in the photomicrograph above).

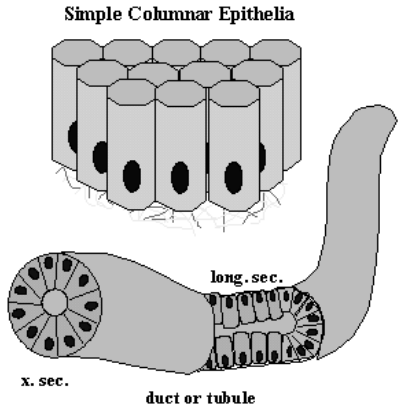
Do not draw separate diagrams of simple squamous and simple cuboidal epithelia, but rather illustrate the relationship between the two epithelia with their relevant basement membranes, the surrounding connective tissue and blood vessel by using only one diagram. This will assist you later with your studies of other basic tissue types and organ systems!

What is the function of the endothelium lining the capillary?

What is the function of the choroid plexus?

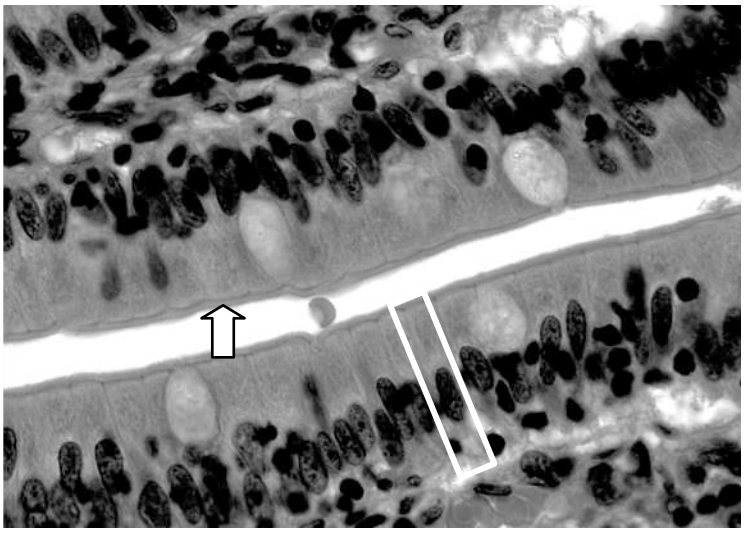
(c) <u>Simple columnar epithelium</u>

Jejunum
Slide: 85
Stain: H&E



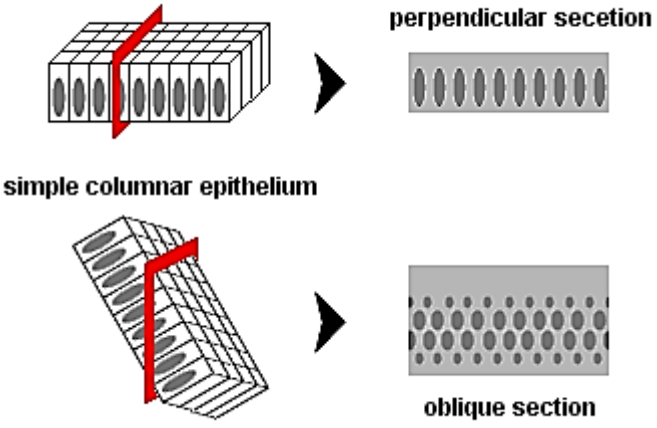
Simple columnar epithelium with striated/microvillus border

The lumen of the jejunum (small intestine) is lined by a simple columnar epithelium (illustrated by the white rectangle in the photomicrograph below). The apical specializations of this epithelium are represented by the regular closely packed microvilli (labeled with a white arrow in the same photomicrograph), which give this apical region a striated appearance (hence the name).



Under H.P. study and draw the striated, microvillus border of this simple columnar epithelium. The microvilli are seen as a solid eosinophilic line on the surface of the epithelium. In addition, draw the microvillus border using suitable electron micrographs in which these specializations are seen as fingerlike projections rather than just a border.

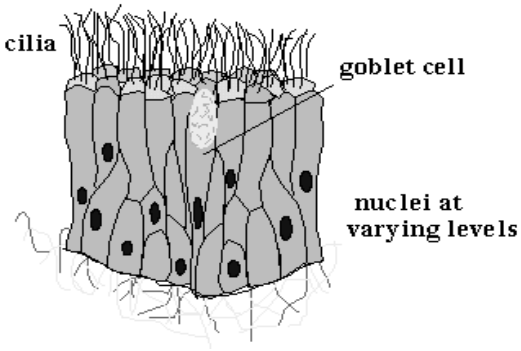
Note: *In some regions the nuclei might appear to be at different levels. Do not make the mistake of classifying this epithelium as pseudostratified; all the cells reach the luminal surface however the section has been cut obliquely and therefore you will see nuclei at different levels. See the diagram below.*



http://www.lab.anhb.uwa.edu.au/mb140/

(d) Pseudo-stratified epithelium

Pseudo-stratified ciliated columnar epithelium with goblet cells



Trachea
Slide: 32
Stain: H&E and elastic

Study and draw under H.P. the columnar epithelium facing the lumen. Show the relationship between the epithelial cells, which lie between the basement membrane, and the tracheal luminal surface.

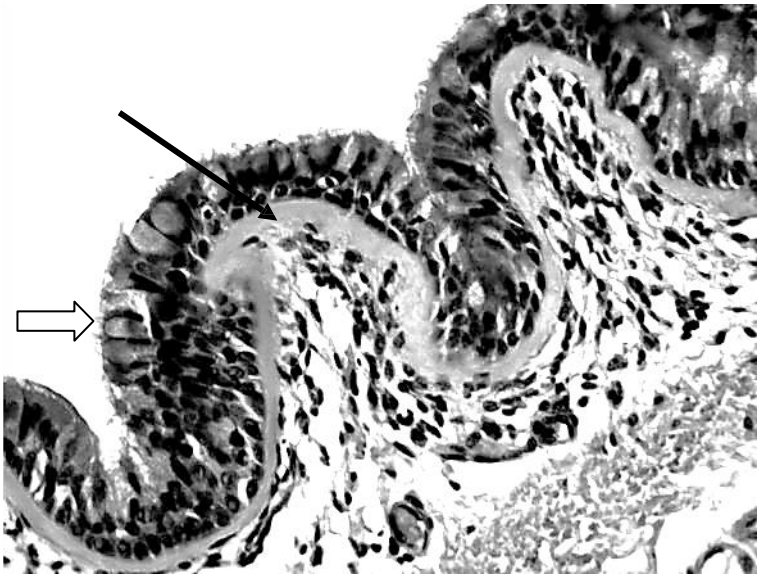
Note: *This epithelium is classified as a* ***simple*** *epithelium as all the cells lie on the basement membrane. They do not however all reach the luminal surface.*

Note:

- Cilia are specializations on the apical surface of these epithelial cells. Briefly describe the structure and function of cilia.

- The numerous goblet cells within the epithelium (what is the function of these cells?)

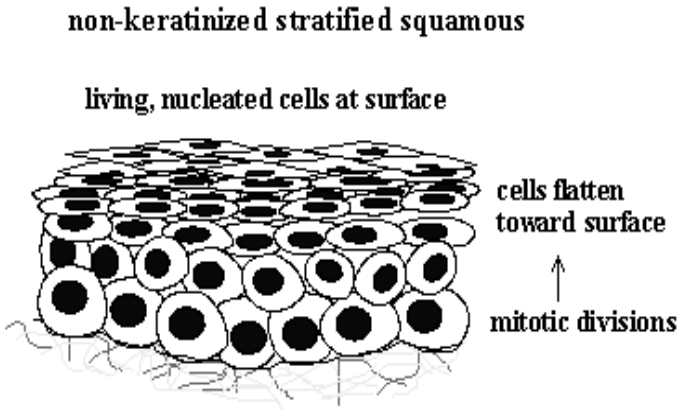
Describe and know the morphological and structural differences between a microvillus border and a ciliated border at both the light and electron microscope level.



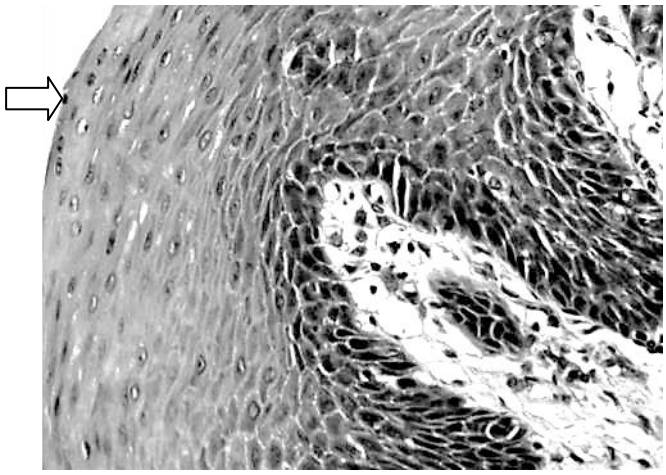
II. STRATIFIED EPITHELIA

(a) Stratified squamous non-keratinized epithelium

Oesophagus
Slide: 32
Stain: H&E



Study the oesophagus by using under H.P. Identify the thick sheet of epithelium lining the lumen, which is classified as stratified squamous non-keratinized epithelium.



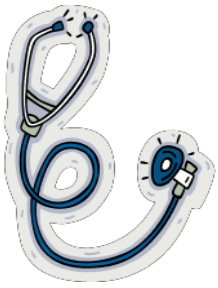
L.P. and H.P.: Study and draw the cells of this epithelium to illustrate how the cells and the nuclei progressively change in shape from the basement membrane to the luminal surface.

Note:

- The presence of nuclei in the superficial cells (labelled with a white arrow in the photomicrograph above. Compare this epithelium with the epidermis of the skin - stratified squamous keratinized epithelium)
- The underlying connective tissue forming papillae between the folds of the epithelium
- The indistinct basement membrane

Why is this type of epithelium called "stratified"? Why "squamous"? Why "non-keratinized"?

Explain the presence of "islands" of connective tissue seen within the epithelium.



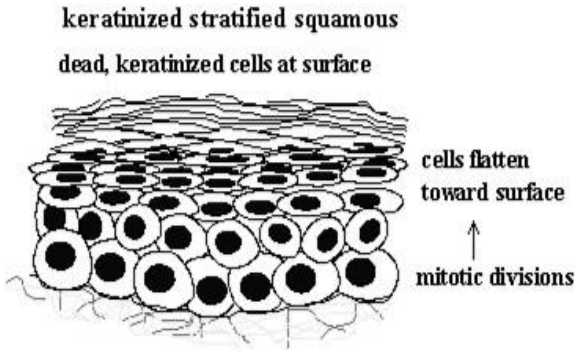
Clinical Correlation

The most common types of cancerous tumours in adults originate from epithelial cells. These cells are known to have a high mitotic index with constant cell renewal, which is taken as their advantage since they are often exposed to mechanical stress and trauma. These cells have close contact with the surface as well as with the lumen of the internal environment, which gives pathogens and carcinogens free access, which often may be harmful. Malignant tumours of surface epithelium are **carcinomas** and those originating from glandular epithelium are **adenocarcinomas**. Knowing a tumour's histological characteristics assist in determining the correct diagnosis but also resolves the cancer stage as well as aids its treatment

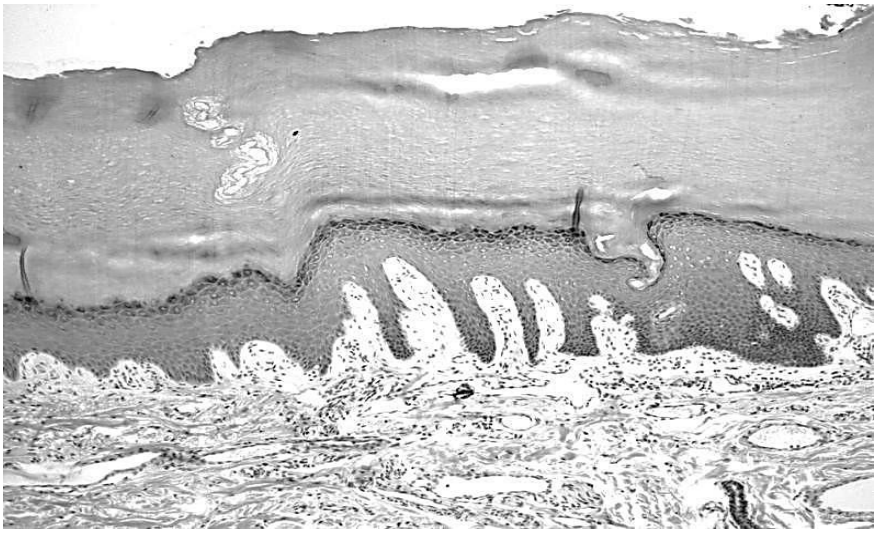
William K. Ovalle and Patric C. Nahirney (2007). Netter's Essential Histology

(b) Stratified squamous (keratinized) epithelium

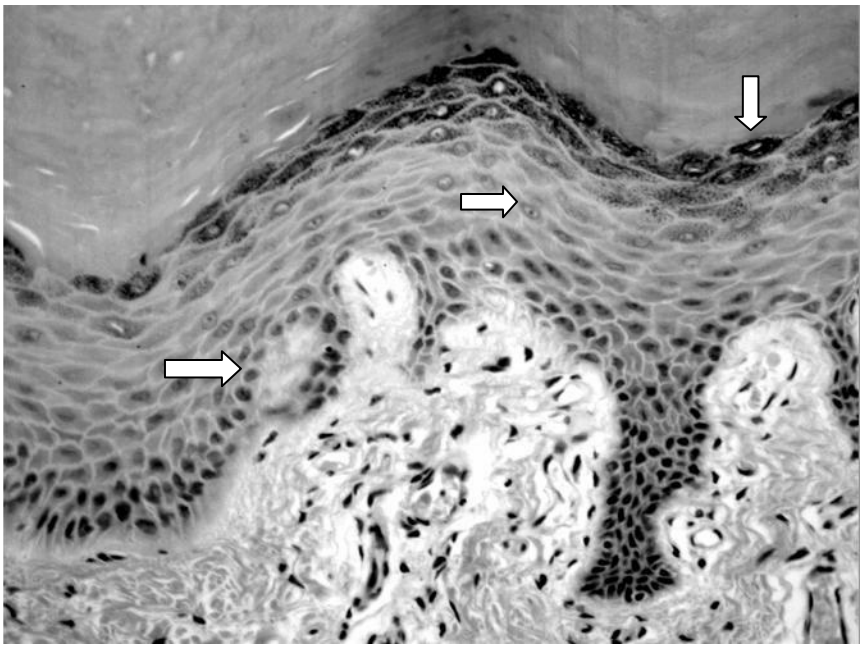
Thick skin
Slide: 35
Stain: H&E



L.P. and H.P.: Study the wide section of the epidermis of the thick skin of the palm (see two photomicrographs of the thick skin shown below).



Study, draw and note how the cells and the nuclei progressively change in shape from the basement membrane to the skin's free surface (see the shape of the cells and their nuclei labelled with white arrows in the photomicrograph below).

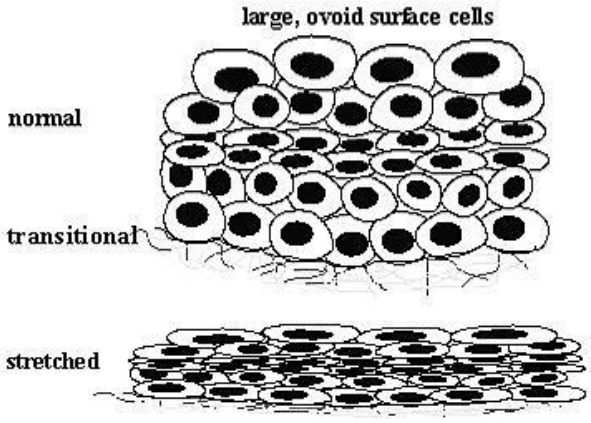


Note the absence of the nuclei in the squamous cell of the most superficial layers of the stratum corneum. Relate this to the classification of this epithelium!

Why is this type of epithelium called "stratified"? Why "squamous"? Why "keratinized"?

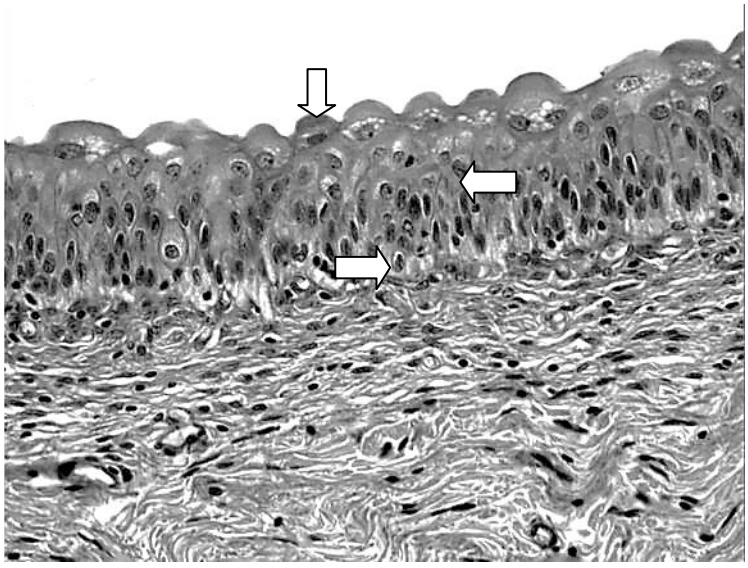
(c) Transitional Epithelium (Urothelium)

Urinary bladder
Slide: 43
Stain: H&E



Locate the epithelium on the luminal surface of the urinary bladder. Using L.P. find a region of epithelium that has been cut perpendicularly. Study and draw the epithelium under L.P. and H.P. to show the:

- Shape and size of the surface cells and their nuclei
- Shape and size of the intermediate cells and their nuclei
- Shape and size of the basal cells and their nuclei
- Apparent absence of a basement membrane



Was the urinary bladder distended or relaxed when this tissue was fixed?

Is transitional epithelium classified as a simple or stratified epithelium? Give reasons for your answer.

Glands: Derivatives of Epithelium

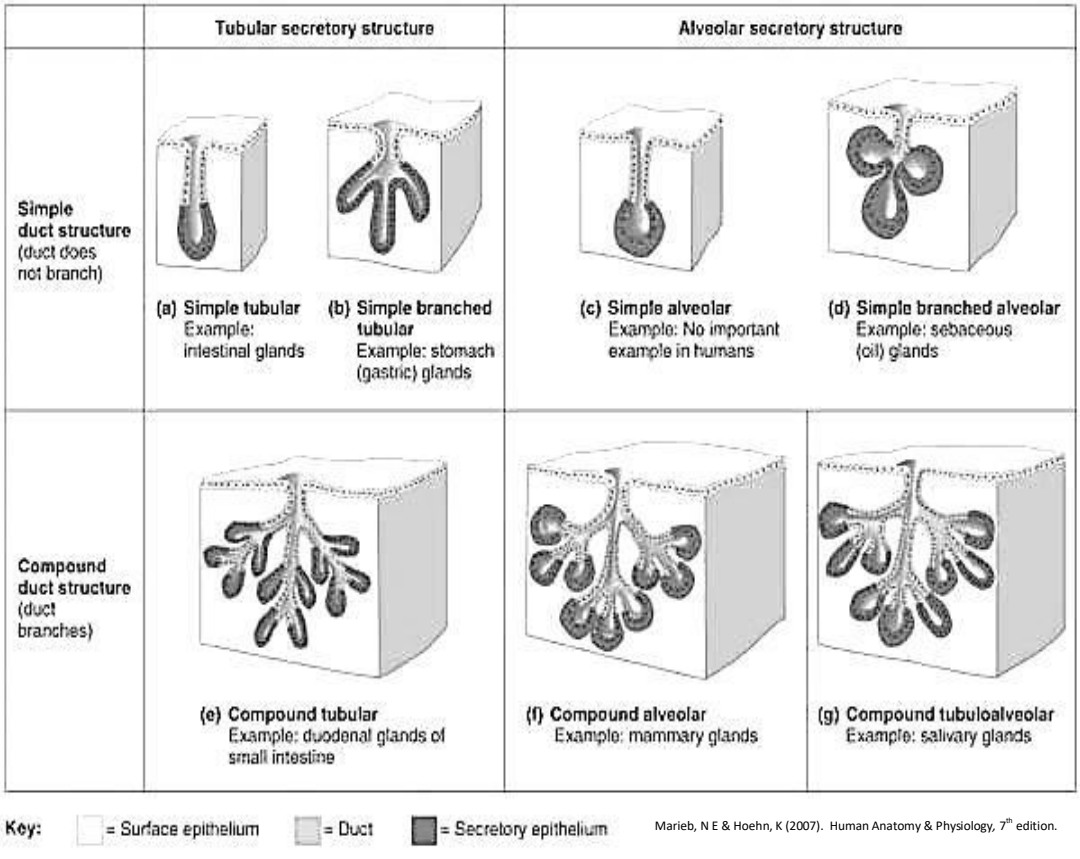
All glands, whether endocrine or exocrine, develop embryonically as epithelial invaginations, which penetrate into the underlying connective tissue. Their most important function is to produce and secrete extracellular products.

Endocrine glands are ductless glands (having lost their connections with the epithelial surface) and they release their products called hormones directly into the blood stream for distribution to the target organs. (Endocrine glands will be studied later in the year).

Exocrine glands, unlike endocrine glands, remain connected to the surface epithelium from which they originated via the ducts, which are used for secretion of their products onto the surface or the lumen of the relevant organ. Apart from the ducts these glands contain the secretory units, made out of the groups of epithelial (secretory) cells continuous with the excretory duct system.

Exocrine glands may be either unicellular or multicellular. Unicellular glands (e.g. goblet cells) are scattered amongst other epithelial cells. Multicellular glands (most common) have either a **single duct** (simple glands) or a **branched duct** system (compound glands) leading from the secretory units - secretory end-piece(s) to the surface epithelium.

Some multicellular, exocrine glands are part of the wall of an organ e.g. glands in the wall of the digestive tract; others are encapsulated and lie outside the organ into which they drain e.g. the liver and pancreas which drain into the duodenum (these multicellular glands will be studied later in the year).



Marieb, N E & Hoehn, K (2007). Human Anatomy & Physiology, 7th edition.

OBJECTIVES

To understand and recognize the main types of glands
To be able to recognise the difference in the histology between the different cell types of the particular gland
To relate structure of the relevant cell with the function of the gland in which it is found

NOTES

Study, draw and label each of the examples of glands below according to the following scheme:

1. **Classify the gland**, i.e. unicellular or multicellular, simple or compound.
2. In compound multicellular glands, note the structure of the cells making up:
   - The secretory end-pieces - mucous, serous, mixed etc.
   - The epithelium of the ducts

3. **Note:**
   - The **nature of secretion** [mucus, serous (protein secretion, e.g. Enzymes) or lipid secretion etc.]
   - Staining reactions of the secretory cells in routine H and E sections.

   Note: *Having a look at the staining reaction can assist you a lot with your answer about the function of the cell hence the whole gland!*

   - The **shape(s) of the secretory end-pieces** (tubular, tubulo-alveolar / tubulo- acinar, or alveolar / acinar – Note: these terms are part of the full classification and are thus very important)
   - **The mode of secretion** (merocrine, apocrine, holocrine)

PRACTICAL WORK

I. UNICELLULAR GLANDS

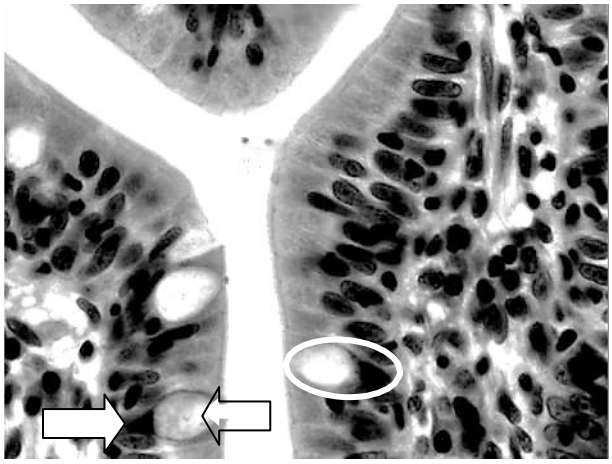
Goblet cells in simple columnar epithelium

Jejunum
Slide: 85
Stain: H&E

Identify the pale-staining goblet cells scattered among the simple columnar cells of the epithelium (encircled in white).

Note, draw and label using L.P. and H.P.:

- The expanded (goblet like) apical end of the goblet cells (right arrow) filled with pale droplets of secretion (what do these glands secrete?)



- The pyknotic (this is the irreversible condensation and thickening of the nuclear material into a solid, darkly staining mass in cells that undergo apoptosis) nuclei and basophilic cytoplasm located at the basal pole of these cells (left arrow).

Refer to the diagram of the pseudostratified ciliated columnar epithelium to see the unicellular gland (the goblet cell) interspersed in between the columnar cells.

II. MULTICELLULAR GLANDS

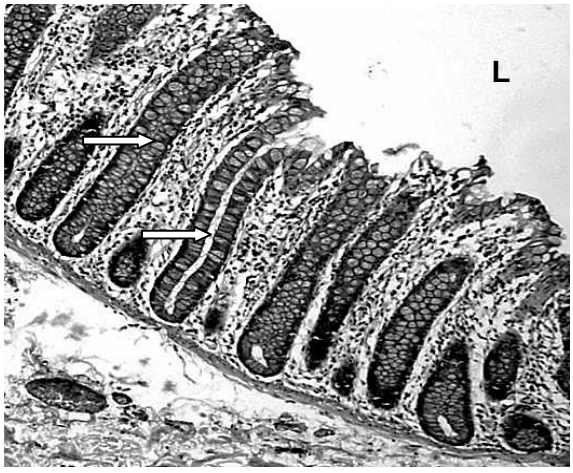
SIMPLE GLANDS

Define a simple gland.

(a) Simple tubular glands e.g. intestinal glands in the colon

Colon
Slide: 91
Stain: H&E

Identify the lumen of the colon and the intestinal glands of the mucosa using L.P. and H.P. Find a region where the gland has been cut longitudinally.



Note, draw and label:

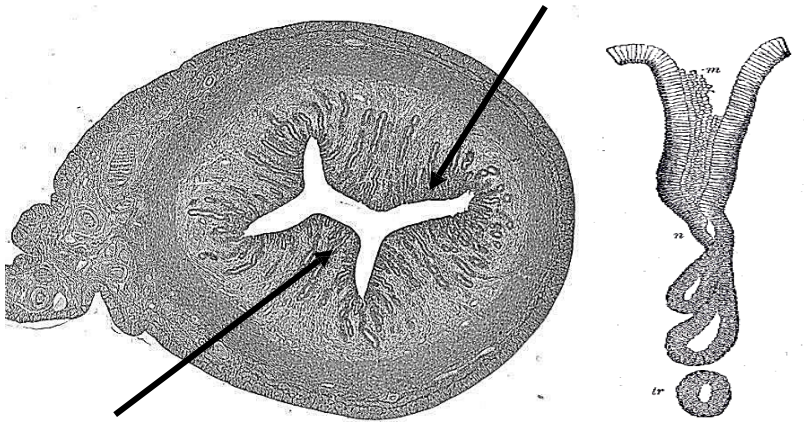
- The shape of the intestinal glands labelled with a white arrow (crypts of Lieberkuhn)
- The numerous goblet cells in the epithelium lining the lumen of the gland.

What is meant by the "lumen" of a hollow structure such as an intestinal gland?

Why are intestinal glands classified as "simple" glands and why "tubular" glands?

Where is the lamina propria (connective tissue) in relation to the glands? Refer to the origin of the gland if you don't know the answer!

Uterus (baboon)
Slide: 10
Stain: H&E



The uterine wall has simple tubular glands too (labelled with a black arrow). Under L.P. identify the lumen of the organ and the simple tubular glands, opening into the lumen.

Study the glands and look for the opening of a gland into the lumen.

H.P.: Study and draw the epithelium and underlying basement membrane. In some regions the nuclei appear to lie at different levels within the cells of the epithelium. Can you explain this in terms of the plane of section?

Only one cell type occurs in this example of simple columnar epithelium. What is the function of these epithelial cells?

(b) Simple coiled tubular glands

Skin of the scalp
Slide: 36
Stain: H&E

Eccrine sweat glands

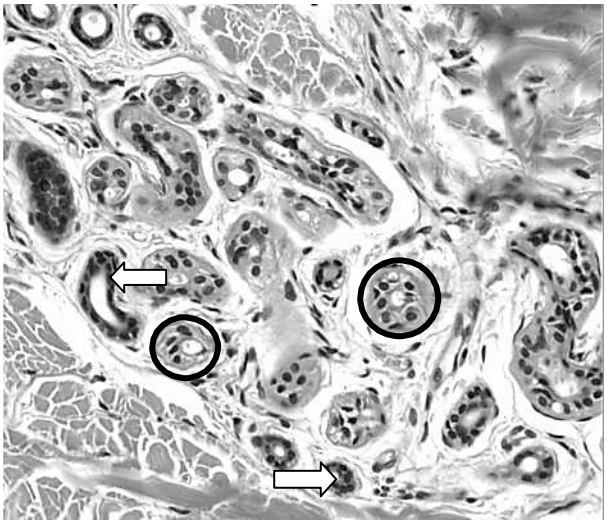
Using L.P. locate the duct (labelled with a white arrow in both photomicrographs below) and secretory end-piece (encircled in black in both photomicrographs) of an eccrine sweat gland of the dermis (connective tissue below the epidermis) and **study and draw** them by using H.P.

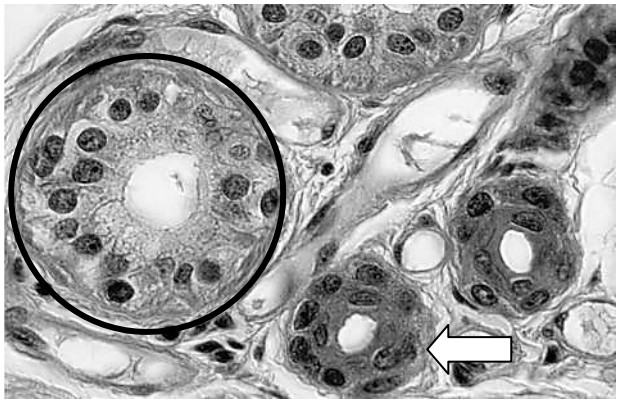
In the secretory end piece (encircled in black in both photomicrographs) note the:

- Small lumen
- Secretory cells - what shape?
- Myoepithelial cells - location?

In the duct (labelled with a white arrow in both photomicrographs) note the:

- Narrow lumen
- Two layers of cells lining the lumen





Classify the epithelium which lines the duct.

Classify the epithelium which lines the secretory end piece.

Classify the sweat gland.

What is the chemical nature of sweat and what is its function?

Simple branched alveolar gland

Skin of scalp
Slide: 36
Stain: H&E

Sebaceous glands

Using L.P. a sebaceous gland, which releases its product via a duct into the hair follicle (see the photomicrograph below).

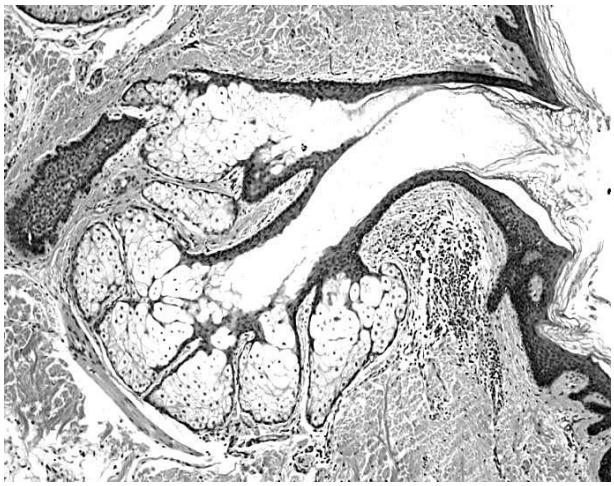
Study and draw using both L.P. and H.P. (see both photomicrographs below):

- The flattened cells of the basal layer of the gland (labelled with a thin black arrow in the second photomicrograph 'B' below).
- The change in shape of the secretory cells from the base of the gland to the duct region.
- The frothy appearance of the cells (compare the histology of these cells with the goblet cells and mucous secreting cells of the submandibular gland – see compound glands section).
- The debris in the lumen of the duct.

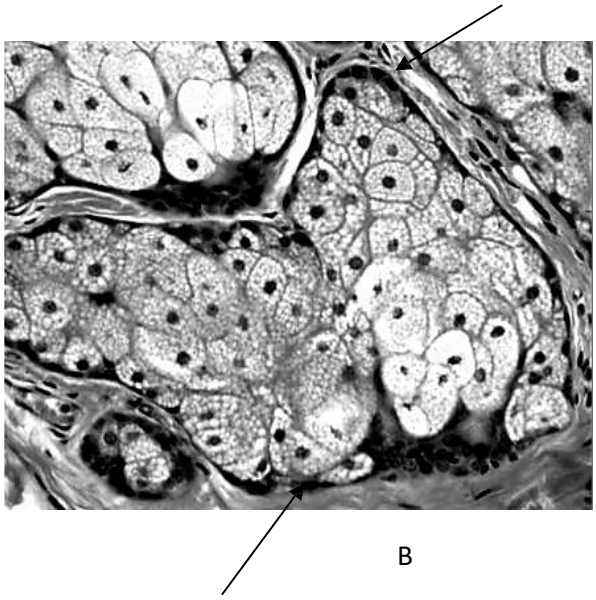
Classify the sebaceous gland.

What is the nature of secretion of this gland and the function of the secreted product called sebum?

What is the mode of secretion of the sebaceous gland?



A

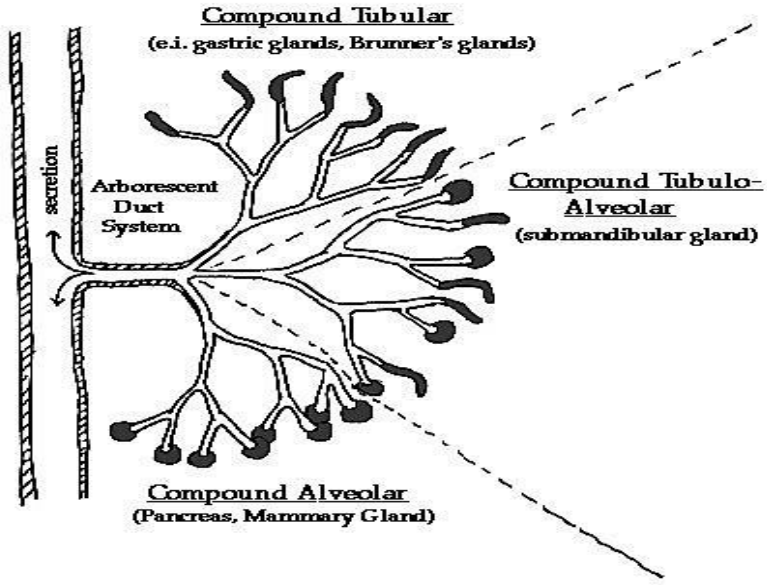


B

COMPOUND GLANDS

Define a compound gland.

Identify three types of compound glands and discuss the morphological differences between them?



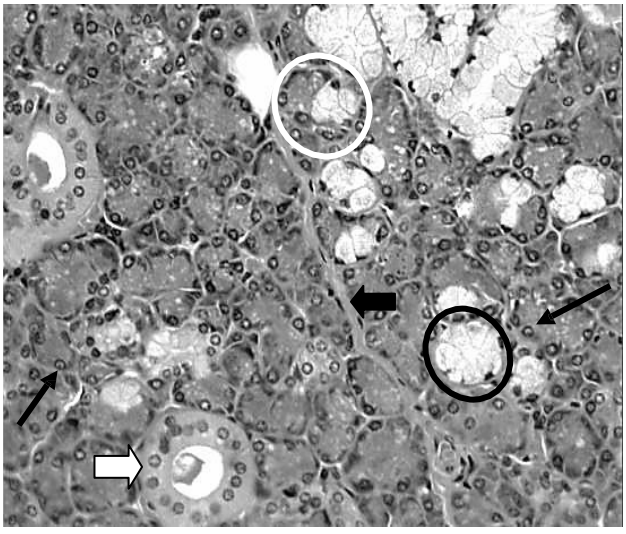
Types of Exocrine Glands in Man (Wheater's Functional Histology 4th edition)

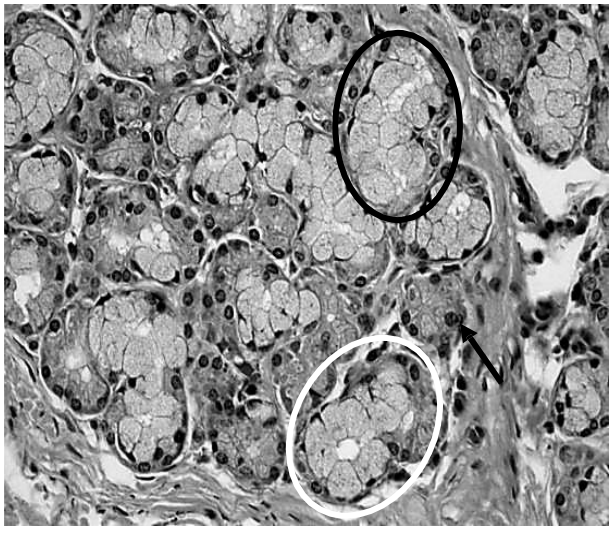
(a) Compound tubulo-alveolar glands

Submandibular gland
Slide: 2
Stain: H&E

Note:

- The eosinophilic capsule and septae (labelled with a black arrow in the photomicrograph below) dividing the gland into lobes and lobules.
- The ducts in the parenchyma of the gland (labelled with a white arrow) and blood vessels lying in the septae.
- The parenchyma of the gland (shown in both photomicrographs below).
- Define parenchyma.





Note and draw using L.P. and H.P.:

- The numerous eosinophilically stained alveolar, serous secretory end-pieces lined by cuboidal cells with spherical nuclei (labelled with a thin black arrow in both photomicrographs above).
- The few palely stained tubular mucous secretory end-pieces with flattened nuclei (encircled black in both photomicrographs).
- Compare the histology and staining reaction of the serous secretory end-piece with that of the mucous secretory unit.

- A mucous secretory end-piece with a serous demi-lune (encircled white in both photomicrographs).
- Myoepithelial cells in the secretory end-pieces.
- Branches of the ducts both within the parenchyma (white arrow upper photomicrograph) and in the connective tissue septae (thick black arrow upper photomicrograph).

What are the distinguishing features of the serous and mucous secretory end-pieces?

What is the function of the serous and mucous secretion in the submandibular gland (relate the structure/staining reaction with the function of that particular cell)?

Why is this gland classified as a "compound" gland and why "tubulo-alveolar gland"?

How did you identify the serous demi-lunes in the secretory portion of the gland? What do they secrete in the mucosa of the lip?

RECAPITULATION
EPITHELIA AND GLANDS

EPITHELIA

1. Define “epithelium” and list the features common to all epithelia.

2. How are epithelia classified?

3. Give three **surface specialisations** of epithelia. For each give an example of where it is found in the body and its function.

SURFACE SPECIALISATION	EXAMPLE	FUNCTION

4. Complete the following table of comparison for epithelia:

FEATURE	SIMPLE			PSEUDO-STRATIFIED	STRATIFIED	
	SQUAMOUS	CUBOIDAL	COLUMNAR	COLUMNAR	SQUAMOUS	TRANSITIONAL
CELL SHAPE						
NUCLEUS SHAPE & POSITION						
SURFACE SPECIALI-SATIONS						
EXAMPLES						
FUNCTION						

GLANDS

1. Explain why glands are classified as epithelial tissues.

Glands may be classified according to several different criteria. For each of the criteria A to D below, give the different types of glands and give at least one example of where they may be found in the body.

A. PRESENCE OR ABSENCE OF A DUCT SYSTEM	
1. Duct system present:	**E.g.**
2. Duct system absent:	**E.g.**

B. SHAPE OF SECRETORY END PIECE AND STRUCTURE OF DUCT SYSTEM	
1.	**E.g.**
2.	**E.g.**
3.	**E.g.**
4.	**E.g.**
5.	**E.g.**

C. MODE OF SECRETION	
1.	**E.g.**
2.	**E.g.**
3.	**E.g.**

D. CHEMICAL NATURE OF THE SECRETION	
1.	**E.g.**
2.	**E.g.**
3.	**E.g.**

Connective Tissue

OBJECTIVES

To understand and be able to recognize the main connective tissue types in tissue sections and in light and electron micrographs:

The main connective tissue (ct) types are:

Areolar or loose ct (e.g. mesentery, omentum, lamina propria or mucous membranes; underlies all epithelium)
Dense irregular ct (e.g. dermis)
Dense regular ct (e.g. tendons and ligaments)

Specialised CT:

Adipose tissue (e.g. hypodermis)
Cartilage (hyaline and articular – e.g. synovial joints; elastic – e.g. ear; fibrocartilage – e.g. intervertebral disc)
Compact and spongy bone
Blood
Haemopoietic tissue
Lymphatic tissue
*Reticular tissue (*e.g. *basement membranes)*
*Elastic tissue (*e.g. *aorta)*

To understand and be able to recognize the main connective tissue fibres in tissue sections and in light and electron micrographs:

The main connective tissue fibres are:

Collagen
Reticular (not seen in routinely stained (H&E) tissue sections)
Elastic (not seen in routinely stained (H&E) tissue sections)

To understand and be able to recognize cell types found in tissue sections and in light and electron micrographs of connective tissue

Cells specific to connective tissue/fixed cells:

Fibroblasts and fibrocytes (e.g. in ct proper and dense regular ct)
Smooth muscle cells (e.g. tunica media of the aorta, and elastic tissue)
Adipocytes (most connective tissues)
Chondroblasts and chondrocytes in cartilage

Transient cells/ wondering cells

Macrophages
Mast cells
Plasma cells
Granulocytes
Lymphocytes

NOTES

These Notes are compiled from Ross and Pawlina; Histology: A Text and Atlas. 5th Edition. 2006. Lippincott, Williams and Wilkins.351 West Camden St, Baltimore, MD 21201.

The distinction of ct and other tissues with a ct component is not always clearly defined. For example peripheral nerve tissue consists of nerve fibre, (axons) and Schwann cells held together in a loose ct matrix. Only the axons and Schwann cells are really nervous tissue as they arise from the neural ectoderm in the embryo. The ct, (endoneurIum, perineurium and epineurium) arises from mesoderm in the embryo and are therefore ct. Most authorities regard the entire peripheral nerve as nervous tissue with a connective tissue component.

Most ct arises from the embryonic mesoderm.

More confusion arises because the **connective tissue fibres and components of the ct matrices can be produced by different cell types.**

In most fibrous ct, for example loose areolar, tendon, ligament and dermis the entire tissue of ground substance and fibre meshwork are produced by fibroblasts. In bone marrow and the lymphoid organs the **reticular fibres are produced by reticulocytes.** In the **tunica media of the aorta, the smooth muscle cells produce all the components of the matrix** including the prominent elastic lamina.

Connective tissue consists of cells and extracellular fibres in an amorphous ground substance. The ground substance and the fibres are together referred to as the " extracellular matrix". Connective tissues are the binding and supporting tissues of the body.

All the soft body parts except the central nervous system have a supporting framework **(stroma**) of connective tissue.

All epithelia and epithelial derivatives including hair follicles and the parenchyma of glands structures are devoid of blood vessels and lymphatic's and the supply of metabolites come from the vessels in the surrounding ct. So that for example waste products or products for secretion or absorption taken up by the epithelia are passed into the stroma and then taken up by the blood vessels. Experiments have shown that it is not the epithelia alone that are the functional unit but **the epithelium and the associated connective tissue.**

There are three main categories of epithelium/connective tissue combinations:

1. **Mucosae or mucous membranes line all tracts and cavities of the body that communicate with the outside of the body.** Examples include the respiratory, alimentary, urinary and genital tracts. The components are the epithelium, its basement membrane, and underlying loose ct called the lamina propria. The lamina propria may contain the secretory portions of glands that have grown out from the epithelium during development**.**

2. **Serosa or serous membranes.** These line the body cavities that have **NEVER** had any communication with the outside of the body. (The exception is the in the female where the uterine tubes open into the coelomic cavity). These include pleural, pericardial and peritoneal cavities. The epithelium component is a mesothelium a single layer of

flattened epithelium lying on a basal lamina rather than a basement membrane and a loose fibrous ct that lacks glands. The mesenteries that suspend viscera from the body wall and omentum, which connect the viscera together, are double serosae because they consist of two mesothelia with a thin layer of connective tissue between them.

3. **Skin.** Covers the outside of the body. It consists of the epidermis, which is a stratified keratinised squamous epithelium. Lies on a basement membrane and the underlying dermis containing hair follicles, sweat glands and sebaceous glands in the dermis (these structures may also be found partly in the hypodermis, which is not classified as part of the skin). The mammary glands are also of epidermal origin and are modified sweat glands.

PRACTICAL WORK

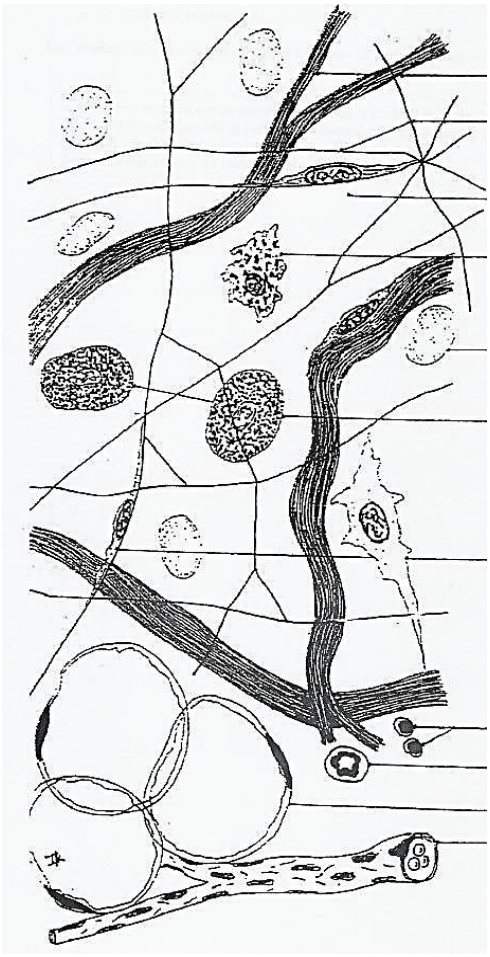
CONNECTIVE TISSUE PROPER

Remember: Matrix = cells and fibres in an amorphous ground substance

Note: *We are going to classify the connective tissue by the type of matrix it possesses, the types of cells present, extracellular fibre types present and their arrangement*

Also Note: *the staining reactions of the cells, fibres and ground substance and the distribution of cells in relation to fibres.*

Loose (areolar) Connective Tissue



I. Connective Tissue with a Semi-Solid Matrix

I. LOOSE (AREOLAR) CONNECTIVE TISSUE

Connective tissue spread or mesentery spread (rat)
Slide: 120
Stain: Haematoxylin, acid fuchsin, and elastic stain

If you have a mesentery spread, remember to look for the mesothelial cells. A mesentery is a thin layer of tissue suspending the gut from the body wall. It is covered on both sides by a simple squamous epithelium, the mesothelium, continuous with that enfolding the gut and lining the peritoneal cavity. You will therefore be studying a "sandwich" consisting of two outer layers of simple squamous epithelium, or mesothelium, and a thin "filling" of loose connective tissue between them. Remember, the spread has NOT been sectioned, so when you look through this thin "sandwich" you will see the nuclei of the two epithelial (mesothelial) layers as well as those of the connective tissue cells.

Note: *Elastic fibres are stained blue by a special technique.*
Macrophages may have ingested carbon particles as some rats were injected with India ink.

Select a thin area of the spread:

Under H.P. study and draw:

- Collagen and elastic fibres, fibroblasts, macrophages (histiocytes), mast cells, blood cells and adipocytes.
- Mesothelial cell nuclei (epithelial cells not connective tissue cells) will also be seen.

Is there any evidence that ground substance is present in loose (areolar) connective tissue?

Compare the distinguishing features of elastic and collagen fibres.

How did you identify macrophages and mast cells?

Explain the presence of mesothelial cells.

Estimate the amount of ground substance present.

Classify this connective tissue into one of the three-epithelia tissue categories given above.

Explain what is meant by "Spread".

II. & III. DENSE IRREGULAR CONNECTIVE TISSUE AND ADIPOSE TISSUE

Non-lactating mammary gland
Slide: 122
Stain: H&E and in some slides, elastic stain

AND

Axillary Sheath
Slide: 26
Stain: H&E and in some slides, elastic stain

Macroscopic: Identify the large, very palely stained areas of adipose tissue and the more darkly eosinophilic areas of dense irregular connective tissue

Study and draw under L.P. and H.P.:

- The dense irregular connective tissue noting that the nuclei of the fibroblasts associated with the collagen fibres are darkly stained and that the fibres are extra-

cellular. (Note the arrangement of the fibres). Are any other cell types other than fibroblasts present in the section?

- Adipose tissue noting that the lipid droplet in each cell has been dissolved out during preparation of the section.

Why is adipose tissue classified as a connective tissue?

What types of connective tissue fibres are found in adipose tissue?

IV. DENSE REGULAR CONNECTIVE TISSUE

(a) Musculo-tendinous junction

Slide: 50
Stain: H&E

Macroscopic: The band of palely eosinophilic tissue is tendon. The fibres inserting into the tendon obliquely are skeletal muscle fibres.

Study and draw the tendon under L.P. and H.P.

Histologically distinguish between dense regular and dense irregular connective tissue?

Relate the function of dense regular connective tissue to its histological structure.

List the similarities and differences between: dermis of skin, tendon and skeletal muscle

Estimate the amount of ground substance present in the tendon.

Suggest another type of dense regular connective tissue.

(b) Elastic tissue

Aorta
Slide: 28
Stain: Elastic

Examine the slide

(c) Reticular tissue

Liver
Slide: 25
Stain: Silver impregnation method

Examine the slide and give the function of elastic and reticular tissue

Clinical Correlation

Invasive carcinoma of the breast is divided into six main histological types named according to the histological pattern presented. To diagnose and understand this set of diseases it is important to be able to identify the connective tissue (and epithelium) seen in the normal breast.

Definition:
Desmoplastic: *The stromal, or 'desmoplastic', responses seen histologically in primary breast carcinomas can vary from being predominantly cellular (fibroblasts/myofibroblasts) with little collagen to being a dense acellular tissue. The mechanisms underlying the stromal response are complex; paracrine activation of myofibroblasts by growth factors is important as well as possibly cytokines/chemokines. It also appears that platelet-derived growth factor (PDGF) is the initiator of the desmoplastic response.* (**Rosemary A Walker** The complexities of breast cancer desmoplasia *Breast Cancer Res* 2001, **3**:143-145)

These are:
Invasive ductal carcinoma: (55%) Tumour cells invade breast tissue and after a desmoplastic response a dense fibrous stroma is seen (lump).

Invasive lobular carcinoma (10%). The tumours are often multifocal within the breast often with bilateral breast involvement. This type of cancer is also associated with a desmoplastic response. The tumour cells typically the tumour cells are compressed into narrow cords, described as an Indian file pattern of invasion

Mixed ductal and lobular carcinoma (30%) A mixture of both types described above.

Special Types (5%):
Tubular carcinomas (2%) Present as small tubular structures that look like small ducts. There is usually a desmoplastic response with a fibrous stroma forming a stellate pattern. Although only 2% of all invasive carcinomas they account for 20% of carcinomas detected by mammography.
Mucoid carcinoma Usually seen in postmenopausal women. Mucins are secreted into the stroma creating a shiny appearance.
Medullary carcinoma. Usually seen in postmenopausal women forming soft fleshy masses without the desmoplastic response.

Alan Stevens, James S. Lowe, & Ian Scott. Core Pathology, 3rd Edition

RECAPITULATION
CONNECTIVE TISSUE WITH A SEMI-SOLID MATRIX

1. In a table, summarise the features of all the connective tissues with a semi-solid matrix. Relate the variations in structure to differences in function.

2. Summarise the distinguishing features of each of the connective tissue cell types studied so far.

II. Connective Tissue with a Fluid Matrix

OBJECTIVES

BLOOD and LYMPH are considered connective tissues, as they are composed of cells, or parts of cells, as well as an extracellular component. Blood is the fluid connective tissue that circulates through the cardiovascular system while lymph is the fluid that is collected from the tissues and returned to the blood stream. Lymphatic organs and the formation of lymphocytes will be studied later in this course.

The objective of the following practical is to:

Identify and describe the characteristic features of each of the main cell types found in a normal blood smear (i.e. Erythrocytes, Leukocytes and Platelets)
Identify and describe the characteristic features of each of the different leukocytes
Describe the main function of each of different cells types found in a normal blood smear

NOTES

These notes are compiled from Ross and Pawlina; Histology: A Text and Atlas, 6th Edition, 2011. Lippincott, Williams and Wilkins and Victor P. Eroschencho; di Fiore's Atlas of Histology with functional correlations, 7th Edition, 1993. Lea & Febiger.

Blood is a specialized form of connective tissue that consists of formed elements (erythrocytes, leukocytes and platelets) suspended in a protein rich extracellular matrix (plasma). It primarily serves as a transport medium for gases, nutrients, waste products, antibodies, hormones and various other substances but also play an important role in the maintenance of homeostasis within the body by acting as a buffer and taking part in coagulation and thermoregulation.

The various cells found in blood are formed by the process of hemopoiesis. This process involves the multiplication, differentiation and development of pluripotential stem cells into various types of mature blood cells. Blood cells (i.e. the formed elements) include:

Erythrocytes (red blood cells):
These anucleate, biconcave cells are the most abundant cell type found within blood and they are often used as a size reference for other cell types. They mainly function in the transport of oxygen and carbon dioxide.

Leukocytes (white blood cells):
These nucleated cells can be subdivided into two categories (granulocytes and agranulocytes) according to the shape of their nucleus, the absence or presence of specific cytoplasmic granules and the staining reaction of their granules. Leukocytes in general function as a defence system against foreign material.

Platelets (thrombocytes):
These are anucleated cytoplasmic fragments derived from megakaryocytes and are the smallest of the formed elements and functions in blood clotting.

PRACTICAL WORK

(a) Blood Smear

Normal adult (human) peripheral blood
Slide: 70
Stain: Romanowsky-type bloodstain & Leishman's techniques

A few notes on the staining technique:

The formed elements of blood all have specific functions and characteristic structural features. The latter can be shown up by special staining techniques, such as a Romanowsky-type stain. This stain is a mixture of dyes including a basic dye (methylene blue), an acidic dye (eosin, pink in colour) and a metachromatic basic dye, azure (blue in colour). Certain cell inclusions may change the colour of this metachromatic dye from azure blue to purple. In a smear we can describe structures within the cells as being basophilic (staining blue), eosinophilic, (staining pink), neutrophilic (staining lilac with both the basic and the acidic dye), azurophilic (reddish-purple) and metachromatic (purplish-blue).

Formed elements

Under LP find each of the blood cells listed below and then study, draw and label each under HP paying particular attention to:

- The relative sizes of the different cell types in comparison to erythrocytes (erythrocyte diameter ± 7.8 m in a smear)
- The size and shape of each cell
- The size and shape of the nucleus (i.e. number of lobes) and the degree of chromatin clumping
- The staining reaction, size and shape of the cytoplasmic granules (leukocytes)
- The relative abundance of cytoplasm within the cell (leukocytes)

I. ERYTHROCYTES

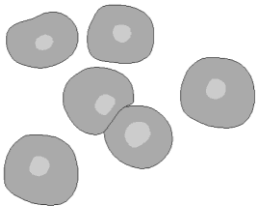
These anucleated cells are the most abundant cell type and they are typically used as a size reference for other cell types.

Blood

Plasma
- Liquid extracellular matrix
- Composition: Water (91-92%), protein (7-8%) and other solutes (1-2%)
- 55% of whole blood volume

Formed Elements
- Suspended in plasma
- 45% of whole blood volume

Erythrocytes (Red blood cells)
- Anucleated cells
- Diameter: 7.8 µm



Leukocytes (White blood cells)
Nucleated cells

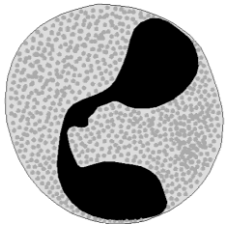
Thrombocytes (Platelets)
- Anucleated cells
- Diameter: 2-3 µm

Granulocytes
Polymorphonuclear
- Complexly *lobed* nucleus
- *Specific* granules in cytoplasm

Agranulocytes
Mononuclear
- Single, *rounded/indented* nucleus
- Lacks *specific* granules in cytoplasm

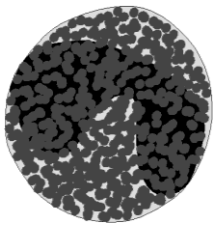
Neutrophil
- 50-70% of circulating leukocytes
- Diameter: 10-12 µm

Monocyte
- 3-8% of circulating leukocytes
- Diameter: 18µm



Eosinophil
- 2-4% of circulating leukocytes
- Diameter: 10-12 µm

Lymphocyte
- 20-40% of circulating leukocytes
- Diameter: 6-15µm



Basophil
- <1% of circulating leukocytes
- Diameter: 10-12 µm

* Respective diameters are estimated values and refer to the sizes of these cells in blood smears, in which cells are spread and appear larger than they would in the blood

Clinical Correlation

Anemia is the most common disorder associated with blood. This condition is usually the results of a reduction in the number of healthy red blood cells or a reduction in the normal quantity of hemoglobin in the blood. This is typically caused by excessive blood loss (hemorrhage), accelerated red blood cell destruction (hemolysis), reduction in red blood cell production (insufficient hemopoiesis) or production of red blood cells with insufficient hemoglobin. However, there are several types of anemia, each caused by a variety of different factors giving rise to different sets of clinical symptoms. The most common symptoms associated with anemia include fatigue, weakness and loss of energy.

Ross and Pawlina (2011). Histology: A Text and Atlas, 6th Edition.
Harhold Sheedlo (2005). USMLE Road Map: Histology.

II. LEUKOCYTES

These nucleated cells are typically divided into two main groups:

2.1. Granulocytes (granular leukocytes)

Granulocytes are characterized by the presence of **specific** granules in their cytoplasm. There are three types of granulocytes:

Eosinophils: Characterized by large, refractile, eosinophilic cytoplasmic granules

Neutrophils: These are the most numerous leukocytes. These cells are characterized by their multilobed nucleus and fine cytoplasmic granules which may be difficult to distinguish

Note: *A small drumstick shaped appendage is usually present on one of the nuclear lobes of the neutrophils of females. This appendage is known as a Barr body which is the condensed, quiescent (inactive) X chromosome.*

Basophils: Characterized by coarse, intensely basophilic cytoplasmic granules typically obscuring the nucleus. As these granules are water soluble, they may not be very well preserved. There are very few basophils (they make out <1% of circulating leukocytes). Don't waste too much time trying to find these, look at the demonstration.

Clinical Correlation

Leukemias, a cancer of the blood or bone marrow, are typically characterized by a progressive proliferation of leukocytes. It is broad term used to describe a variety of diseases. Leukemias are therefore divided into several main groups. The first division is based on whether the disorder is acute or chronic. Acute leukemias are characterized by a rapid increase in the number of immature leukocytes (most commonly seen in children) whereas chronic leukemias are characterized by an excessive accumulation of abnormal, relatively mature leukocytes (mostly seen in older individuals, but can affect any age group). The second division of the disorder is based on the type of cell that is affected. The disorder will be classified as a lymphoblastic or lymphocytic leukemia when lymphocyte precursor cells are affected, whereas myeloid or myelogenous leukemias are typically malignancies of granulocyte, red blood cell or platelet precursor cells. Combination of these two divisions thus leads to the four main categories of leukemias: Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), Acute Myelogenous Leukemia (AML), Chronic Myelogenous Leukemia (CML).

Ross and Pawlina (2011). Histology: A Text and Atlas, 6th Edition.
Harhold Sheedlo (2005). USMLE Road Map: Histology.

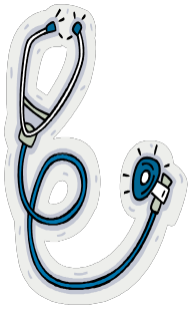
2.2. Agranulocytes (agranular leukocytes)

Agranulocytes are characterized by the absence of **specific** granules in their cytoplasm. There are two types of agranulocytes:

Monocytes:	Largest leukocyte. Characterized by a large, indented, eccentric, nucleus and extensive, pale basophilic cytoplasm
Lymphocytes:	Most common agranulocytes, characterized by an intensely stained spherical nucleus that may also be indented. The cytoplasm of these cells is typically scant and appears as a thin rim around the nucleus. Both small and medium sized lymphocytes occur in the circulation. Small lymphocytes are not much bigger than red blood cells and medium lymphocytes are roughly the size of neutrophils.

III. PLATELETS

These are anucleated cytoplasmic fragments derived from megakaryocytes.
Find a cluster of platelets and note their structural features.



Clinical Correlation

Hemophilia is a bleeding disorder in which it takes a prolonged period of time for blood to clot or coagulate. This is due to a reduction in the normal levels of blood clotting factors essential for a normal clotting process. Hemophilia is a recessive sex-linked, X chromosome disorder affecting mostly males. Hemophilia A is the most common form of this disease and is caused by a deficiency in clotting factor VIII, which is one of the factors involved in fibrin generation while hemophilia B is caused by a deficiency of or a defected clotting factor IX.

Ross and Pawlina (2011). Histology: A Text and Atlas, 6th Edition.
Harhold Sheedlo (2005). USMLE Road Map: Histology.

RECAPITULATION
CONNECTIVE TISSUE WITH A FLUID MATRIX

1. Why is blood considered to be a type of connective tissue?

2. Are there any fibres in blood? If so, how are they formed and what is their function?

3. Which cell types typical of blood and of lymph are to be found in the connective tissue outside the vascular system? Which of these cell types did you see in the connective tissue spreads?

4. Why are the granules of the granular leucocytes called "specific" granules?

5. What are "aspecific" granules and in which blood cell types are they found?

6. How is it possible to "sex" a blood smear?

7. What are the functions of blood?

8. What is plasma?

9. What is serum?

10. What is lymph and where is it formed?

11. Complete the table comparing the different cell types found in blood. Which of these is strictly speaking not a type of "cell" and why?

Cell Type	Size	Shape of the Nucleus	Cytoplasm (Granular or Agranular)	Type/s of Granules	Staining Reaction	Main Function
Erythrocyte						
Leukocytes: ***Neutrophil***						
Eosinophil						
Basophil						
Monocyte						
Lymphocyte						
Platelets						

III. Connective Tissue with a Solid Matrix

OBJECTIVES

To identify, classify and describe the histological structure and function of the different types of bone and cartilage.

NOTES

There are two forms of connective tissue with a solid matrix: cartilage and bone. As in connective tissue bone and cartilage are composed of cells, fibres and ground substance. The cells occupy small cavities within the solid matrix; these small spaces are known as lacunae. Cartilage is composed of cells called chondrocytes and a highly specialized extracellular matrix. Bone tissue is classified as either compact or spongy (also known as cancellous bone). A mineralized extracellular matrix characterizes both types of bone. The cells of bone are called osteocytes.

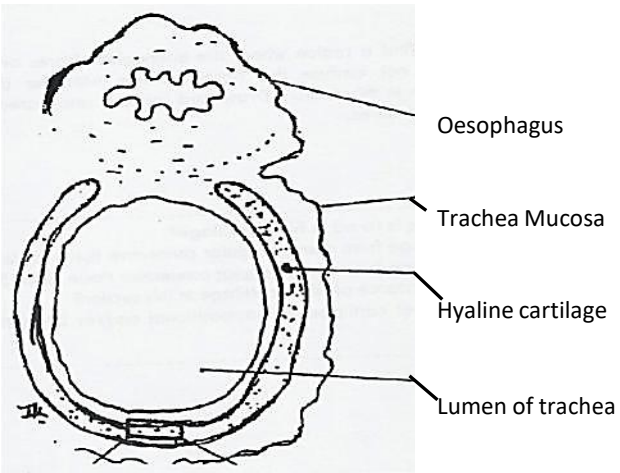
PRACTICAL WORK

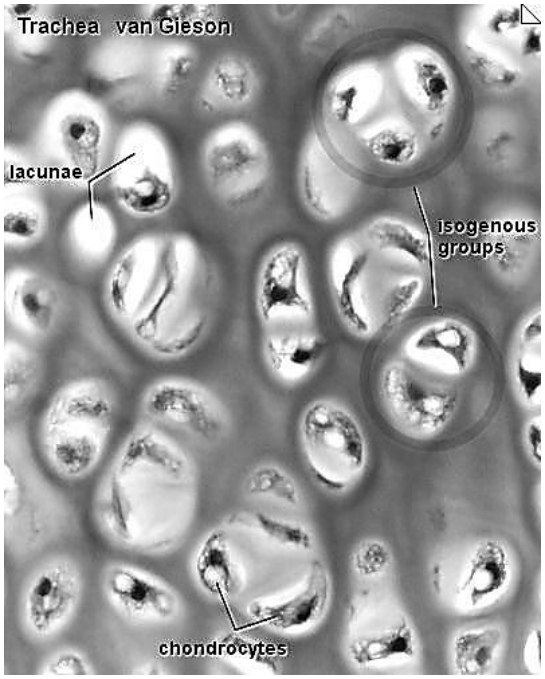
I. CARTILAGE

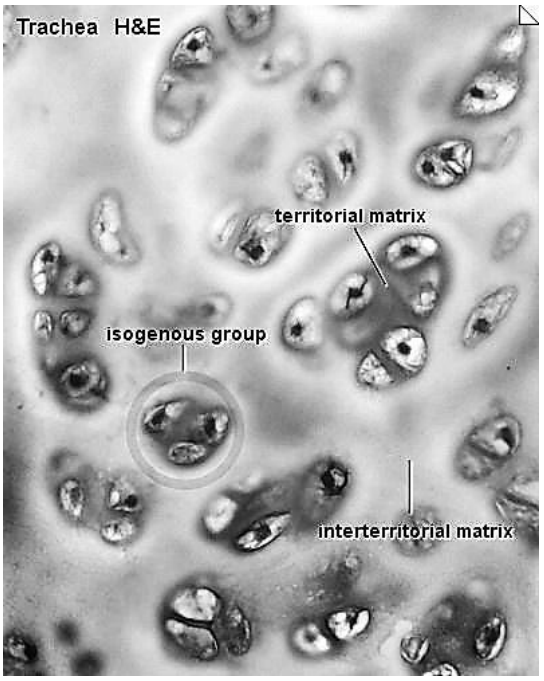
(a) Hyaline cartilage

Trachea and oesophagus
Slide: 32
Stain: H&E

This slide is a cross section through the trachea and oesophagus. You need to orientate yourself around this section, as there are two structures on this slide. The oesophagus consists of a folded lumen and it is smaller in diameter. You will learn about this structure later in the year when you study the histology of the GIT. The trachea will be identified by the basophilic (blue-staining) ring of cartilage (C-shaped).





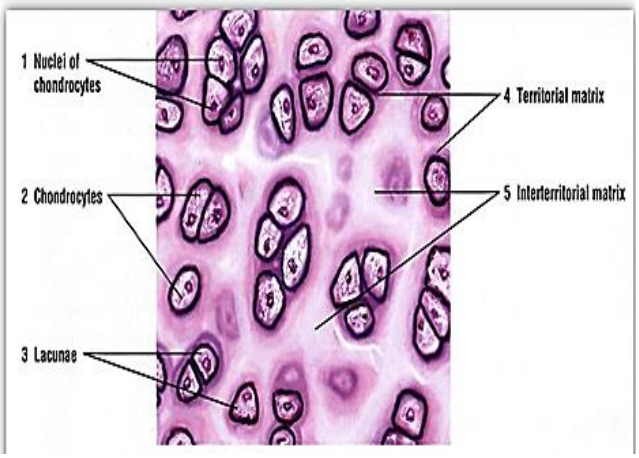


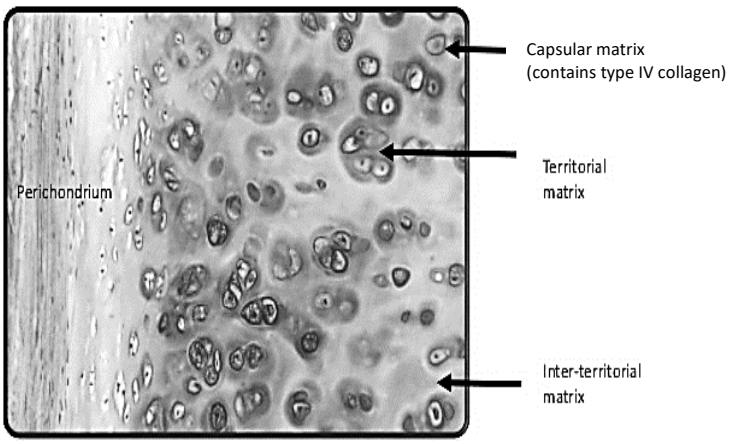
http://www.lab.anhb.uwa.edu.au/mb140/

Study and draw the cartilage under L.P. and H.P.

Note:

- The fibrous perichondrium (what type of connective tissue is this?)
- What types of cells are found in the perichondrium?
- Is perichondrium present in articular cartilage? Give reasons for your answer.
- The lacunae (variation in size and shape from the outer surface to the centre of the cartilage plate) and arrangement of the chondrocytes within the lacunae (single or in "nests"/isogenous groups)
- The shrunken chondrocytes (cartilage cells) within the lacunae
- The matrix and capsules of the lacunae. Distinguish between the territorial, interterritorial and capsular matrices.

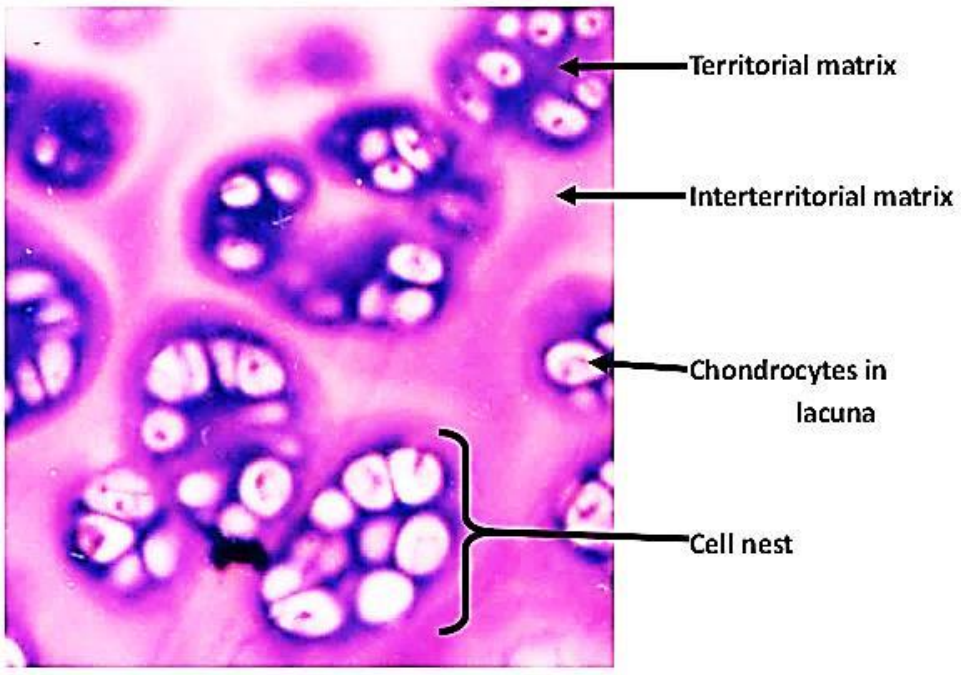




***Darker staining indicates higher (proteoglycans)**

https://www.slideshare.net/rajeshkmcic/histology-of-cartilage

Hyaline cartilage- magnified



http://slideplayer.com/slide/10720112/

Cartilage consists of collagen fibres within its matrix. (Comment on the apparent absence of fibres within the matrix).

Why is this type of cartilage called “hyaline”?

What is the function of hyaline cartilage and where else do we find it?

What are chondroblasts? Where do they lie in growing cartilage?

Describe the evidence seen in this section that "appositional" and "interstitial” growth have taken place in the cartilage plate.

How do the living chondrocytes acquire their nourishment?

(b) Elastic cartilage

External auditory meatus
Slide: 51
Stain: H&E and elastic

Examine this slide

(c) Fibrocartilage

Meniscus of knee
Slide: 79
Stain: H&E

This slide is a section through the meniscus of the knee.

Fibrocartilage is a combination of dense regular connective tissue and hyaline cartilage. This type of cartilage is found in intervertebral disks, the symphysis pubis, articular disks of the sternoclavicular and temperomandibular joints, menisci of the knee joint, the triangular fibrocartilage complex of the wrist, and certain places where tendon attach to bones.

Under L.P. and H.P. find a region where the eosinophilic fibres are particularly densely woven. Do not confuse the fibrocartilage with the dense regular ct seen in this section. Draw and label a small area to show the details of the cells and fibres.

What type of connective tissue fibre is found in fibrocartilage?

How do you distinguish fibrocartilage from dense irregular connective tissue?

How do you distinguish fibrocartilage from dense regular connective tissue?

What do you see in the ground substance of fibrocartilage in this section?

Is there any evidence in this type of cartilage that appositional and/or interstitial growth have taken place? Give reasons for your answer.

Clinical Correlation

Chondromas are benign tumours of hyaline cartilage which may arise either on the surface of bone or within the medullary cavity where they are known as endochondromas. These tumours are composed of nodules of benign hyaline cartilage. The neoplastic chondrocytes are benign, with no dysplastic features and are embedded in a well-formed cartilage matrix.

Chondrosarcomas are malignant cartilage tumours, which mainly arise, in middle-aged and elderly individuals. In this tumour, the chondrocytes are enlarged and may be binucleate.

Young, B., Stewart, W., O'Dowd, G. (2011). Wheater's basic pathology, 5th Edition.

II. BONE

The matrix of bone is impregnated with inorganic salts, principally calcium phosphate (constituting the hydroxyapatite crystals), and thus it is very hard. Because of the hard nature of bone, the bone tissue that you will see has been either decalcified or ground down. Typically a bone consists of bone tissue and other connective tissues, including hemopoietic tissue, fat tissue, blood vessels, and nerves. If the bone forms part of a synovial joint, hyaline cartilage is present.

(a) Compact bone (Decalcified)

Long bone
Slide: 1
Stain: Decalcified, H&E

This is a section through a decalcified long bone. Note the many Haversian canals in the centre of a Haversian system, (more or less circular in outline)

Under L.P. note and illustrate:

- The periosteum
- The arrangement of lamellae - circumferential, interstitial, and concentric in the Haversian systems (where are these lamellae found within compact bone?)

- Haversian canals containing remains of blood vessels
- Volkmann's canals (what is their key feature in histological identification?)

- The central marrow cavity and endosteum
- The cement lines

Under H.P. draw a single Haversian system to show:

- The arrangement of lamellae
- The lacunae, canaliculi and remnants of osteocytes
- The central Haversian canal

What is the main organic constituent in bone matrix?

(b) Compact bone (Ground bone)

Slide: 3
Stain: Ground bone and silver impregnation

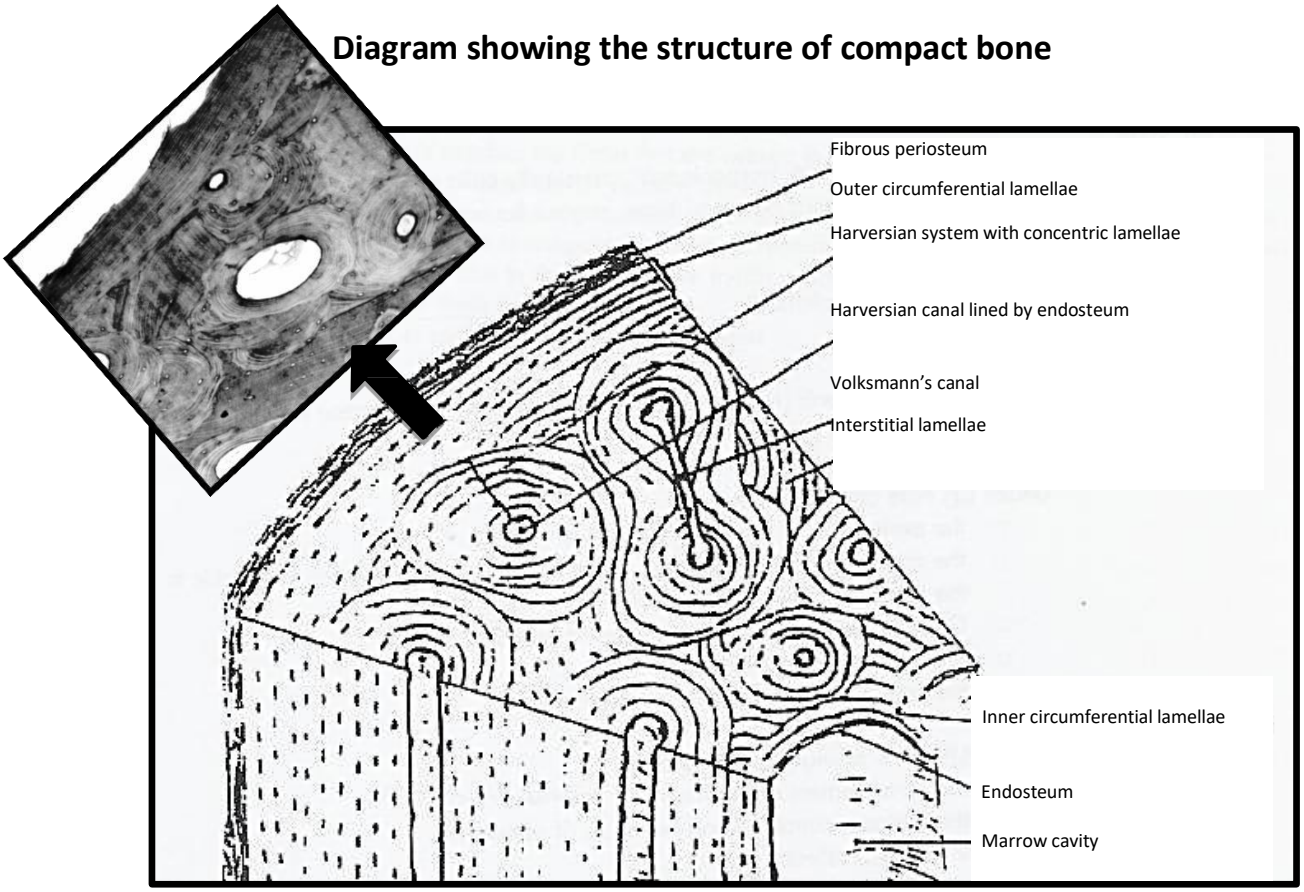
Note and draw under L.P. and H.P.:

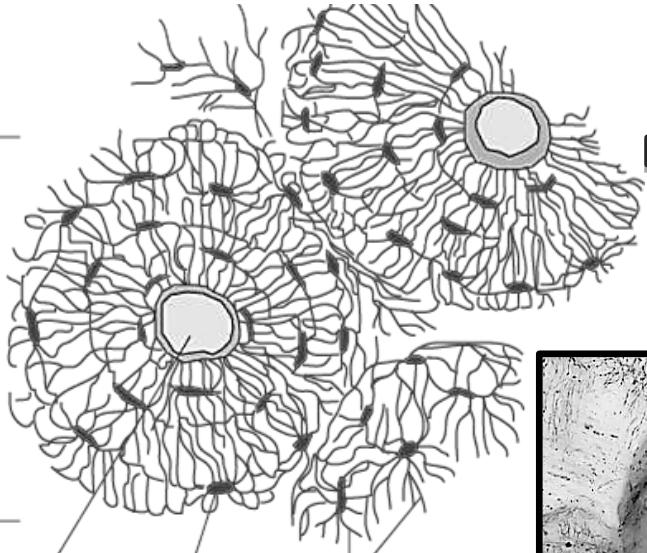
- The concentric lamellae of bone on the outside and inside of the section. Explain the term "lamella "of bone.

- The Haversian canals and Volkmann's canals, which are blackened due to the silver impregnation technique.
- The arrangement of the lamellae surrounding the Haversian systems
- The lacunae situated between the lamellae. What do they contain?

- The canaliculi radiating from the lacunae. What do they contain?
- The interstitial lamellae.

Diagram showing the structure of compact bone

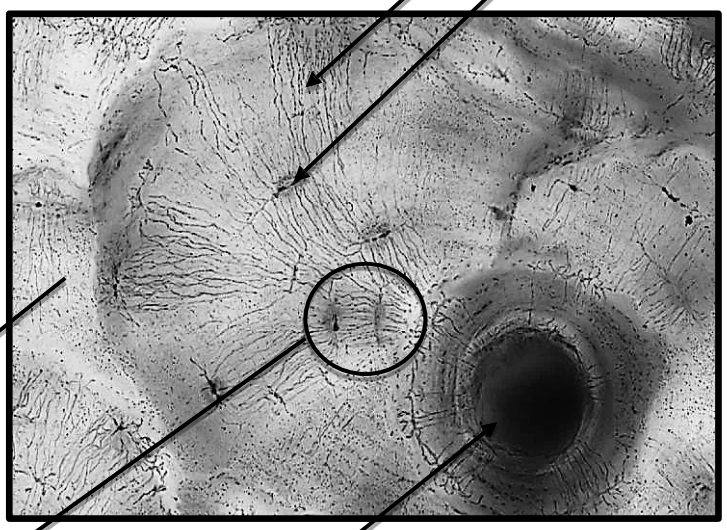




Also referred to as a Haversian System

Canaliculi – black "thread-like" or "hair-like" structures running perpendicular to the circumferential lamellae within the osteon

Osteocyte



Housed in lacunae
Lacuna is a small space containing an osteocyte (bone) or chondrocyte (cartilage)

Interstitial lamellae

Note how two osteocytes communicate with each other via cytoplasmic process, which are sent via canaliculi (tunnels in the bone)

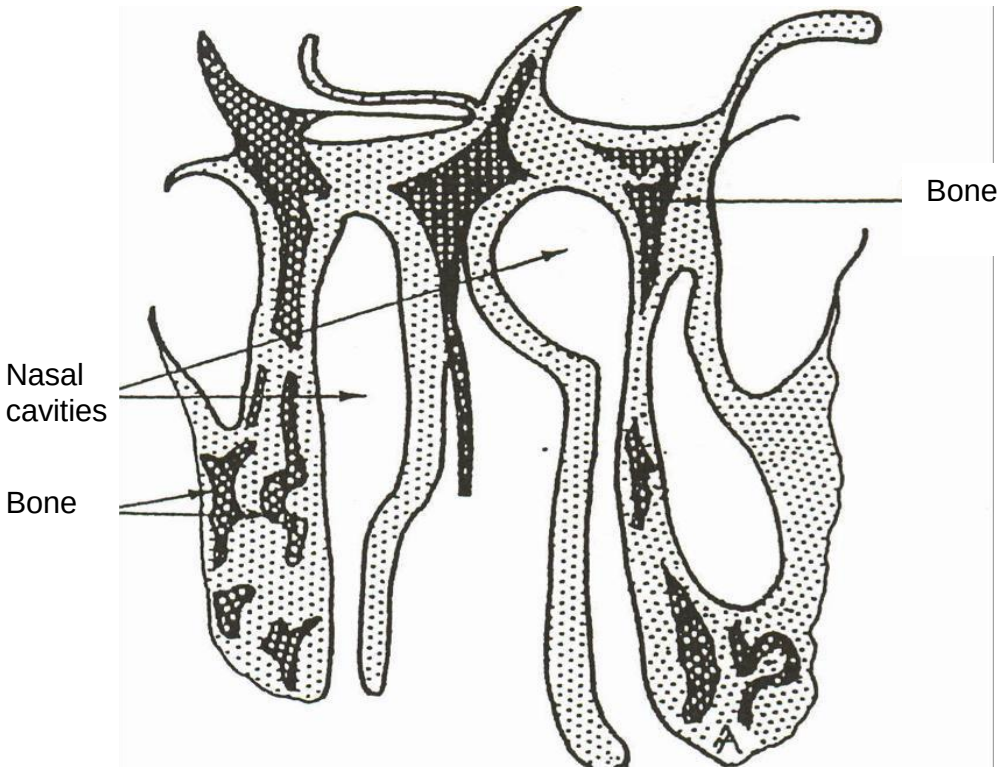
Haversian canal

http://www.wikiwand.com/en/Haversian_canal

(c) Cancellous or spongy bone

Nasal cavity and air sinuses
Slide: 67
Stain: Decalcified, H& E

Look for a trabeculum (spicule) of cancellous bone in any of the positions indicated in the diagram below. The bone has been decalcified and stains intensely eosinophilic.



Bone in *section* through nose and air sinuses

Under L.P. and H.P. study and illustrate a trabecula (spicule) of cancellous bone to show

- The periosteum, with eosinophilic fibres
- The arrangement of lamellae
- The contents of the marrow spaces
- The shape and position and contents of the lacunae

Clinical Correlation

Bone is a frequent and important site of **haematogenous metastatic spread of malignant epithelial tumours,** particularly carcinomas of bronchus, breast, kidney, thyroid and prostate. Metastatic tumour deposits usually destroy bone trabeculae, although carcinoma of the prostate sometimes stimulates excessive new bone formation, resulting in osteosclerotic rather than the more usual osteolytic deposits.

Osteoid osteoma is a benign but painful tumor of bone.

Osteogenic sarcoma or osteosarcoma is the most common malignant primary tumour of bone. It occurs mainly in children and adolescents

Young, B., Stewart, W., O'Dowd, G. (2011). Wheater's basic pathology, 5th Edition.

RECAPITULATION
CONNECTIVE TISSUE WITH A SOLID MATRIX

1. Why is bone considered to be a connective tissue?

2. Which type of fibre is found in the matrix of bone?

3. What inorganic salts are responsible for the hardening of bone?

4. Complete the following table to compare the different types of cartilage

FEATURE	HYALINE CARTILAGE	FIBRO-CARTILAGE	ELASTIC CARTILAGE
CELLS			
FIBRES			
GROUND SUBSTANCE			
PERICHONDRIUM			
GROWTH			
LOCATION/ FUNCTION			

5. Draw two **fully labelled diagrams** to show the differences and similarities between **compact and spongy bone**.

   Include the cell types present, their structure and arrangement, lamellae, periosteum and endosteum, marrow cavities, blood vessels.

   Add a note on the sites where each type is found and relate this to its function.

6. List the similarities and differences in structure between cartilage and bone

7. Give some examples of sites in the body where compact and cancellous bone occurs.

IV. Osteogenesis

OBJECTIVES

In this practical you will study the process of bone formation.

The objectives of this practical are thus:

To identify the histological features of the cell types involved in osteogenesis (please refer to glossary)

To understand the function of these cell types

To relate the embryonic development of bone with its function

To understand and describe the growth of long bones

To understand the role of mesenchyme, heamatopoietic tissue, cartilage and osteoid in the process of osteogenesis

NOTES

The term "osteogenesis" refers to the development and growth of bone. This takes place in the foetus and after birth until the growth in length of the body ceases and during repair processes. Bone formation occurs via two distinct processes, initially – endochondral ossification and intramembranous ossification.

Some bones e.g. the flat bones of the face and the skull, develop directly from mesenchyme ("intramembraneously"); others, such as the vertebrae and the long bones of the limbs, develop from mesenchyme but upon a cartilage model. This is known as "endochondral" ossification. Immature bone is also known as ‘woven bone’ or ‘bundle bone’ due to the non-lamellar arrangement of collagen fibrils. Remodelling of woven bone results in the production of mature ‘lamellar’ bone.

Intramembranous and endochondral ossification will be studied under the present heading.

A note on the staining of bone

Calcium forms an insoluble precipitate with haematoxylin; calcified bone and cartilage matrix therefore stains basophilically with haematoxylin. Decalcified bone matrix and matrix that is not yet calcified ("osteoid") are eosinophilic due to the collagen content.

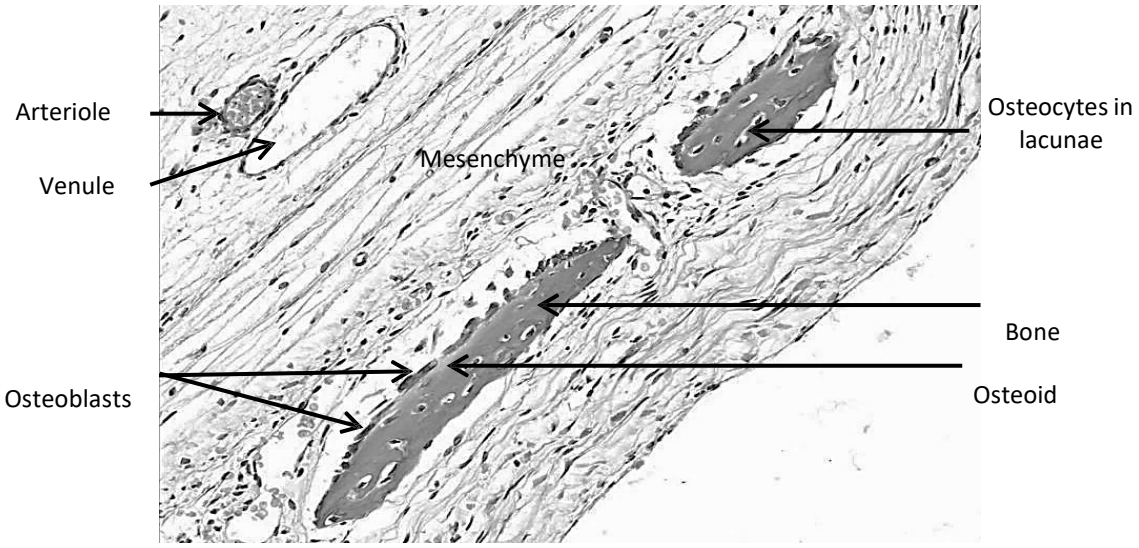
PRACTICAL WORK

I. INTRAMEMBRANOUS OSSIFICATION

The following slides are coronal sections of foetal rat heads at different stages of early embryonic development.

(a) Foetal skull (early)

Slide: 109
Stain: H&E



Note:

- The mass of palely-staining, elongated mesenchymal cells underlying the developing skin
- The numerous blood capillaries within the mesenchyme
- A small spicule of bone with osteocytes lodged within its lacunae. At this stage there is little apparent organisation of the bone matrix into lamellae. Why?

- Osteoblasts covering the surface of the spicules of bone. What is their function? How can you distinguish osteoblasts from the surrounding mesenchymal cells?

- The palely eosinophilic osteoid secreted by the osteoblasts.

(b) Foetal skull (late, decalcified)

Slide: 110
Stain: H&E

Note:

- The changes in the histological structure of the tissue with progressive development (compare with Slide 109). The developing skin with epidermal and dermal layers, as well as hair follicles, are now evident.
- Slightly larger spicules in which cavities between the spicules of bone can be seen. These cavities are forerunners of marrow cavities and contain capillaries and mesenchymal cells.

II. ENDOCHONDRAL OSSIFICATION & GROWTH

Endochondral bone growth begins during the foetal period and continues into early adulthood. Growth in length of long bones is dependent on the presence of epiphyseal cartilage.

(a) Foetal knee (decalcified)

Slide: 52
Stain: H&E

This is a longitudinal section of a human foetal knee joint, with the developing femur, patella and tibia. Describe the section under L.P. and H.P.

At each end of the long bones is a pale staining mass of cartilage – the *epiphysis.* In the region of the *diaphysis*, there are deeply stained trabeculae of bone. The epiphyses are separated from the diaphysis by a narrow zone – the metaphysis where bone is laid down on the degenerating cartilage.

Note that the developing bone is ensheathed by a periosteum and perichondrium. Is there any histological difference between the periosteum and perichondrium?

What is the periosteal collar and what type of bone formation occurs here?

What is the role of the periosteum in increasing width of bone?

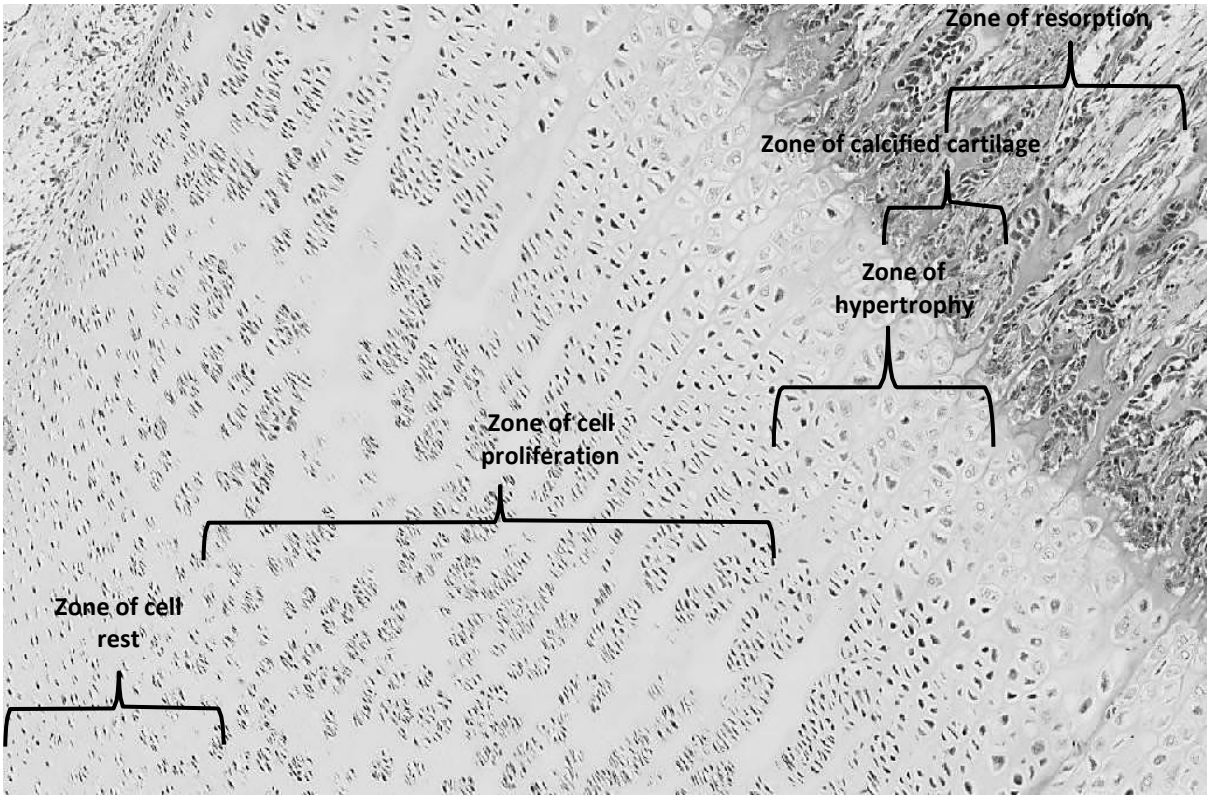
Note:

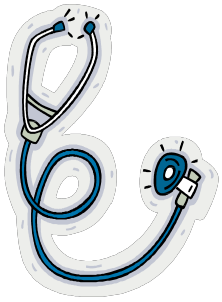
- The hyaline cartilage of the epiphysis.
- The connective tissue "islands" containing blood vessels within the cartilage. These are extensions from the perichondrium and will ultimately play a part in endochondral ossification of the epiphysis itself.
- Identify the following zones within the epiphyseal cartilage:
    - The zone of reserve cartilage, which exhibits small, isolated cells.
    - The zone of proliferation (or proliferating cartilage) where the cells are arranged in longitudinal nests.
    - The zone of hypertrophy where cells are significantly larger than those of the preceding zone.

    - The zone of calcified cartilage where the hypertrophied cells begin to degenerate and the cartilage matrix itself calcifies. Note the pale basophilic appearance of the calcified cartilage.
    - The zone of resorption where the walls of the unoccupied chondrocyte lacunae breakdown and become continuous with the marrow spaces of the metaphysis.
- Within the diaphysis, the spicules of calcified cartilage surrounded by osteoblasts (derived from osteoprogenitor cells sourced from the invading blood vessels)
- Mixed trabeculae containing the basophilically staining cartilage, surrounded by the eosinophilically stained osteoid secreted by the osteoblasts.
- Progressive enlargement and remodelling of the bony trabeculae from the metaphysis towards the centre of the diaphysis.
- Osteoclasts located within Howships lacunae in direct apposition to trabeculae of bone. How would you distinguish between osteoblasts and osteoclasts on a structural basis?

- Osteocytes within the calcified osteoid.
- The contents of marrow spaces between the bony trabeculae. What are the functions of marrow?

- The eosinophilic collagenous fibres of the periosteum





Clinical Correlation

Osteogenesis imperfecta
A group of diseases where a mutation in a gene encoding for the α chains of type I collagen causes a reduction in the amount and quality of secreted collagen. Patient presentation includes fragile bones susceptible to fracture.

Osteoporosis
A metabolic disorder characterised by decreased bone mass caused by an in imbalance in osteoblast-mediated bone deposition and osteoclast-mediated bone resorption. Patient presentation includes fragile bones prone to fracture. Postmenopausal osteoporosis results from excessive osteoclast activity. A risk factor in postmenopausal women is the reduced level of oestrogens.

Underwood and Cross, (2009). General and Systemic Pathology 5th Edition.
Ross & Pawlina, (2011). Histology A Text and Atlas 6th Edition.

RECAPITULATION
BONE AND OSTEOGENESIS

1. What are the distinguishing histological features of:
   a) Osteoblasts

   b) Osteocytes

   c) Osteoclasts

2. Which of the cells in bone secrete:
   a) The fibres

   b) The ground substance

3. What are the components of:
   a) The fibres

   b) The ground substance of bone

4. From where is the calcium of bone obtained and on which component of the bone is it laid down?

5. What is the function of bone?

6. Bone is in a state of "dynamic equilibrium". Discuss the implications of this statement.

7. What is the function of the bone marrow in:
   a) The foetus

   b) The growing child and adolescent

   c) The adult?

8. Where are the cavities in compact bone and how do they differ from those in cancellous bone? What do these cavities contain in living bone?

9. Which sites in the developing diaphysis of a long bone, e.g. the femur, give rise to:
    a) The passageways of the nutrient blood vessels?

    b) The Haversian canals of the adult bone?

10. How do the interstitial lamellae of compact bone arise?

11. What is meant by the terms "intramembranous" and "endochondral" ossification?

12. Give some examples of bones that are formed by:
    a) Intramembranous ossification.

    b) Endochondral ossification.

13. The clavicle can be considered a "long" bone. What is unique about its development and growth?

14. At what age do the primary centres of ossification in the femur and tibia appear?

15. Where do the secondary centres of ossification appear in:
    a) The femur?

    b) The tibia?

16. At what ages do the secondary centres of ossification appear in:
    a) The distal end of the femur?

    b) The proximal end of the tibia?

17. What is the embryological origin of bone?

18. What is the embryological origin of cartilage?

Muscle

OBJECTIVE

Muscle fibres are elongated cell(s) specialized for contraction. There are three types of muscle fibres:

Skeletal (striated)
Cardiac (striated)
Smooth

The objective of the following practical is to study each type of muscle fibre according to the:

Size, shape and presence or absence of branching
Number, shape and position of nuclei
Presence or absence of cross striations

After studying the histological structure as well as the ultrastructure of muscle, you should be able to classify, identify and describe the **structure and function** of the different types of muscle (i.e. skeletal, cardiac and smooth muscle).

NOTES

These notes are compiled from Ross and Pawlina; Histology: A Text and Atlas, 6th Edition, 2011. Lippincott, Williams and Wilkins and Victor P. Eroschencho; di Fiore's Atlas of Histology with functional correlations, 7th Edition, 1993. Lea & Febiger.

Muscle tissue is primarily composed of individual muscle cells (fibres) whose principle function is contraction. This contraction results in the movement of the body, the beating of the heart, peristaltic and other movements as well as changes in the shape and size of internal organs. Muscle contraction results from the interaction of the myofilaments, actin (thin filament) and myosin (thick filament), which occupy the majority of the sarcoplasm of the muscle cell.

Muscle tissue is classified on a structural basis as:

Striated
At light microscopy level these muscle cells present cross striations, which are produced by the specific cytoarchitectural arrangement of both the actin and myosin myofilaments.

Striated muscle can further be subdivided into three categories based on their location:

- *Skeletal Muscle*
  This is the major type of muscle tissue found in the body. It is under voluntary control and usually attached to bone. Its primary function is movement of the appendicular and axial skeleton and it also plays an essential role in the maintenance of position and posture of the body.

- *Visceral Striated Muscle*
  This muscle type is restricted to soft tissues such as the tongue, pharynx, diaphragm (lumbar part) and oesophagus (upper two thirds). It is morphologically identical to skeletal muscle and plays an important part in speech, breathing and swallowing.

- *Cardiac Muscle*
  This particular type of striated muscle is found in the walls and septa of the heart as well as the walls of the large veins that are attached to the heart. Cardiac muscle, unlike skeletal muscle, is under involuntary control and is responsible for the automatic and rhythmic contraction of the heart.

Smooth

Smooth muscle cells do not contain any visible cross-striations. This is due to the high lability of the myosin-containing myofilaments as well as the myofilaments within the sarcoplasm of smooth muscle cells that do not reach the same level of specific cytoarchitectural arrangement, as they do in striated muscle. Smooth muscle is under involuntary control and usually exhibits spontaneous activity in a wave like fashion, passing in a slow, sustained contraction over the entire muscle. Smooth muscle primarily lines the walls of hollow viscera, such as the organs of the gastrointestinal tract, and blood vessels.

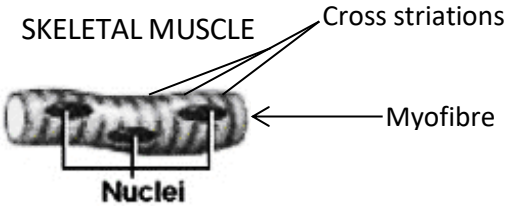
Muscle Terminology

Special terminology is typically used when one refers to, or describes the structures and organelles associated with a muscle cell:

Myofibre/myocyte:	A single muscle cell
Sarcolemma:	Plasma membrane of muscle cell
Sarcoplasm:	Cytoplasm of muscle cell
Sarcoplasmic reticulum:	Endoplasmic reticulum of muscle cell
Sarcosome:	Mitochondria of a muscle cell
Sarcomere:	Contractile/functional unit of muscle cell

○ PRACTICAL WORK

I. SKELETAL MUSCLE



(a) Striated skeletal muscle

Tongue
Slide: 76
Stain: Iron haematoxylin

A few notes on the staining technique:

Iron haematoxylin is blue-black in colour and has been used to demonstrate the cross-striations and nuclei.

Under L.P. note the plane(s) of section of the muscle fibres. Select a longitudinal and a transverse section:

- Study and draw them under H.P
- Name the bands or cross striations
- Compare these striations with those seen in an electron micrograph

Why are the "A" and "I" bands so named?

Where is the Z line?

What is a sarcomere?

What is a myofibril? Is it striated?

Why does the whole fibre appear to be cross-striated?

Name the connective tissue sheath around:

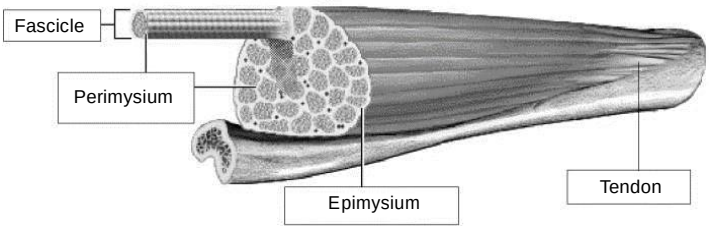
a) An entire anatomical (skeletal) muscle

b) A bundle of skeletal muscle fibres

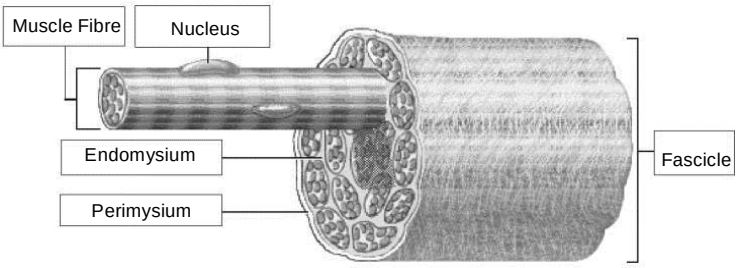
c) Each muscle fibre

How do you distinguish fibroblast nuclei in the connective sheath from the muscle cell nuclei?

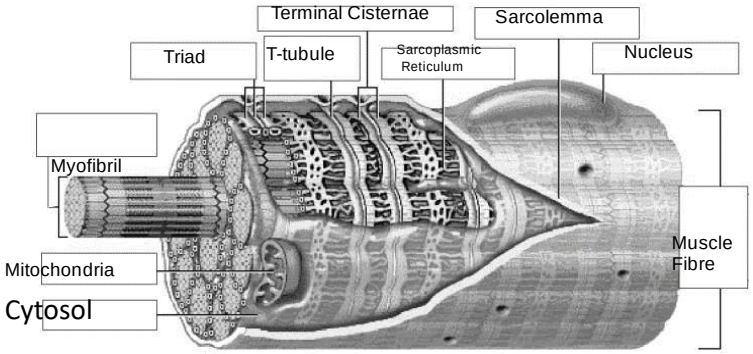
Skeletal Muscle Organization



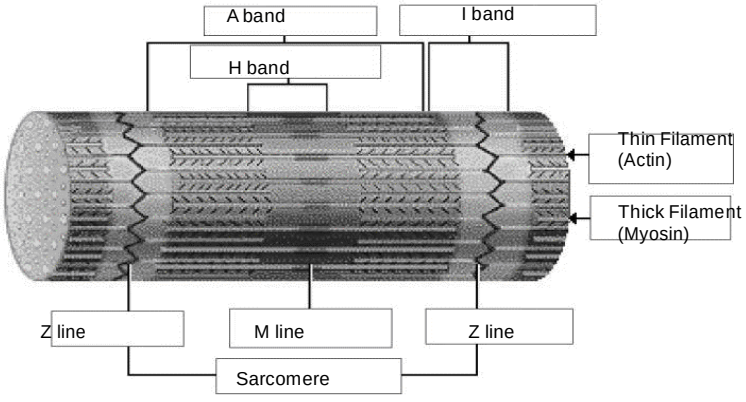
Whole skeletal muscle



Fascicle



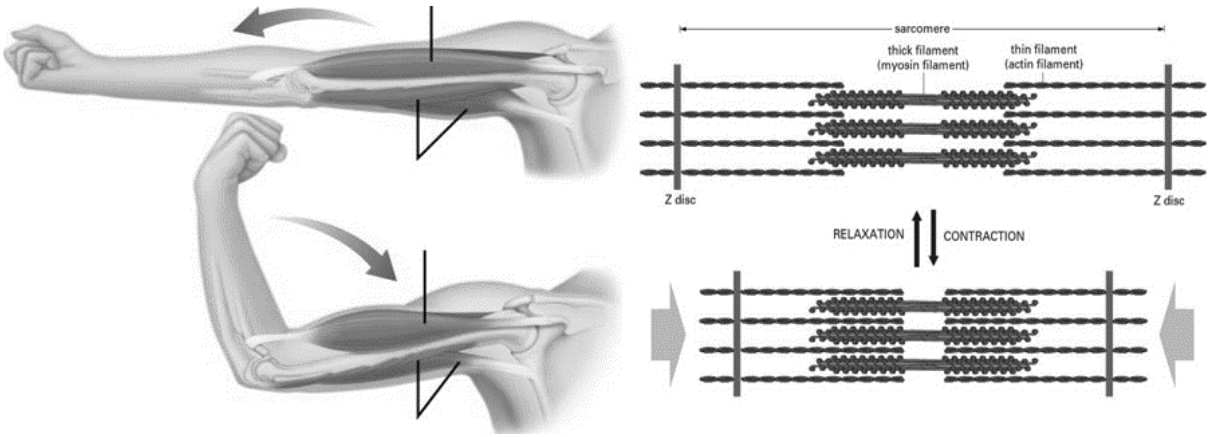
Muscle cell/fibre



Myofibril

http://staff.fcps.net/cverdecc/Adv%20A&P/Notes/Muscle%20Unit/Muscle%20Anatomy/anatomy_review.htm

How muscles contract → the sliding filament model

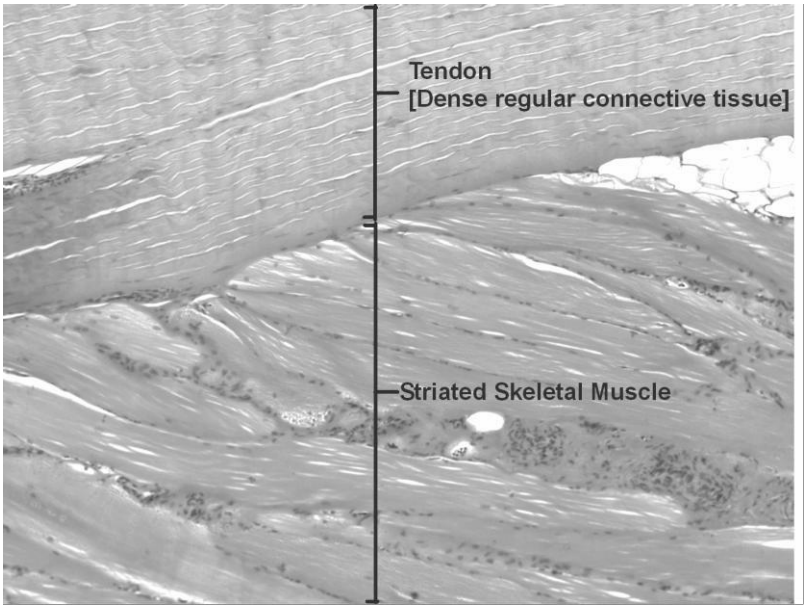


(b) Musculo-tendinous junction

Slide: 50
Stain: H&E

Examine this slide

Recall from your practical on connective tissue, the band of palely stained tissue is tendon (dense regular connective tissue) while the fibres inserting into the tendon obliquely are the skeletal muscle fibres.



How can you distinguish between the tendon and the skeletal muscle? What is the function of this type of junction?

Clinical Correlation

Duchenne's Muscular Dystrophy (DMD) is a form of muscular dystrophy caused by a point mutation of the gene encoding for the protein dystrophin, which is responsible for anchoring the cytoskeleton of the sarcolemma to the extracellular matrix. It is a sex-linked, inherited genetic disorder (X-linked recessive trait) primarily affecting only males. Disease onset is typically between the ages of 3 and 5 and patients typically suffer from muscle wasting that progress rapidly. Patients typically lose the ability to walk at around 12 years of age and have to use a ventilator to breathe by the age of 20. Currently there is no cure for muscular dystrophies.

Ross and Pawlina (2011). Histology: A Text and Atlas, 6th Edition.
Harhold Sheedlo (2005). USMLE Road Map: Histology.

(c) Neuromuscular (myoneural) junction

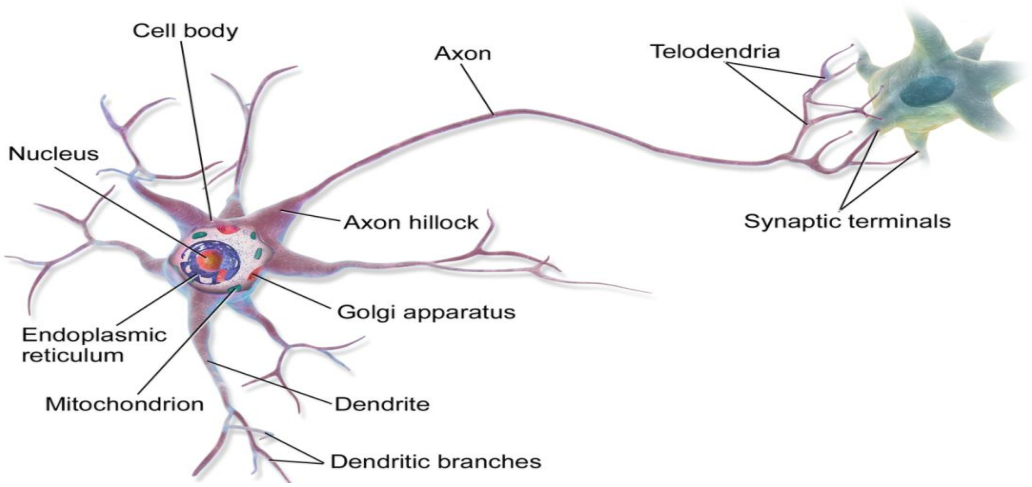
Striated (skeletal) muscle – Snake
Slide: 4
Stain: H&E and gold impregnation

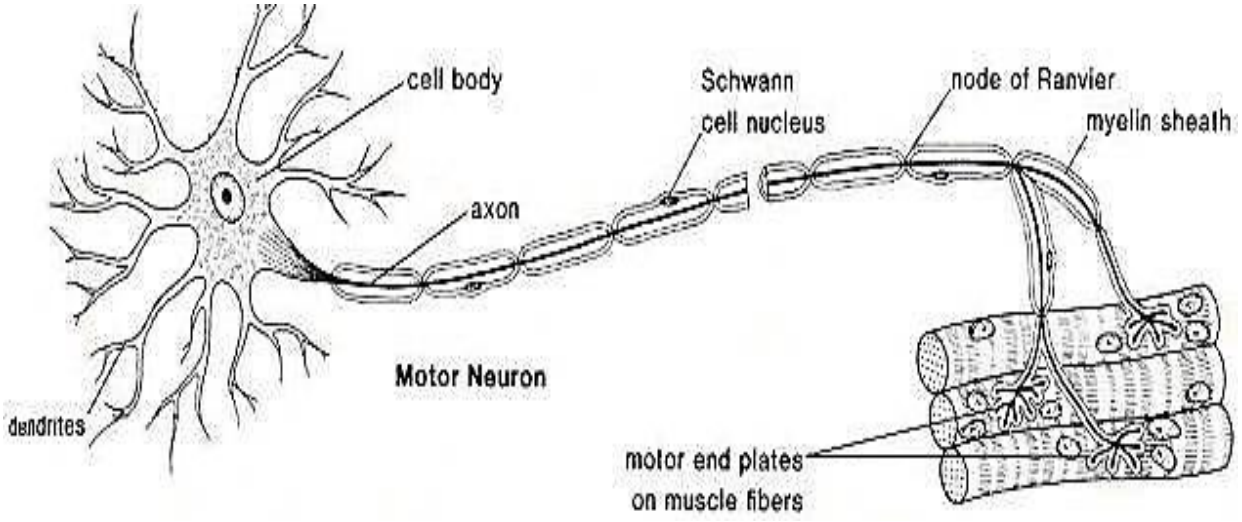
Examine this slide

This is a whole mount of striated skeletal muscle of a snake impregnated with gold to demonstrate nerve fibres.

Note: *This slide will be studied in more detail in the next section on* ***Nervous Tissue***

Motor neurons located in the spinal cord or brainstem innervates skeletal muscle.
When an axon of these motor neurons reaches the muscle it branches and gives rise to several unmyelinated terminal branches that will end on individual muscle fibres. These terminal swellings of the axon are also known as **boutons**, and they are in close contact with the sarcolemma of the muscle fibre. This structural complex is called a **neuromuscular junction** or **motor end plate**.





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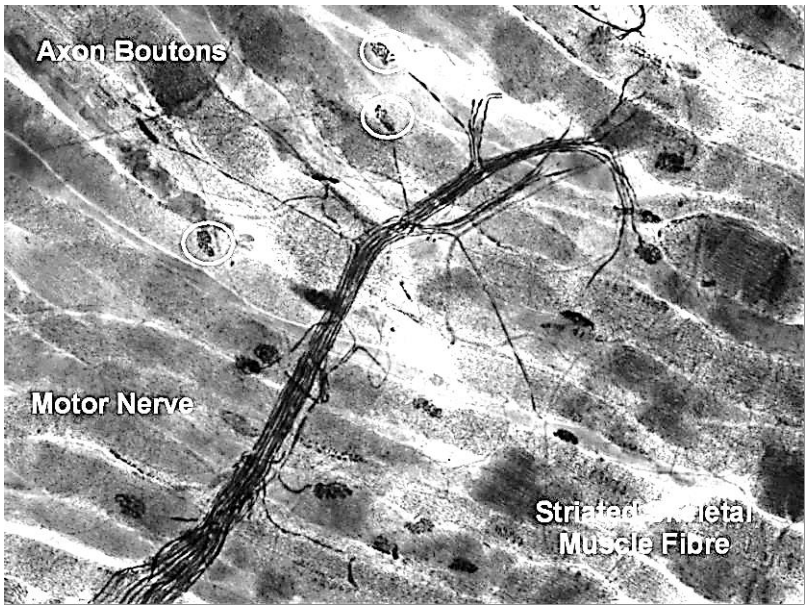
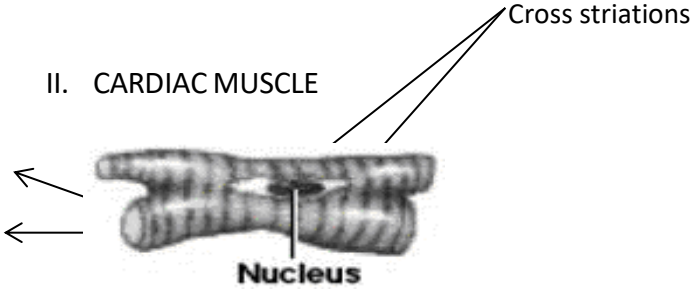


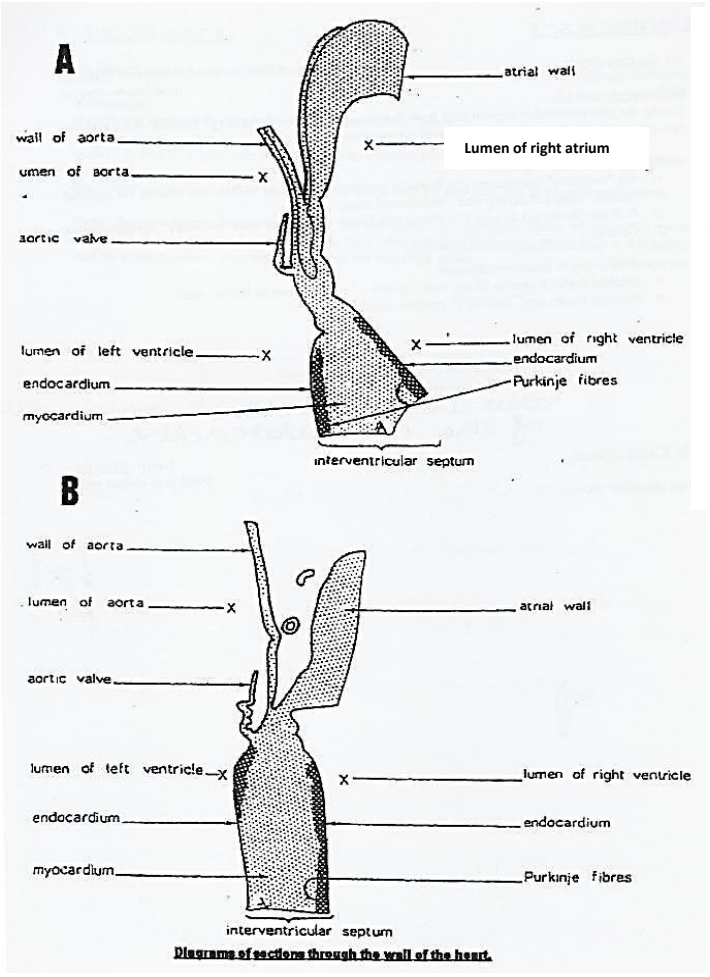
II. CARDIAC MUSCLE

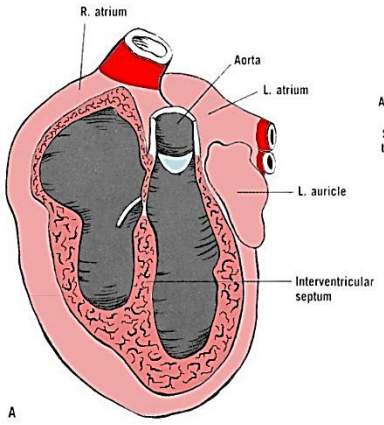


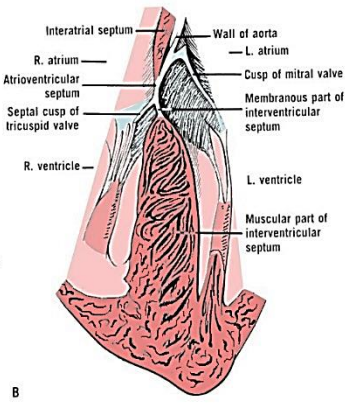
(a) Cardiac muscle

Heart (interventricular septum)
Slide: 57
Stain: Iron haematoxylin

Macroscopic and L.P:
Locate the interventricular septum and look for a region where "ordinary" cardiac muscle fibres are cut longitudinally and banding is evident (see diagram).







Reference for image - Basic Human Anatomy. *Dartmouth Medical School*. https://www.dartmouth.edu/~humananatomy/figures/chapter_23/23-6.HTM

Under H.P. study and draw:

- The "ordinary" cardiac muscle fibres in longitudinal section. Follow the scheme for skeletal muscle. Note branching and intercalated discs.
- Purkinje fibres in L.S. and T.S. (these are larger and paler than "ordinary" cardiac muscle fibres. They also have much larger glycogen spaces than those seen in cardiac muscle fibres).
- The capillaries (with black stained erythrocytes in the lumen) located within the connective tissue between the muscle fibres

(b) Cardiac muscle

Heart
Slide: 86
Stain: H&E and elastic stain

Examine the slide

Complete the table below by listing the difference in structure between Purkinje fibres and "ordinary" cardiac muscle fibres

	PURKINJE FIBRES	**ORINARY CARDIAC FIBRES**
Size (diameter)		
Staining reaction		
Glycogen space		
Striations		
T- tubules		
Intercalated discs		

What is the function of Purkinje fibres?

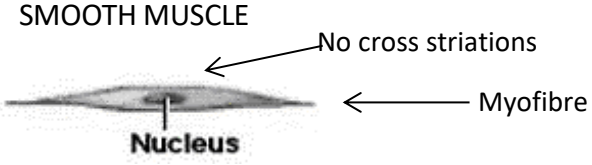
Why do Purkinje fibres appear paler compared to “ordinary” cardiac muscle fibres?

What is an intercalated disc? List the components of an intercalated disc

Name 2 functions of intercalated discs

What is contained within the space around the nucleus of cardiac muscle?

III. SMOOTH MUSCLE



(a) Smooth muscle

Uterus (baboon)
Slide: 10
Stain: H&E

Macroscopic:
Identify the eosinophilic muscle layer in the wall of the uterus.

Under L.P. find a region where smooth muscle fibres are cut both longitudinally and transversely.

Study and draw under H.P. a few fibres cut in L.S. and T.S and note the distinguishing features.

How does smooth muscle cells contract?

What are the caveolae seen in EM graphs of smooth muscle? Suggest a function for them.

Note: *Smooth muscle fibres can easily be confused with collagen fibres of dense irregular connective tissue in H&E preparations. Find some collagen fibres and fibroblasts in this section and list the differences in structure between the two fibre types.*

SMOOTH MUSCLE FIBRES	COLLAGEN FIBRES

Clinical Correlation

MYASTHENIA GRAVIS IS AN AUTO-IMMUNE DISEASE IN WHICH CIRCULATING ANTIBODIES BLOCK ACETYLCHOLINE RECEPTORS AT THE POSTSYNAPTIC NEUROMUSCULAR JUNCTION. IT IS CAUSED BY A REDUCED NUMBER OF FUNCTIONAL ACETYLCHOLINE RECEPTOR SITES; HOWEVER ABNORMALITIES WITHIN THE SYNAPTIC CLEFT CAN ALSO OCCUR. THE DISEASE IS TYPICALLY CHARACTERIZED BY A MARKED REDUCTION IN THE RESPONSE OF THE MUSCLE FIBRE TO A NERVE STIMULUS AND THE NUMBER OF NEUROMUSCULAR JUNCTIONS REDUCES AS THE DISEASE PROGRESSES. THE HALLMARK OF THIS DISEASE IS PROGRESSIVE MUSCLE WEAKNESS AFTER PERIODS OF INCREASED ACTIVITY. MUSCLES MOST SUSCEPTIBLE TO FATIGUE INCLUDE MUSCLES THAT CONTROL EYE AND EYELID MOVEMENT, FACIAL EXPRESSION, CHEWING, TALKING AND SWALLOWING WHILE RESPIRATORY AND POSTURAL MUSCLES MAY ALSO BE AFFECTED. AN EFFECTIVE TREATMENT IS ADMINISTRATION OF ACETYLCHOLINESTERASE INHIBITORS.

ROSS AND PAWLINA (2011). HISTOLOGY: A TEXT AND ATLAS, 6TH EDITION.
HARHOLD SHEEDLO (2005). USMLE ROAD MAP: HISTOLOGY.

RECAPITULATION
MUSCLE

1. a. What is the functional characteristic common to all muscle cells?

   b. Give the two cytoplasmic constituents that are chiefly responsible for this activity.

2. Differentiate between a muscle, a muscle fibre, and a myofibril.

3. What are the epi-, peri- and endomysium and what are they composed of? Are they present in cardiac and smooth muscle?

4. How is the contraction of each of the three muscle types controlled?

5. How are muscle fibres adapted to the functions they perform?

7. Tabulate the following:

	Diad	Triad
Constituents		
Location		
Function		

8. Complete the table by listing the differences between the three types of muscle fibres

	SKELETAL	CARDIAC	SMOOTH
Size and Shape			
Connective tissue components			
Branching			
Nucleus			
Striations			
T-tubules			
Cell-to-cell junctions			
Innervation			
Location			
Function			

Nervous Tissue

OBJECTIVES

In this practical you will study:

Nerve cell bodies e.g. the anterior horn cells of the spinal cord and spinal ganglion cells
The nerve fibres of a spinal reflex arc
Supporting cells related to these cells and fibres will also be seen.

The objectives of the following practical are thus:

♦To identify the characteristic features of neurons and nervous tissue
♦To identify the different structures of/within a neuron and be able to explain how each structure contributes to its function
♦To describe the structure and function and give a location of multipolar and pseudo-unipolar neurons
♦To distinguish between neurons and their supporting cells
♦To identify sections of peripheral nerves and identify/describe the structural features within peripheral nerves
♦To distinguish between myelinated and unmyelinated nerve fibres
♦To understand the structural organization of peripheral nerves and their connective tissue sheaths (anatomical nerve; nerve bundles; nerve fibres)

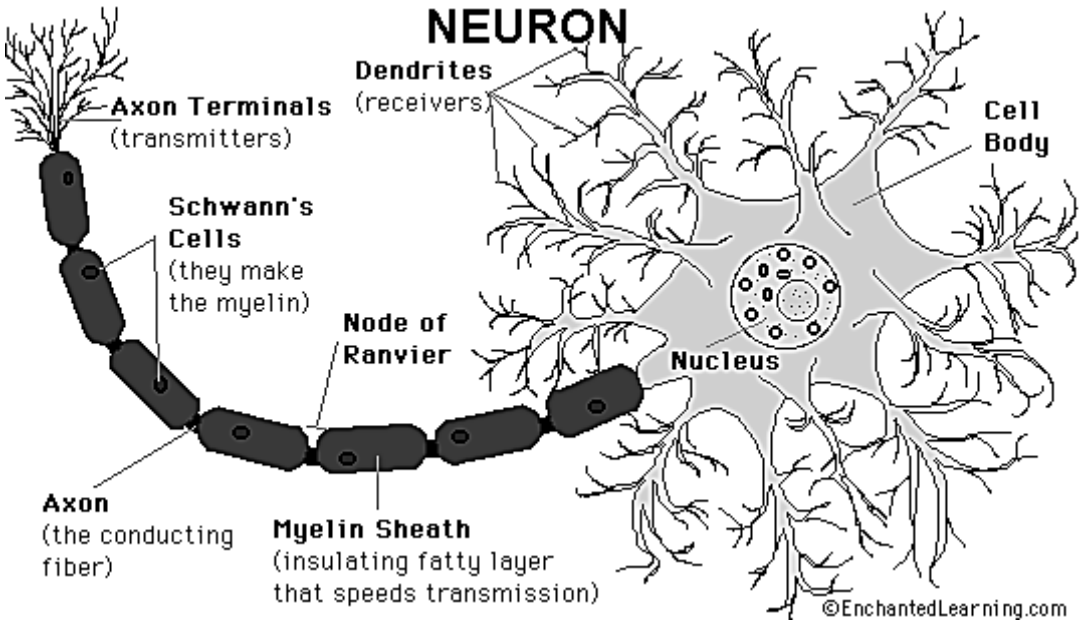
NOTES

Nervous tissue is made up of neurones or nerve cells, and their supporting cells.

Note: *The cell body of the neurone is frequently referred to as the "nerve cell"; whereas the cytoplasmic processes, i.e. the dendrites and the axon, with their neurilemmal (Schwann) cells and endoneurial sheaths, are referred to as "nerve fibres".*

The specific structural features of nervous tissue are poorly visualized with the standard H&E stain. Special stains are therefore used when specifically looking at nervous tissue. However you should still be able to identify nervous tissue, later on, when looking at organs stained with H&E.

The functional connections between neurons are usually not seen, you are therefore required to think more deeply about how the cell bodies, axons, dendrites, supporting cells and synapses are related to each other, where the impulses will go to and from where they have come.



www.enchantedlearning.com/.../brain/Neuron.shtml

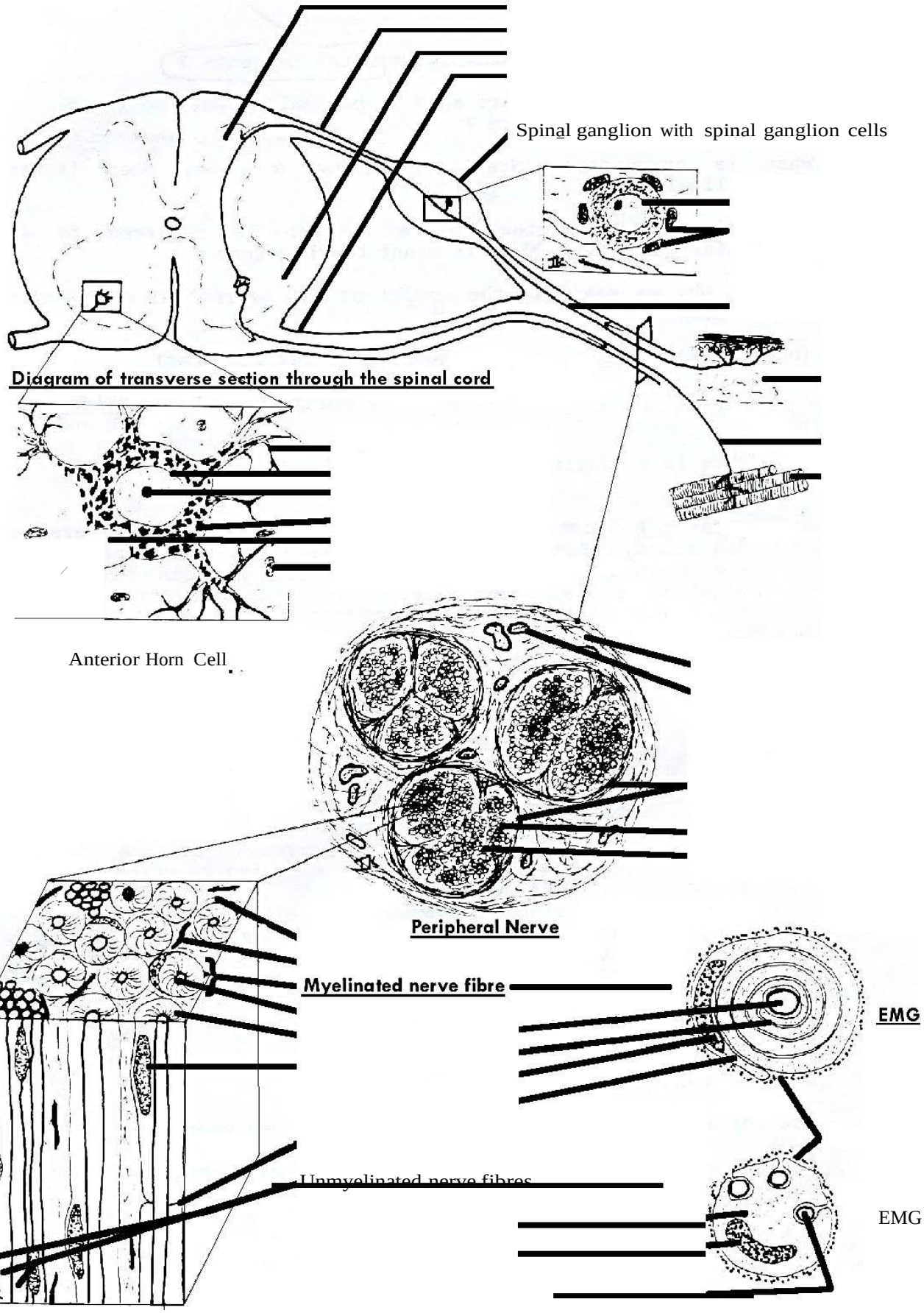
PRACTICAL WORK

The diagram overleaf of a simple reflex arc illustrates the basic construction of a spinal nerve with both sensory (afferent) and motor (efferent) nerve fibres. It also shows the location of the sensory and motor nerve cell bodies (neurones) associated with these fibres.

Label the diagram using the following labels:

- Afferent nerve fibre
- Anterior (ventral) horn
- Anterior (ventral) root (motor, efferent fibres)
- Axon hillock
- Axon
- Blood vessel
- Dendrites
- Efferent nerve fibre
- Endoneurium
- Epineurium
- Fibroblast nucleus
- Muscle, myelin sheath
- Nerve fibres
- Nissl bodies
- Node of Ranvier
- Nucleolus
- Nucleus of neuroglial cell
- Nucleus, perineurium
- Posterior (dorsal) horn
- Posterior (dorsal) root (sensory, afferent fibres)
- Satellite cells
- Schwann cell cytoplasm
- Schwann cell nucleus
- Skin

It will be useful to relate where the cells studied in this section, are found in relation to this diagram.



Nerve fibres in Transverse and Longitudinal Section

I. NEURON TYPES

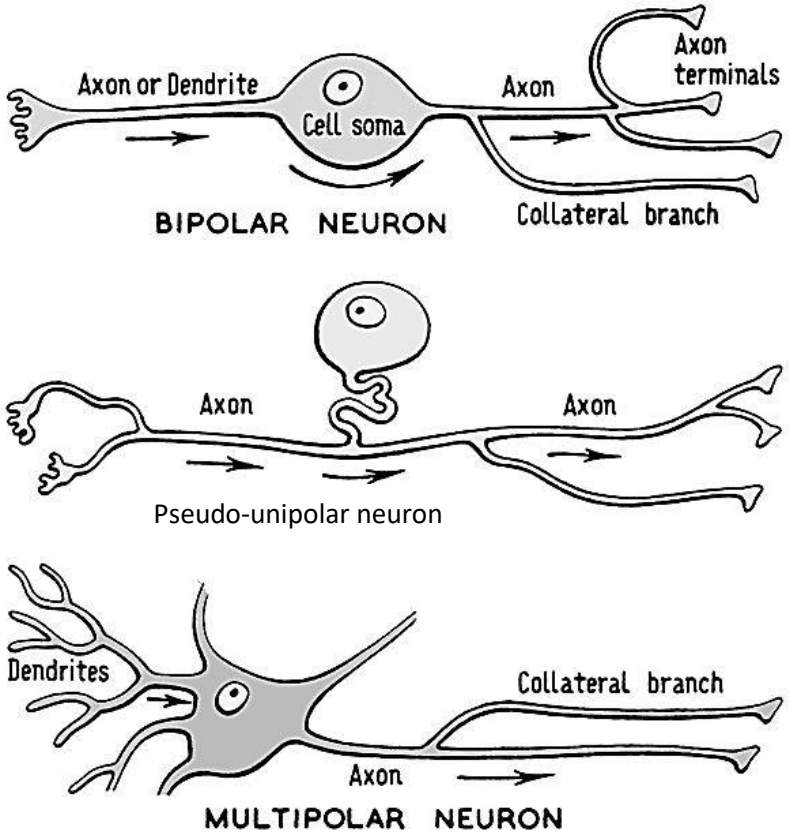
Neurons are the functional unit of the nervous system and are classified as bipolar, multipolar or pseudo-unipolar.

The cell bodies (perikaryon) of all neurons are characteristically large, in comparison to other cells, and therefore easily found in microscopy sections, even under LP. This is related to the heavy demands of the cell body by the excessive length of many neurons – the axon that innervates your little toe has its cell body in the base of the spinal cord.

The nuclei of all neurons are also very characteristically large and very palely stained (often referred to as a vesicular or euchromatic nucleus), such that its nucleolus will be clearly visible.

Cytoplasm of nerve cell bodies contain basophilically stained Nissl bodies characteristic of protein producing cells.

The cytoplasmic processes (dendrites and axon), extending from the cell body, are relatively thin and weave in and out of the plane of section. You will not be able to see the full length of these processes in your section. Thus neurons are mainly identified by their cell bodies.



vanat.cvm.umn.edu/neurLab1/neuron.html

Write short notes on the characteristic features of each neuronal type.

(a) Anterior horn cells (multipolar neurons)

Spinal cord (vervet monkey)
Slide: 64
Stain: Kluver and Barrera technique

A few notes on the staining technique:

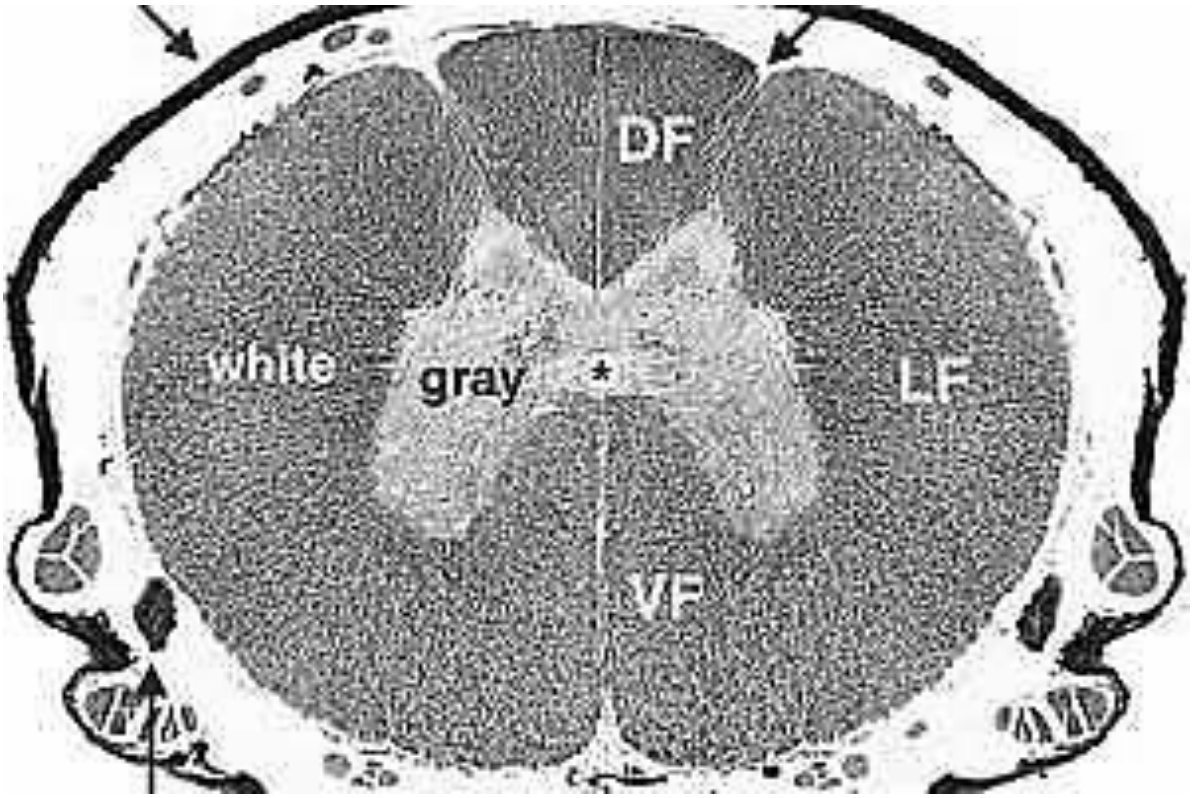
With this technique, myelin stains dark blue and basophilic substances such as rough ER and nuclei stain violet.

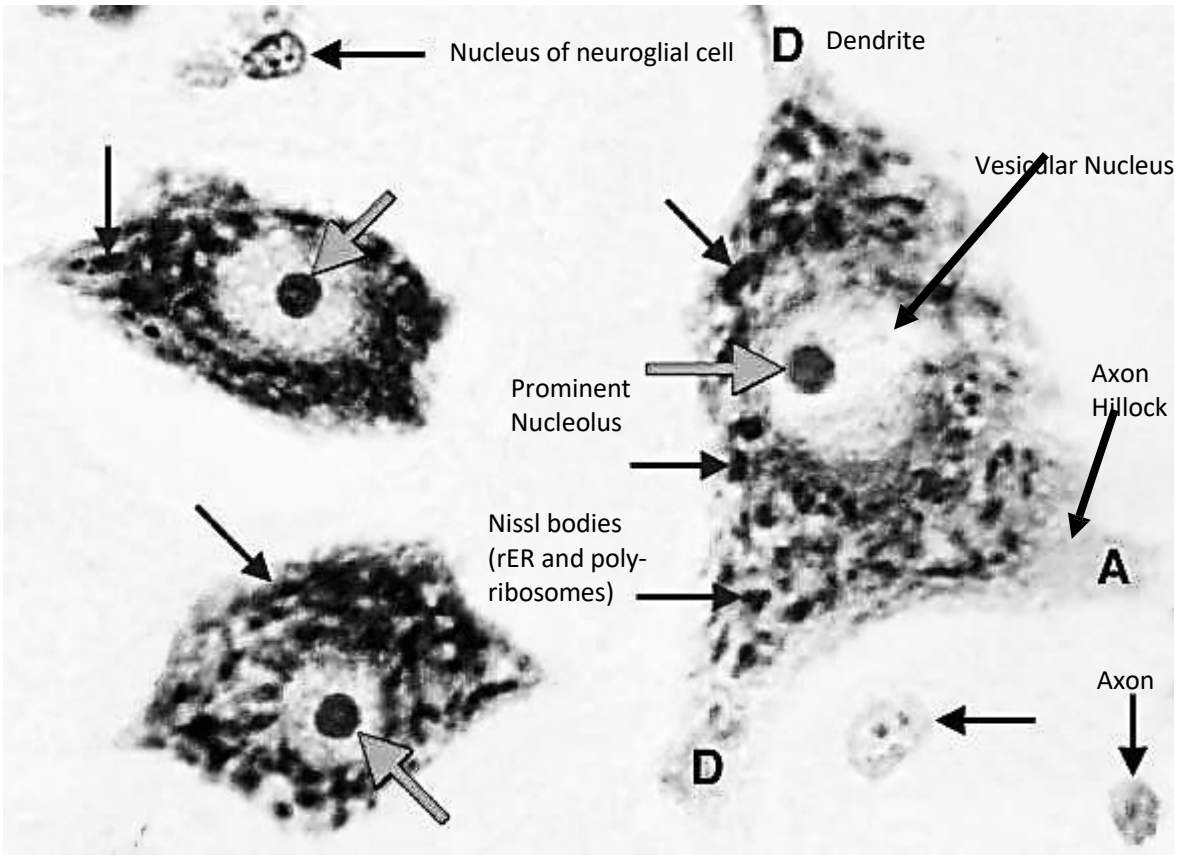
***Note**: In this case you can use "blue" and "purple" to describe the colour of the staining not eosinophilic or basophilic.*

Macroscopic:

Note:

- The palely stained H-shaped grey matter
- The intensely blue white matter
- The central canal (indicated by an * in the diagram on the next page) lined by ependymal cells of the spinal cord, this may be collapsed in your section





http://vanat.cvm.umn.edu/neurHistAtls/index.html

Identify the two narrow dorsal (posterior) horns and the two more widely dilated ventral (anterior) horns.

Which components of neurons are found in white matter and which components are found in grey matter?

Based on this and on the organization of a spinal reflex arc, where would you expect to find the cell bodies of the anterior horn cells?

Under L.P. find the anterior horn cells and the surrounding neuropil.

*The neuropil is the pale blue fibrous looking tissue **between** the cell bodies; it is NOT connective tissue but rather consists of the axons, dendrites and glial processes*

Under H.P. study, draw and describe the structure of a couple of these cells.

Pay particular attention to the following:

- The large cell body (compare the size to other cells in this section and in other slides)
- The shape of the cell body (relate its shape to the schematic diagram drawn earlier)
- The large, round, palely stained (vesicular or euchromatic) nucleus and its prominent nucleolus
- The shape, arrangement and staining reaction of the Nissl bodies in the cytoplasm of the cell body and in the dendrites
- The axon and "axon hillock"

  Note: *Although the cell body is large there is only 1 axon, which can extend from the cell body in any direction. Therefore not every anterior horn cell in your section will be cut through its axon. Try and find at least one anterior horn cell with its axon and axon hillock clearly visible*

- The many dendrites containing Nissl bodies extending from the cell body.

  Note: *It may appear as if some dendrites become devoid of Nissl bodies further away from the cell body. This just indicates that the process is leaving the plane of section, it is therefore important to look at the cytoplasm of the cell body from where that process leaves to identify it as a dendrite or axon*

- The small violet stained nuclei of neuroglia in the grey matter between the anterior horn cells.

  Note*: We do not see the cytoplasm and hence we cannot see the shape or the processes of these cells with this stain*

- The rest of the neuropil containing capillaries with blue stained erythrocytes and the blue "fibrous looking" nerve cell processes of both the neuroglial cells and the neurons

Are anterior horn cells sensory or motor neurons?

The anterior horn cells are classified as "multipolar" neurons. What is meant by this term? Refer to the slide and give reasons as to why they are multipolar?

What are Nissl bodies?

- Why does it stain basophilically?

- What proteins do they produce?

The pale-stained nucleus of a neuron is referred to as a "vesicular" nucleus. What is meant by this term? What does it indicate about the activity of this cell and why?

Return to L.P. Observe the white matter on the outside of the spinal cord.

To which neurons (motor or sensory) do the axons in the white matter surrounding the dorsal horn and ventral horn most likely belong to?

Under H.P. most of the axons seen in the white matter are cut in transverse section, see if you can spot the nuclei of the neuroglial cells, between the nerve fibres.

What types of neuroglial cells would you expect to find here?

Clinical Correlation

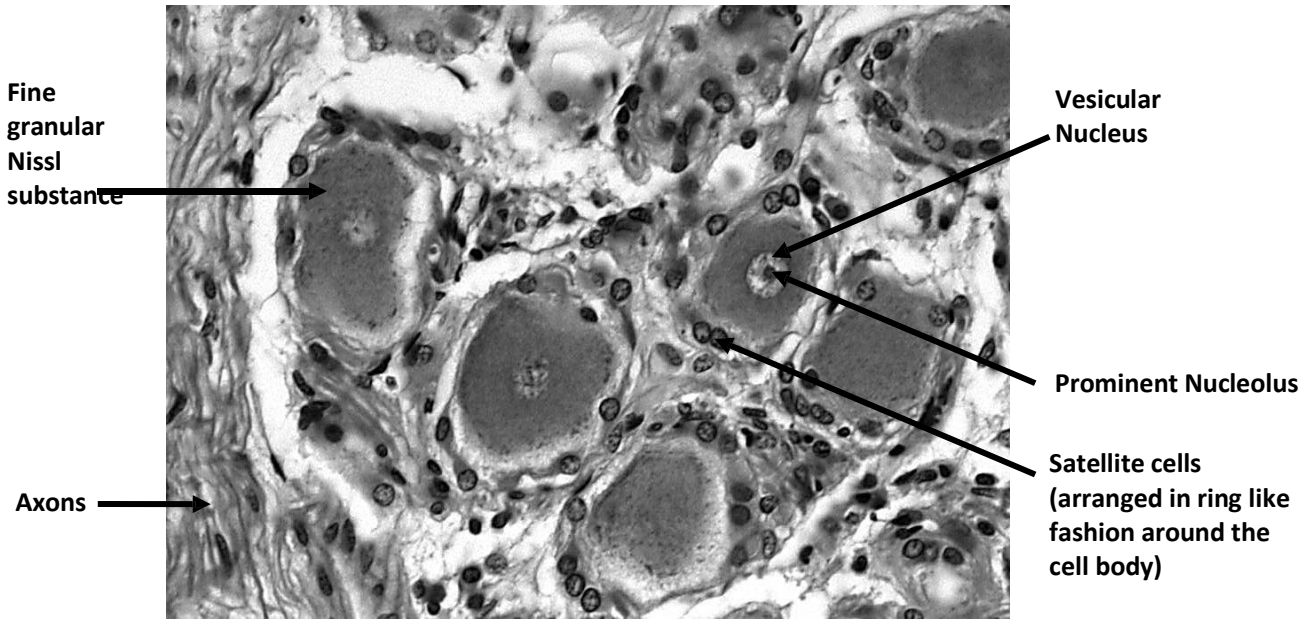
Amyotrophic lateral sclerosis (ALS), or **Lou Gehrig's disease** is characterised by a loss of anterior horn cells of lower motor neurons. This leads to a loss of motor innervation and thus muscle atrophy. People affected by this disease are generally middle-aged males and present with weakness in their extremities. As only motor neurons from the spinal cord are affected their sphincter control, sensation and intellectual capabilities are not affected. Patients generally have shorter life spans due to difficulties in breathing and swallowing. The famous physicist Stephen Hawking and South African rugby player Joost van der Westhuizen, suffer from this disease.

(http://library.med.utah.edu/WebPath/TUTORIAL/CNS/CNSDG.html).

(b) Spinal (Dorsal root) ganglion cells (pseudo-unipolar neurons)

Spinal Ganglion
Slide: 62
Stain: H&E

Note: *The spinal ganglion is also known as the dorsal root ganglion and contains pseudo-unipolar neuron cell bodies. The previous diagram illustrates the location of the spinal ganglion within the simple spinal reflex arc.*



Under L.P. distinguish the nerve cell bodies, which are large and round

Under H.P. study and draw a couple of cell bodies & their surrounding tissue

Pay attention to the following features:

- The size and shape of the cell body
- The centrally placed large round palely stained (vesicular) nucleus with its prominent nucleolus (because of the large size of the cell body, the nucleus may not be visible in every spinal ganglion cell, i.e. only the cytoplasm of the cell body is visible)
- The staining reaction, appearance and arrangement of the Nissl bodies (compare it to the Nissl bodies in anterior horn cells)
- The discontinuous ring of smaller round nuclei belonging to the supporting cells (satellite cells) around the cell body

Compare the structure of pseudo-unipolar neurons to the multipolar neuron as seen in microscopic sections

What is a ganglion?

Are spinal ganglion cells sensory or motor neurons?

Spinal ganglion cells are classified as "pseudo-unipolar" neurons. Explain this term.

What are satellite cells? What is their function?

Later on in the year, compare the structure of these spinal ganglion cells to ovarian follicles – students often confuse the two
Make sure you can distinguish between the two

Return to L.P., and then locate and observe under HP:

- The fine, pale eosinophilic nerve fibres coursing through the ganglion – the details of which can be found below in the peripheral nerve slide
- Identify transverse, oblique and longitudinal sections of these nerve fibres

Where are the "dendrites" and the axons of these spinal ganglion cells?

Which processes of a pseudo-unipolar neuron are myelinated?

Where do the axons of the spinal ganglion cells synapse?

Note the similarities in structure of the nerve fibres to those seen in the slide of peripheral nerve below

Did you know?

Neurons need supporting cells because neurons are so specialized that they are extremely vulnerable to changes in their environment. The supporting cells essentially act like “baby-sitters”, protecting and assisting the neurons in their function. Neuroglial cells also play central roles in CNS injury and disease.

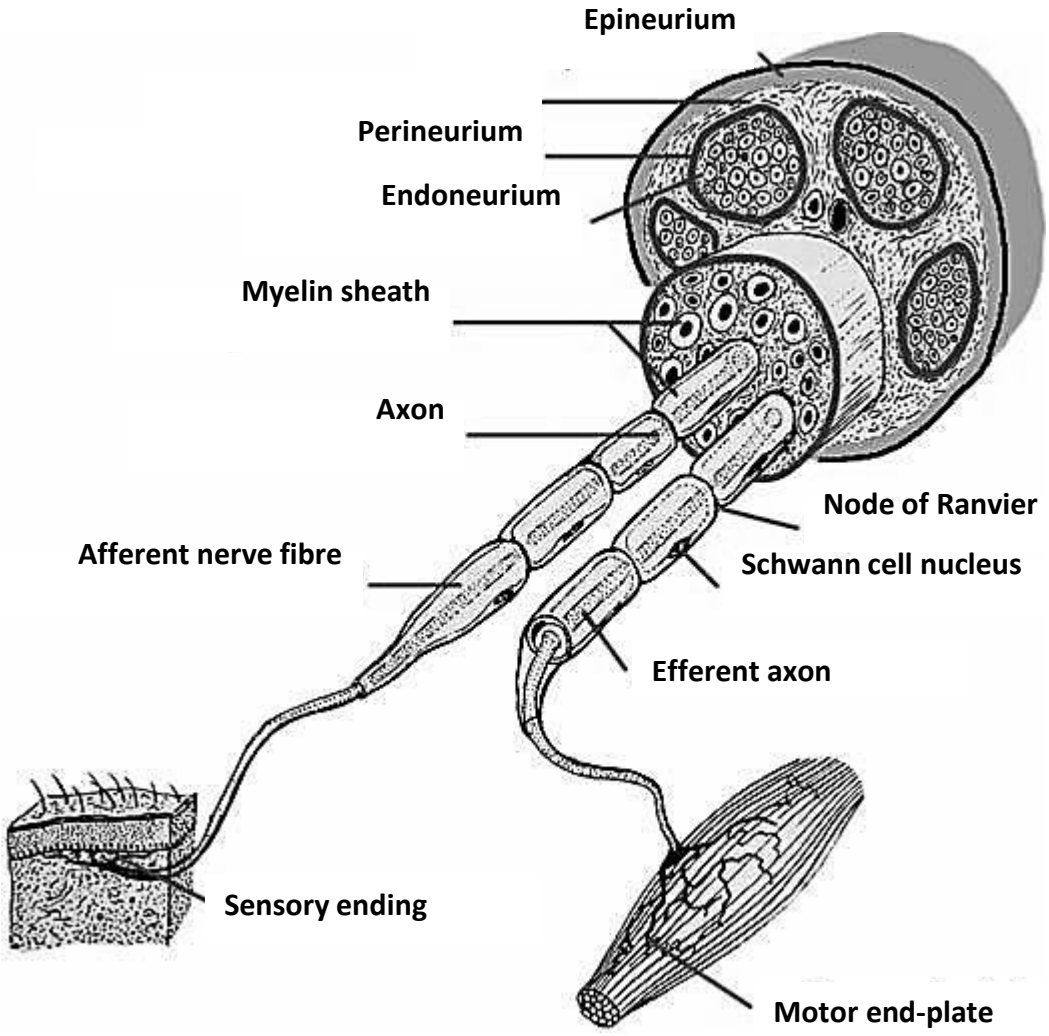
(http://www.vetmed.vt.edu/education/curriculum/vm8054/Labs/Lab9/lab9.htm; Barres, 2008)

II. PERIPHERAL NERVE

Peripheral nerves consist of several nerve fibres (axons with their supporting cells (Schwann cells) and endoneurial sheaths) running parallel to each other and arranged in bundles. Each bundle is surrounded by its own connective tissue sheath (perineurium) and the bundles are also collectively arranged within a connective tissue sheath known as the epineurium. This forms the whole anatomical nerve, which can be seen with the naked eye in gross dissection.

The axons that run within the peripheral nerve all begin and end in different places, thus the nerve acts like a major highway for signal propagation. The individual axons will thus leave the peripheral nerve to innervate their specific effector cell. In most nerves, the action potentials are propagated in the same direction; however some nerves have adjacent axons propagating signals in opposite directions.

Below are examples of peripheral nerves stained with 3 different stains (i.e. Mallory's technique, H&E, and fixation in osmium-tetroxide). They illustrate the different characteristics of peripheral nerves. The nerve stained by H&E is useful to look at, as it will help you to identify this tissue in sections of different organs stained similarly later on in the year.



http://vanat.cvm.umn.edu/neurHistAtls/index.htm

(a) Peripheral branch of a spinal nerve

Median nerve
Slide: 30
Stain: Mallory's technique

These are two sections (T.S. and L.S.) of a peripheral nerve stained by Mallory's technique.

A few notes on the staining technique:

Three dyes are used in Mallory's technique, which results in collagen fibres (such as in connective tissue) staining blue, "neurokeratin" staining red, and nuclei staining reddish-orange. Due to fading of the stain, it is possible that the neurokeratin in your section may appear orange/yellow rather than red.

***Note**: Lipids are dissolved out during the preparation of routine sections. Thus the lipid of the myelin is dissolved out but the protein remains. We call the protein part of the myelin sheath "neurokeratin" which is seen as a red-stained network.*

Remember: Myelin sheath is made up of many layers of the cell membrane of the Schwann cell wrapped around an axon. Cell membranes contain phospholipids and proteins.

Macroscopic and L.P.: Study the longitudinal section first

***Note:** Use the diagram at the beginning of this section to guide you.*

Study, draw and write notes on:

- The blue-stained epineurium around the entire nerve
  - Note the blood vessels within this sheath as well with orange stained eyrythrocytes
- The blue-stained perineurium around each bundle of nerve fibres
  - This sheath appears somewhat more tightly arranged around the bundle than the epineurium

  ***Note**: In both connective tissue sheaths, the red dye in the Mallory stain sometimes gets caught up in the connective tissue, thus the appearance of the connective tissue can sometimes contain bits of red and purple staining as well as the blue*

- The wavy appearance of many nerve fibres running in parallel to each other
  - Note: Because the nerve fibres are not arranged in a straight line (with the plane of section) some parts of the nerve fibre "disappear" then reappear further along the section.

Under H.P. Study, draw and write notes on the nerve fibres, paying attention to the following features:

- The thin, blue-stained endoneurial sheath around each nerve fibre
- The red-stained neurokeratin
  - This is the protein part of the myelin sheath, the lipid part was washed out during preparation, thus leaving the neurokeratin with a frothy appearance
- The central unstained axon
  - Again, due to the plane of section and the wavy nature of nerve fibres, the axon may not be visible throughout the entire length of the nerve fibre, but will appear in parts
- Nodes of Ranvier (the axon at the node of Ranvier may stain reddish-orange)
  - This is where the myelin sheath of one Schwann cell ends and the next one starts on the same axon, it gives the myelin sheath a “pinched” appearance in longitudinal section
- The reddish-orange stained oval nuclei of the supporting cells (neurilemmal/ Schwann cells).
  - They are NOT the nuclei of the nerve cell (neuron). Schwann cell nuclei will be found within the pale red stained "neurokeratin" (on the inside of the endoneurial sheath), whereas fibroblast nuclei, which also stain red, and tend to have a flatter appearance will be found within the blue stained endoneurium.

Now study and draw the transverse section under L.P. and H.P. Identify the same features as listed above.

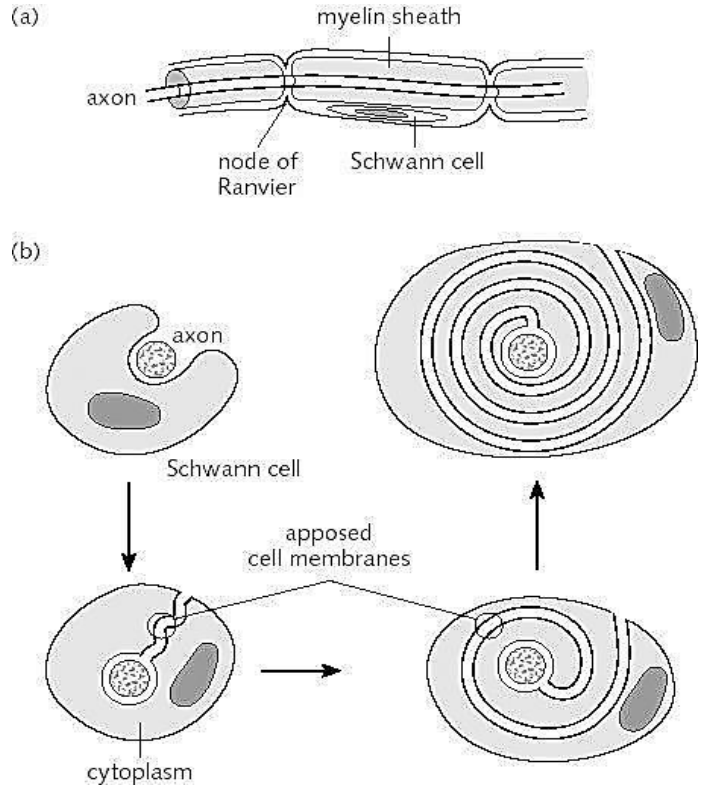
Note the variation in size of the nerve fibres.

In transverse section, the neurokeratin often has a "wheel spoke" appearance. Since the axon at the node of Ranvier stains red, one can identify when the section has been cut through a nerve fibre at the node of Ranvier even in transverse section. Schwann cell nuclei will be found within the neurokeratin (on the inside of the endoneurium) as opposed to fibroblast nuclei.

Are all the smaller nerve fibres myelinated?

How do you identify non-myelinated nerve fibres?
→ they are easier to see in transverse section, try to identify a few.

Diagram illustrating the formation of the myelin sheath by the Schwann cell



http://www.answers.com/topic/myelin

(a) Peripheral branch of a spinal nerve

Neuro-vascular bundle (axillary sheath)
Slide: 26
Staining: H&E

Examine the slide

This is a routine H&E section of a spinal nerve. All the same features mentioned above, will be able to be identified, only the staining reaction will be different. For example neurokeratin stains eosinophilically.

What will the staining reactions of the nuclei and collagen fibres be?

It is a good idea to study this slide in preparation for identifying nerves in organ systems.

When studying this slide note the following:

- The connective tissue sheath (epineurium) forms a distinct boundary around the nerve
- Due to the abundance of lipid in the myelin sheaths and the watery axoplasm, nerves are very palely stained in comparison to other eosinophilic structures. (Muscle, which also has connective tissue sheaths, can look a lot like nerves in H&E sections, but due to the abundance of protein, muscles stain darkly eosinophilic)
- Nerve fibres are tightly packed within a nerve, giving them quite a homogenous appearance
- Nerve fibres are also characteristically wavy in appearance (in L.S.), so that if for some reason the nerve is stretched, it will not snap. As most nerves are cut in oblique section in organs, one will often see numerous tightly packed palely stained structures in a combination of wavy and circular/oval arrangement within a connective tissue sheath
- The nuclei seen tend to be oval in nature, not as flattened as fibroblast nuclei and not as elongated as smooth muscle nuclei in longitudinal section. These nuclei however do not belong to the neurons themselves, but to the supporting cells, namely Schwann cells

(a) Peripheral branch of a spinal nerve

Sciatic nerve
Slide: 65
Stain: Fixed in osmium-tetroxide; unstained

Examine the slide

Lipids and other fats have been blackened by this technique; other components are unstained.

You have now studied three sections of spinal (peripheral) nerve, each stained by a different technique. What can these preparations tell you of the chemical composition of myelin?

Relate this to your knowledge of the formation of myelin.

What is meant by the term “mixed nerve”?

Are the nerve fibres of a spinal nerve efferent, afferent or both? Which are myelinated?

List the supporting cells of the CNS and PNS and the main function of each

Study electron micrographs (EMG's), showing the <u>ultrastructure</u> (very high magnification) of myelinated nerve fibres in both L.S. and T.S. (Explain how myelination occurs – MEDICS ONLY).

Also study the EMG's of unmyelinated nerve fibres in T.S.
Note the difference to the myelinated nerve fibres and note the presence of Schwann cells (nuclei and cytoplasm) in both.

III. NEUROMUSCULAR JUNCTION

Striated skeletal muscle (snake)
Slide: 4
Stain: H&E and gold impregnation

Examine the slide

This is a whole mount of striated skeletal muscle of the snake impregnated with gold to demonstrate nerve fibres (i.e. muscle fibres dissected from the snake were simply teased out on a slide, sections were not cut, hence the tissue is still 3 dimensional).

The nerves are visible as very thin branching fibres running along and across the skeletal muscle fibres. Find

regions where the muscle fibres are at their thinnest. One will be able to see the terminal branches of the axons and the terminal boutons (seen as very darkly staining dotted structures on the muscle)

Read up about the <u>fine structure</u> of the motor end plate in your textbook.

Did you know?

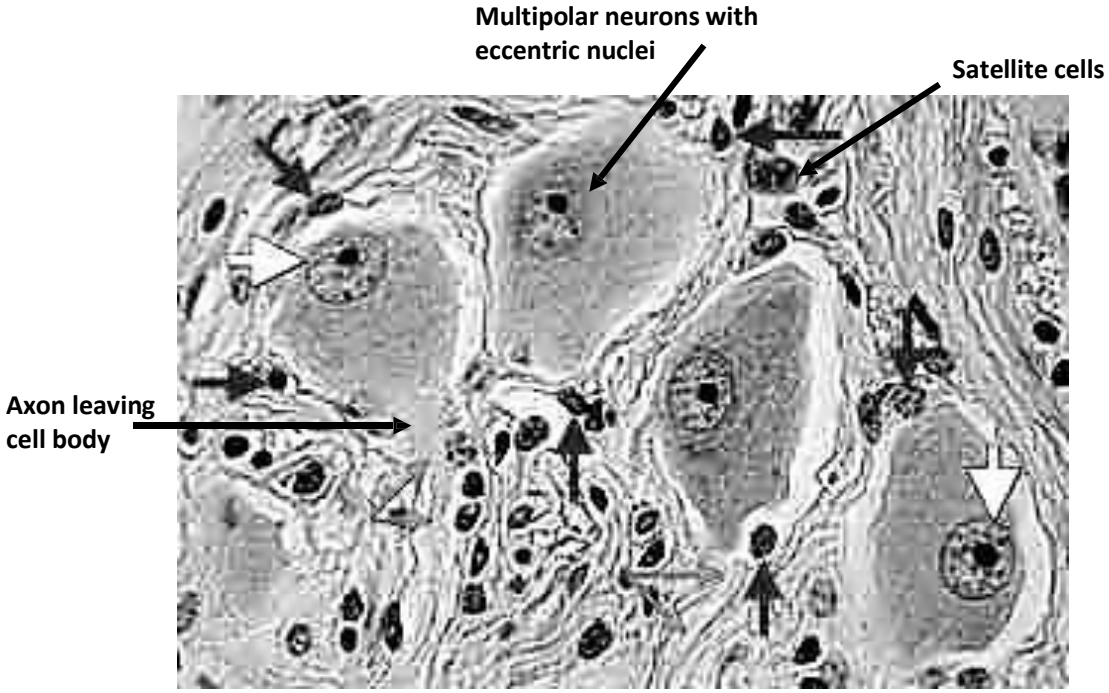
When reattaching severed limbs to patients, surgeons often use the epineurium to hold onto and stitch through when trying to reattach nerve ends.

(www.siumed.edu/~dking2/ssb/neuron.htm)

IV. AUTONOMIC NERVE AND GANLIA

Wall of vagina
Slide: 54
Stain: H&E

Examine the slide



Clinical Correlation

Parkinson's disease is characterised by the slow progressive degeneration of dopamine secreting neurons in the substantia nigra and basal ganglia of the brain. At the microscopic level one can see that the typical pigmentation of these neurons is lost and degeneration of the cell bodies in this area as well as gliosis (increase of neuroglial cells), in the area. At ultrastructural level, the degenerating neurons also display Lewy bodies which are intracellular inclusions caused by the accumulation of intermediate neurofilaments in association with certain proteins.

Ross & Pawlina (2006). Histology: A Text and Atlas

RECAPITULATION
NERVOUS TISSUE

1. Give three structural characteristics of neurons

2. What is the difference between a nerve, a nerve cell and a nerve fibre?

3. Describe the arrangement of white matter and grey matter in the spinal cord

   Note: *The term grey matter comes from the appearance of this tissue in fixed or dead brain tissue, before staining. Living grey matter is actually pink due to the abundance of capillaries through which blood flows. The term white matter comes from the appearance of both living and fixed brain tissue due to the abundance of lipid (fat) in myelin sheaths*

4. Tabulate the similarities and differences between ganglion cells and anterior horn cells.

5. Other than the spinal cord and ganglia, where else in the body would one expect to find nerve cell bodies?

6. What is the structure and function of the Nissl bodies?

7. List the supportings cells of the PNS and give their main function

8. Describe the relationship between the Schwann cells and nerve fibres in myelinated and non-myelinated nerve fibres.

9. Name the connective tissue sheaths of a peripheral spinal nerve, e.g. the median nerve.

10. Distinguish peripheral nerve from muscle and connective tissue fibres.

11. How would you be able to distinguish between nerve cells (cell bodies) and epithelial, connective tissue and muscle cells i.e. from other primary tissue cells?

RECAPITULATION
THE FOUR PRIMARY TISSUES

1. What is a tissue? (as opposed to a cell, an organ or a system)

2. What are the four "primary tissues"? Give the embryonic derivation(s) in each case.

3. What are the distinguishing features of each of the "primary tissues"? Relate the distinguishing features to their main functions.

4. How are the "primary tissues" related to the structure of an organ?

5. Tabulate the differences between epithelia and connective tissues.

6. List the similarities between muscle and nervous tissue.

NOTE:

IT IS IMPORTANT TO KNOW THE ANSWERS TO THESE QUESTIONS AS A KNOWLEDGE OF THE "PRIMARY TISSUES" IS BASIC TO THE STUDY OF THE ORGAN SYSTEMS