The Open University

Histology, microscopy, anatomy and disease

Contents

Week 1: Histology

Introduction

Welcome to this Open University course on histology. This course is designed for students studying human biology at school or university, medical laboratory scientists and anyone interested in biomedical science.

Histology is the study of tissues and their structure, and histopathology labs are found in most hospitals. In the course you will see a histology department in action and discover how histological slides are produced.

Watch the following video in which David Male describes what you'll be learning about over the next four weeks.

Video content is not available in this format.

David has been a Professor of Biology at the OU since 1999, and has a particular interest in the use of technology for teaching science. You will have a chance to experience one such approach during the course, by using a virtual microscope to examine a number of biomedical samples, just as scientists and medical professionals would explore such samples in a lab.

Before you start, The Open University would really appreciate a few minutes of your time to tell us about yourself and your expectations of the course. Your input will help to further

improve the online learning experience. If you'd like to help, and if you haven't done so already, please fill in this optional survey.

1 What is histology?

Histology is the study of tissues and their structure. The structure of each tissue is directly related to its function, so histology is related to anatomy and physiology.

Similarly, histopathology is the study of tissues affected by disease. This is something that can be very useful in making a diagnosis and in determining the severity and progress of a condition. Disease processes affect tissues in distinctive ways, which depend on the type of tissue, the disease itself and how it has progressed.

Histopathology units are found in most hospitals and there are also independent private laboratories. The services provided by these laboratories can be accessed by healthcare professionals, such as general practitioners (GPs).

Because of the great variety of tests that are available, and the high level of skill that is needed to carry them out and interpret them, many laboratories specialise in particular tissues or types of diagnosis. For example, a neuropathology laboratory will focus on understanding diseases that affect the nervous system.

Histology is also used extensively in biomedical research, to identify the causes and possible treatments for disease. This type of research may take place in a hospital laboratory but it is more often carried out in universities, research institutes and pharmaceutical companies.

The conventional view of a histopathologist is someone looking down a microscope. Most histological work does indeed involve the preparation of tissues for microscopy, observation of sections and reporting of the findings. However, a pathologist can often tell a great deal about a tissue without using a microscope.

For example, the brain of a person affected by multiple sclerosis has distinct lesions (areas of damage or injury) that are a few millimetres across. These are called plaques, and can readily be seen in a tissue sample with the human eye (see the darker areas highlighted with arrows in Figure 1 below).

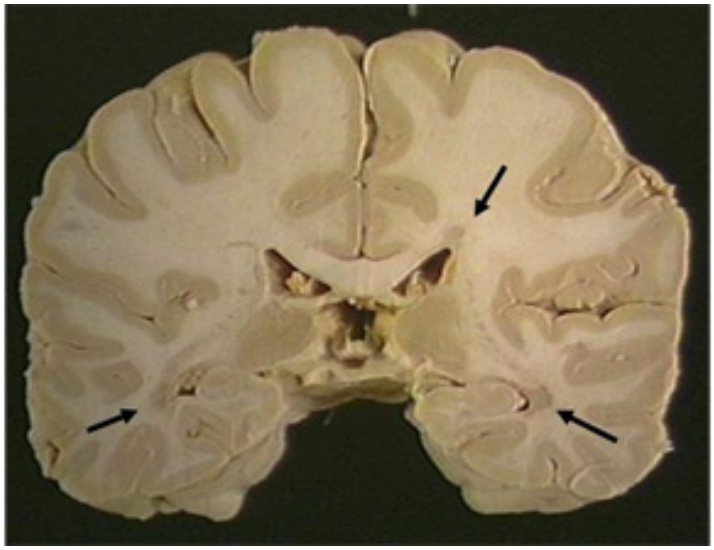


Figure 1 A cross-section through a human brain, with arrows indicating the presence of lesions (Caroldoey, 2015).

Such large specimens that can be examined macroscopically (by eye) are usually only available post-mortem (after death) or following surgical removal of tissue. In contrast, biopsy specimens, which consist of just a needleful of cells or a flake of tissue, can be extracted at any time, but can only be examined microscopically.

In this course we will first teach you how to use a basic light microscope and then show some sections of various human tissues, presented via a virtual microscope, which mirrors the functions of a real microscope.

Once you are familiar with the normal appearance of different tissues, we will start to introduce sections from diseased tissues, and relate their appearance to the normal physiology of the tissue and pathological changes that have occurred.

Note that the virtual microscope tool will work in all modern browsers on desktop computers, laptops and tablet devices. However, we recommend completing the course on a desktop computer or laptop for better viewing of the sections and to enable integration of microscopy with the course text, images and video in separate windows.

1.1 A histology department in action

The following video shows the work of a busy histology department at Milton Keynes Hospital, in central England.

In the video, Peter Mooney, the Laboratory Manager for the department, takes you through the different steps involved in the production of sections, staining and initial analysis of slides for diagnostic histopathology. These steps will be described in more detail later in the course.

Please note that this video is around 17 minutes long, so you may prefer to watch it in stages.

Video content is not available in this format.

1.2 How histological slides are produced

A number of distinct steps are involved in producing histological slides, ready for analysis. Some of these steps are described below.

Fixation

As they die, cells release enzymes that start to break down components of the tissue. This is a process termed autolysis.

The degree of breakdown depends on the tissue and what has happened. For example, post-mortem tissue is not usually taken until several hours or days after the person has died, and this would have undergone much more autolysis than a surgical specimen. Also, different components of tissue vary in their sensitivity to enzymatic digestion. A histologist has to be aware of all these processes and distinguish between changes that are due to a disease and those due to tissue autolysis.

However, it is important that the original structure of the tissue is preserved before it reaches the histology laboratory, so that it can be fully analysed. To minimise tissue breakdown, samples are often placed in a solution of fixative, in a process known as fixation.

Different fixation procedures are required depending on what method is needed for a later step in this process, namely staining. (You will read about staining shortly.) Because of this, it is important to know what techniques will subsequently be applied to a tissue sample when it is taken.

In addition to preventing autolysis, fixation may serve to retain the structure of the tissue and limit microbial growth that could otherwise make analysis more difficult.

Embedding

Tissue that has been received in the laboratory then needs to be prepared for sectioning (the process of cutting it into very thin sections so it can be viewed with a microscope). A variety of instruments are used to cut the sections and the protocol depends on the application.

In most cases the tissue requires embedding in a medium, which allows thin sections to be cut cleanly. Most tissues for routine histology are embedded in wax. This occurs when water is removed from the tissue and progressively replaced by wax, which can be solidified later to make a tissue block.

The tissue is progressively dehydrated by immersing it in successively higher concentrations of alcohol before transfer to the organic solvent (e.g. xylene) and finally embedding in wax. In a large histology laboratory, much of this tissue processing is automated, to save time and to produce consistent results.

Sectioning

A number of devices are available for cutting tissue into sections for analysis:

- a microtome: cuts thin sections (1–50 μm) from fixed tissue
- a vibratome: uses a vibrating blade to cut thicker sections (100–200 μm) from fresh or fixed tissue
- a cryostat: cuts sections from deep-frozen blocks, usually of unfixed tissue.

Most sectioning in routine histopathology departments is done with a microtome producing sections of ~3 μm thickness, from tissue that has been embedded in wax, as Figures 2 and 3 below show.

Figure 2 A histologist examines a tissue specimen in a wax block to establish its orientation.

Figure 3 The wax block containing the tissue is sectioned on a microtome.

1.3 Staining techniques

Most cells and cellular elements are virtually transparent, so it is difficult to distinguish individual cells and cellular structures when viewed with a microscope. However, this issue can be countered if staining agents are applied to the sample while it is being made ready for analysis.

Histologists have developed a wide variety of different staining techniques to identify different elements within tissues when they are looked at with a microscope. This methodology is referred to as histochemistry.

The most commonly used stain for medical diagnosis uses a combination of the dyes haematoxylin (which is blue) and eosin (which is red). This approach is usually abbreviated to 'H&E' staining. An example of some tissue stained using H&E is shown in the slide image below.

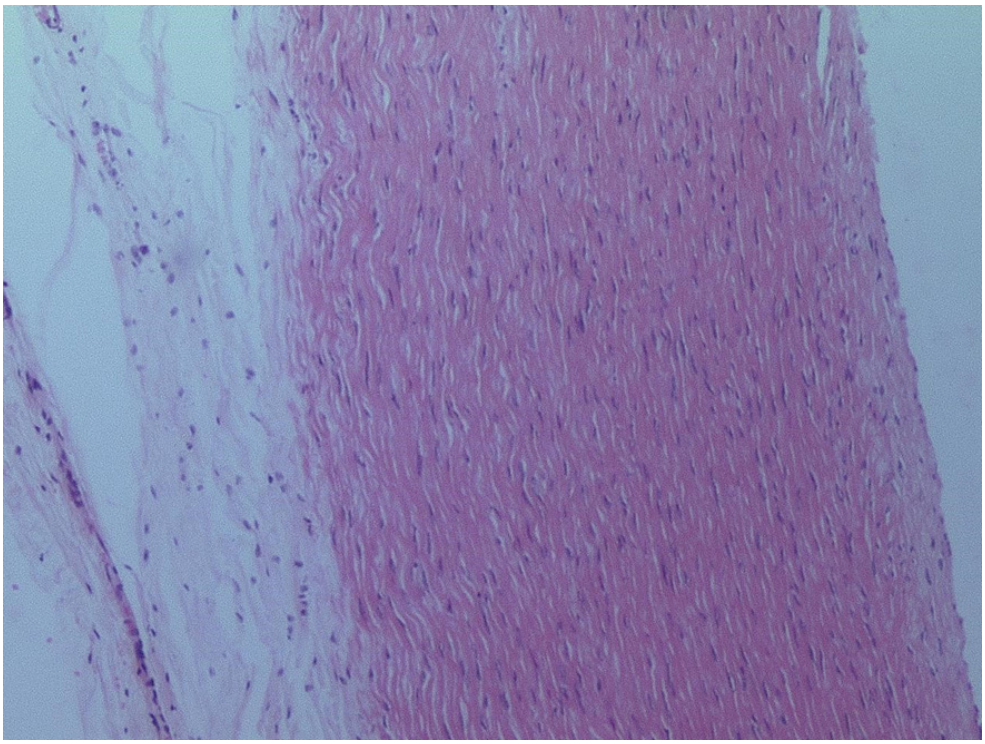


Figure 4 Section across the wall of the human aorta stained conventionally with haematoxylin and eosin.

However, there are some structures that do not stain well with H&E, so different stains have been developed that allow the identification of particular tissue elements (e.g. collagen). These alternative stains include Giemsa, Masson's trichrome and elastic Van Gieson (see the slide below, Figure 5), and they are described in more detail shortly.

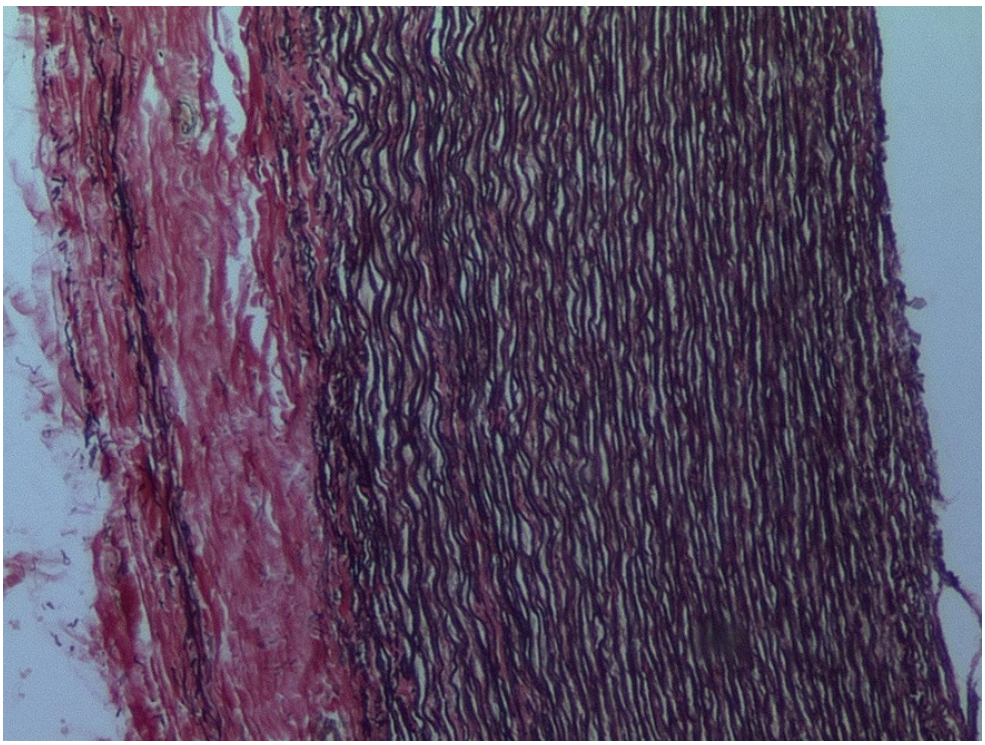


Figure 5 Section across the wall of the human aorta stained with elastic Van Gieson, which identifies the elastic fibres (black) in the media of the artery and the collagen (red) in the adventitia.

A wide variety of other histochemical stains are also available, each of which can help identify particular structures. Some are relatively simple to apply, merely requiring that the section is dipped in the stain for a set time. Others require a number of sequential steps, and in some cases the results can be surprisingly variable or unpredictable. For example, the silver staining technique, originally developed by Camillo Golgi and shown in Figure 6, is notably variable.

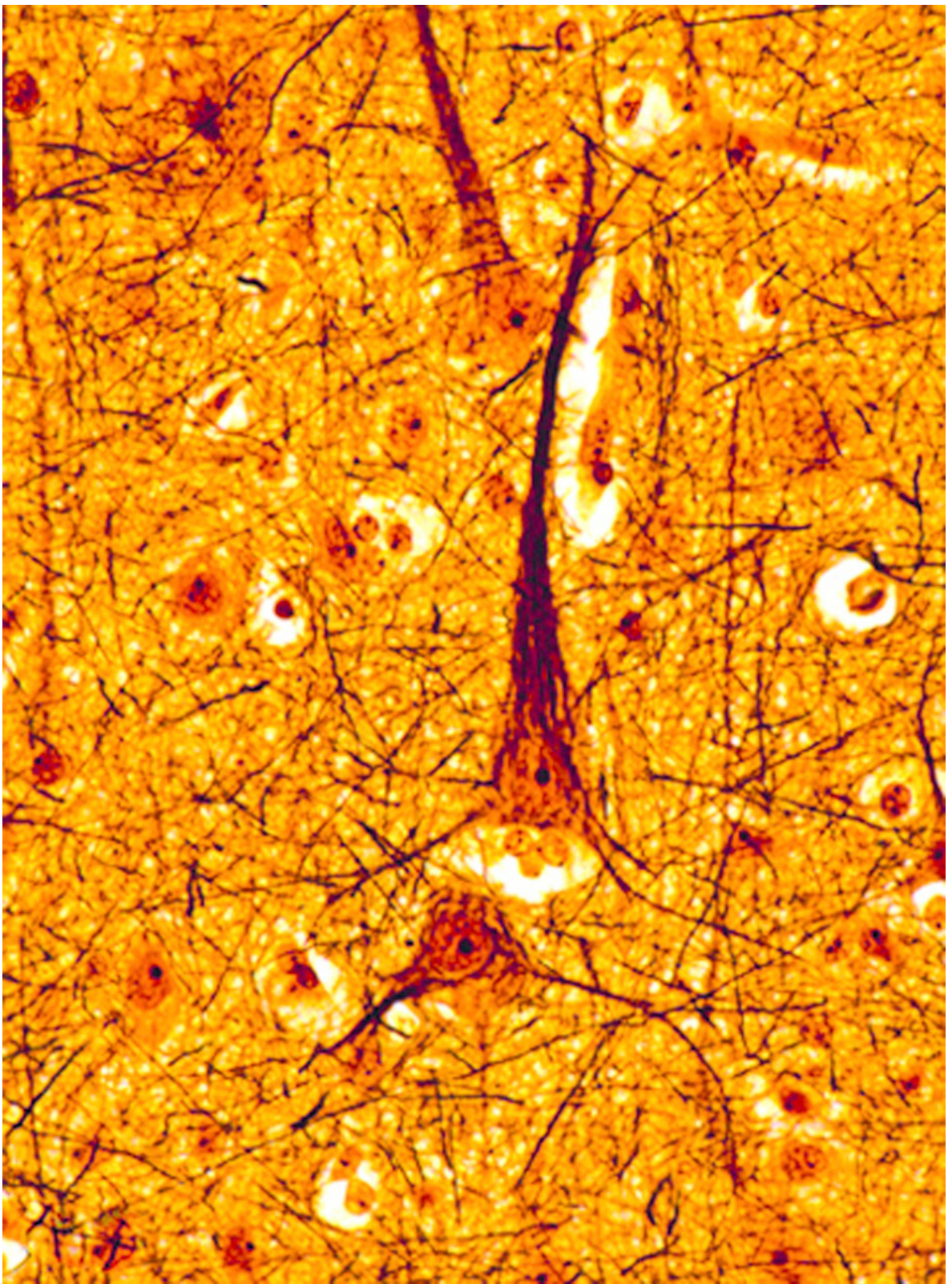


Figure 6 Neurons visualised with a modification of the Golgi silver staining method.

Some of the more commonly used staining techniques are outlined below.

- Giemsa stain consists of a mixture of methylene blue and eosin. It is mostly used on blood films, where it stains red blood cells (erythrocytes) pink and the different types of white blood cells (leukocytes), allowing their identification according to the size

and shape of their nuclei. It also binds to some pathogens (e.g. spirochaetes and syphilis) and it is, therefore, particularly useful if infection is suspected.

- Luxol fast blue/cresyl violet is used to identify myelin, a component of the nervous system which insulates the nerves and stains blue, while other elements of the nervous system stain pink or violet.
- Masson's trichrome stain uses three dyes to stain different structures. It is valuable for distinguishing elements of connective tissue. Typically the cell cytoplasm, muscle and keratin are stained red, nuclei are stained black and collagen is stained blue.
- Periodic acid schiff (pas) stains carbohydrates magenta, including components of the basal lamina, surface glycoproteins on cells, and intracellular carbohydrates, such as glycogen in hepatocytes. Cells that secrete mucus are also strongly stained.
- Congo red is used to identify deposits of protein in tissue called amyloid.
- Toluidine blue is a particularly versatile dye that stains nuclei blue, and can be used to differentiate between different types of granules (e.g. within mast cells).
- Van Gieson stain binds to collagen in the extracellular matrix, staining it pink. It is often combined with a stain for elastic fibres (elastic Van Gieson) which stains black, allowing the two major elements of connective tissue to be differentiated.

1.4 Immunohistochemistry

In the last 30 years, staining methods using antibodies have been increasingly developed. When applied to staining for the light microscope, these techniques are collectively referred to as immunocytochemistry (ICC) for the staining of cells or immunohistochemistry (IHC) for the staining of tissues.

Antibodies are proteins synthesised by one of the cell types of the immune system, but their use for staining other cells has revolutionised histology, as they allow the identification and localisation of individual molecules. Antibodies specifically bind and detect individual proteins. They can therefore be selected to identify the presence or location of a single protein that identifies a single cell type, or is diagnostically discriminating.

A molecule that binds to, and is recognised by, an antibody is called an antigen, and, in the context of histology, such antigens are often referred to as markers, since they act as ways of recognising a particular cell.

Staining with antibodies is particularly valuable for distinguishing between different cell types in a tissue. For example, different classes of lymphocyte appear virtually identical in size and shape, but they can be distinguished through staining according to their surface markers. Similarly, in Figure 7 some nerve cells (neurons) are clearly visible among other cells in the cortex of the brain as a result of this type of staining technique.

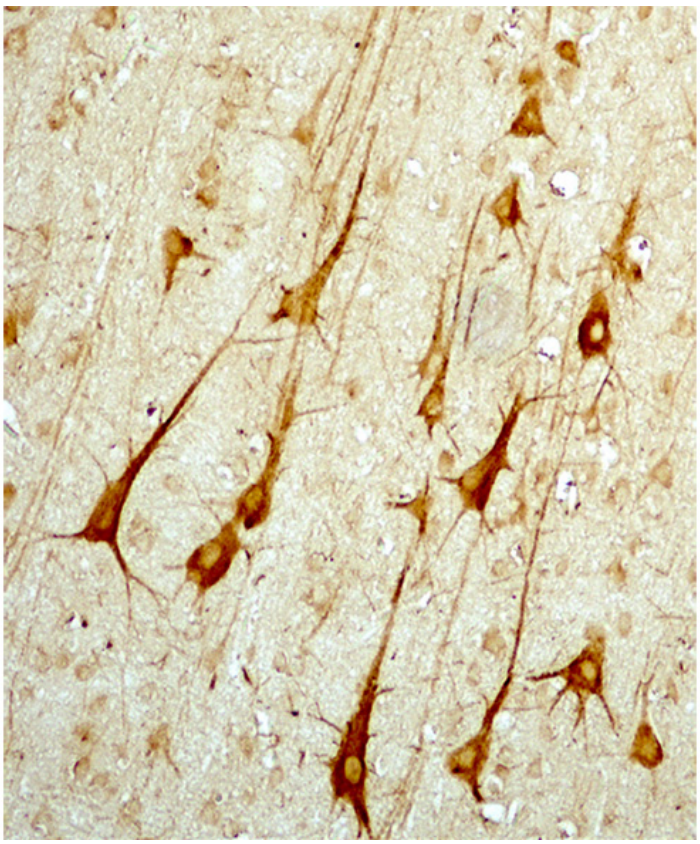


Figure 7 Neurons in the cortex of the brain are identified by immunohistochemistry using an antibody directed against neurofilaments.

1.5 Cells and tissues

Cells have distinctive shapes and functions which depend on how they have developed and their interactions with other cell types. It is difficult to be precise, but there are probably at least 200 different types of cell within the body.

Disease processes affect individual cells in many ways. They may cause them to:

- die
- change their shape
- divide
- move
- invade other tissues.

Any of these changes also affect the anatomy of the tissue.

Understanding the changes that are characteristic of a disease requires a detailed knowledge of the normal appearance of cells and tissues, and the range of normality. Many tissues change considerably with age, so that a feature that is normal in an adult would not be normal in a child. For example, the thymus gland gradually decreases in size with age, so a large thymus in an old person could indicate some underlying pathology.

Primary and secondary changes

The histological changes seen in a tissue may be primary, a cause of the disease process, or secondary, a consequence.

For example, if blood pressure is high due to vascular disease, it may cause an increase in the volume of the heart muscle, as the heart finds it more difficult to pump blood into the circulation. A further consequence may be a corresponding decrease in the volume of the chambers of the heart so that less blood is pumped with each contraction.

Interpreting the changes seen histologically requires a sound understanding of the underlying disease processes.

Identifying cells and structures

As you have read, it is difficult to see many cellular structures using a light microscope, so dyes are often applied to samples to stain the contents and make them visible. There are two main ways of staining cells.

- The traditional method involves the use of dyes that selectively bind to different structures within the cell.
- More recently immunohistochemistry techniques have emerged that use antibodies to stain individual molecules.

Most of the sections you will view in this course have been stained with haematoxylin and eosin (or H&E). The cell nucleus containing DNA binds haematoxylin and is, therefore, blue, as can be seen in Figure 8 below. Inactive cells usually stain dark blue, while more active cells are paler. Dead cells have shrunken compacted nuclei which may be fragmented.

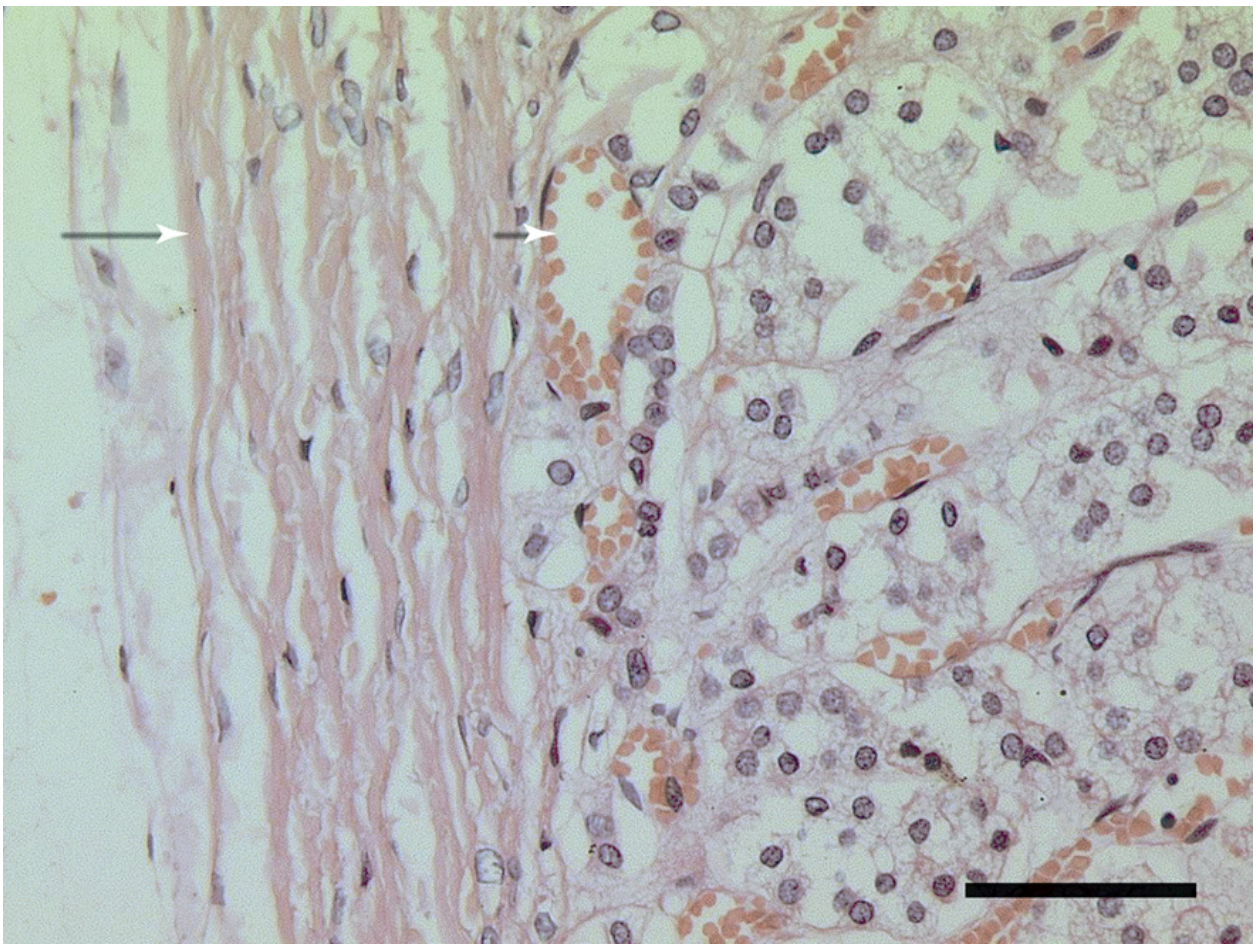


Figure 8 A section of the adrenal gland (cortex), stained with haematoxylin and eosin. Cell nuclei are stained deep blue and the cytoplasm is pink. The capsule, the outer envelope, of the organ (large arrow) is also pink. The erythrocytes (red blood cells) seen within small blood vessels (small arrow) are red. Scale bar = 50 µm.

The cytoplasm of a cell stained with H&E may range from pink to purple. More active cells have diffuse purple staining ribonucleic acid (RNA) in the cytoplasm indicating that they are producing protein. Many cells also have granules which are acidophilic (pink) or basophilic (purple) depending on whether they have taken up eosin or haematoxylin.

The cytoplasm may also contain non-staining vacuoles. For example, fat cells (adipocytes) have a large central vacuole containing fat.

2 Using a light microscope

The remainder of this week focuses on the use of a light microscope for histological purposes. You will have plenty of opportunity to put your learning into practice by using the virtual microscope tool to explore some real samples, and later on to take measurements from them.

Histology departments use light microscopes with transmitted light. Microscopes come in many different types, each of which has distinct functions and applications. However, in a routine histology department, virtually all of the work is done using a light microscope with

transmitted light. This means that the light passes through the section to the objective lens, as shown in Figure 9.

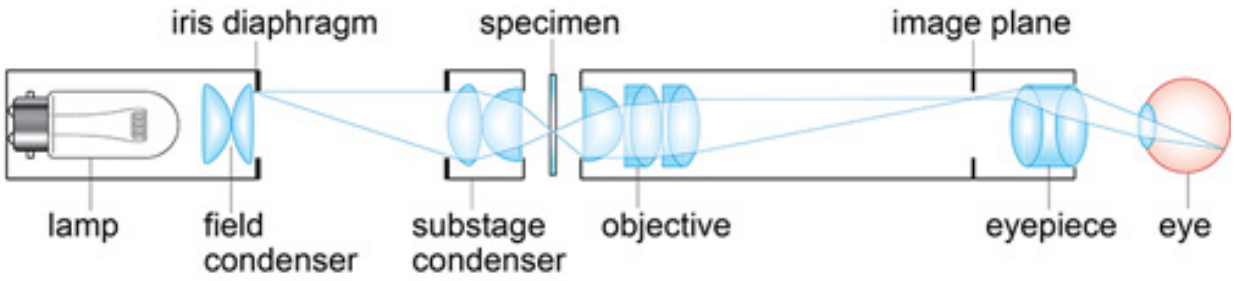


Figure 9 Elements of a light microscope.

Here, light from the lamp is focused on the specimen by the substage condenser. The objective collects light from the specimen and focuses it to form an image within the barrel of the microscope. The eyepiece allows this image to be viewed as if it were a projection in the plane of the section.

Resolving power

It is important to understand some of the limitations of a light microscope.

First, the absolute limit of resolution of a light microscope is determined by the wavelength of light being used (λ) and the properties of the optical components of the microscope in question (A_n). The limit of resolution is defined as the smallest distance (d) at which it is possible to distinguish two separate items.

Optics tells us that the relationship between these properties is:

d = λ /(2 x A_n)

Using the best optical components the value of A_n is approximately equal to 1. Consequently, the resolving power of a microscope is approximately equal to $\frac{1}{2}$ of the wavelength of light passing through the sample.

So for example, green light has a wavelength of 550 nm. This means that structures such as the nucleus (~5000 nm), mitochondria (~1000 nm) and large vesicles (>200 nm) can be resolved by a light microscope. Smaller structures, however, cannot be resolved.

Another limitation of a light microscope is the degree of magnification. No matter how the optical system is configured, increasing the overall magnification beyond 1000x cannot resolve further detail of the cell structure. For this reason, the highest objective found on light microscopes is typically 100x. When used in association with a 10x eyepiece, it gives the maximum of 1000x magnification.

Finally, be aware that the quality and thickness of the section affects what can be resolved. In general, thicker sections allow lower resolution than thin sections. The sections produced for diagnostic pathology are generally about one cell thick, which allows a clear view of the structure of a single cell.

2.1 Basic functions of the virtual microscope

The following video shows you the basic functions of the virtual microscope that you will use throughout this course. It demonstrates the processes of stage movement, focusing,

illumination and the use of different objectives (the parts of the microscope that focus the light into an image at a given magnification).

Video content is not available in this format.

In the next section you will have your first opportunity to use the virtual microscope for yourself.

2.2 Normal blood smear

As you work through this course you will be directed to the virtual microscope to examine lots of different types of samples. You'll start by looking at a normal blood smear.

Activity 1

Open the virtual microscope in a new browser window or tab. This is helpful so you can operate it while still having access to the instructions you are reading now.

Load Slide 1 ('Normal blood smear'), which is available from the Week 1 collection in the drop-down menu above the slides and familiarise yourself with the basic controls, including the slide box, the focus and lighting controls, the stage movement controls and the objectives.

When you first access the slides you will find that they are blurry. This mirrors the experience that you would have when you initially looked at a slide under a light microscope, and can be easily remedied by changing the focus settings and the light level.

If you are struggling to focus a given slide, you can click on the spanner icon and click the checkbox for 'Load slides in focus', and this will clarify the image. We urge you to try focusing the image manually first though, if possible.

Once you have mastered the microscope's basic controls, use the legend to identify different cell types in the blood from Slide 1 ('Normal blood smear'). The predominant cell type is the red blood cell (erythrocyte), which carries oxygen to the tissues, and helps to remove carbon dioxide. Leukocytes, or white blood cells, are involved in immune defence against infection.

There are a variety of different types of leukocytes, all with distinct functions. You will learn more about these cells, and how to identify them, later on in the week. You might like to keep the virtual microscope open as you work through the week.

2.3 Malaria

Having explored the virtual microscope in the previous activity and looked at a normal blood smear, you will now use it to start comparing different samples. Specifically, you will be identifying infected red cells in a blood sample.

Activity 2

First, open the virtual microscope in a new browser window or tab.

Spend a few minutes comparing the normal blood smear (Slide 1 in Week 1) with the sample marked 'Malaria' (Slide 2 in Week 1), in the virtual microscope. Identify similarities and differences between them.

Note down the differences you notice.

Provide your answer...

Discussion

One difference that you may have noticed is that of the colours of cells in the samples. Red blood cells do not normally contain a nucleus or nucleic acids so they do not stain blue. However, infected cells containing the malaria parasite (*Plasmodium*) are stained blue. The appearance of an infected cell depends on how far the parasite has progressed through its developmental stages.

Now take a brief look at the slide for 'Sample 3' (Slide 3). How is this blood smear different from 'Normal blood smear' (Slide 1) and 'Malaria' (Slide 2) samples that you have just looked at?

Make a note of any differences that you observe.

Provide your answer...

Discussion

You may have observed that there are similarities between the 'Malaria' slide and the slide for 'Sample 3'

The reason for this is that 'Sample 3' is another example of malaria in a blood smear. However, in this case, more red cells have been infected than in the 'Malaria' slide, and there are numerous parasites within each cell.

From this evidence you could deduce that ‘Sample 3’ represents blood taken at a later stage of infection than that of the ‘Malaria’ slide. At this later stage the infected cells are called ‘schizonts’, and you can see an example of them at position (4718, 2868) in Slide 3.

2.4 Considerations for interpreting sections

Activity 2 introduced you to the idea of comparing the content of different slides, but there are a number of other considerations to take into account when interpreting samples.

Orientation

Most histological sections are 2D slices, from a 3D piece of tissue. Exactly what will be seen on the microscope slide depends on the plane of the section; that is, the position of the microtome cut, in relation to the anatomical structures in the tissue.

Clinicians attempt to cut tissues in a plane that allows the underlying structure of that tissue to be seen and interpreted most easily. For example, sections of the intestine and skin are usually cut in transverse section (across the tube), so that all layers of the tissue are visible. Similarly, skin sections are cut perpendicular to the surface of the skin.

Positioning the tissue before embedding and knowing its orientation are, therefore, critically important, so that the sectioning is done in the correct plane. For biopsy specimens there is no latitude for deciding the plane of the section, as the direction of the needle tract determines what tissue is available and how it is oriented.

Pathologists expect to view sections in their best orientation as this then gives them the best opportunity of identifying abnormalities. Many anatomical structures are not readily visible in thin, 2D sections, simply because most of the structure is not present. In this case, the 3D structure needs to be interpreted from what can be seen.

In order to give you some insight into this problem, spend a few minutes looking at the three structures in the figure below and thinking about what the different sections would look like if they were taken along the planes indicated by lines shown (i.e. A–A’, B–B’ and C–C’). You can try making simple diagrams of the sections that would result from sectioning along these lines, if you wish.

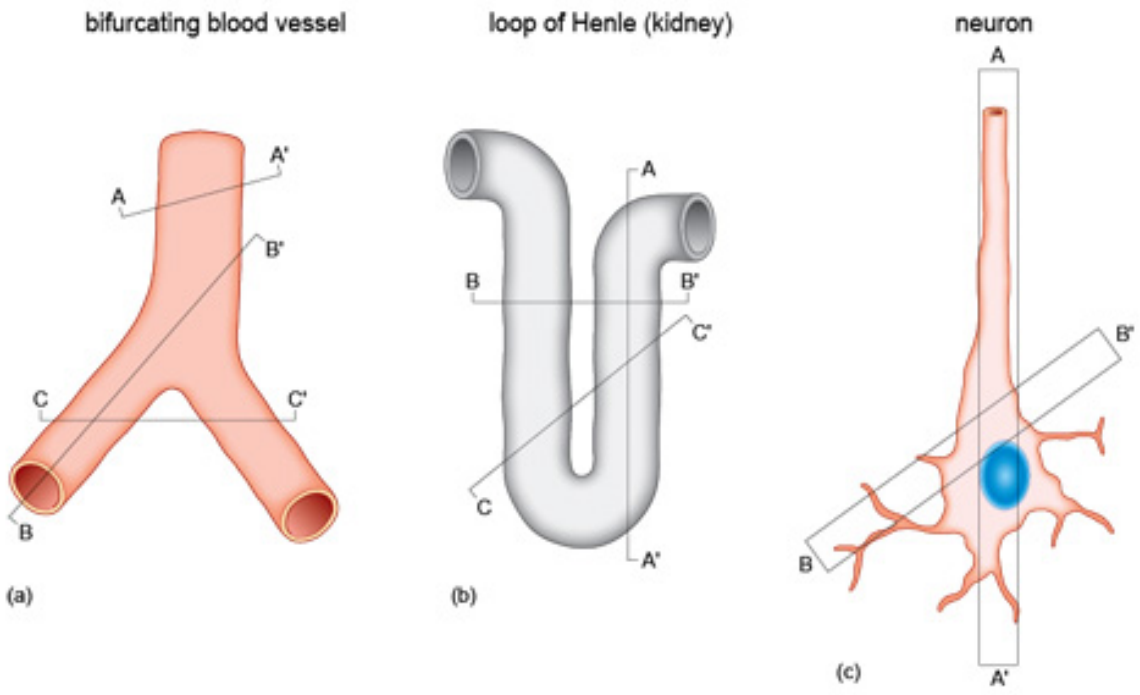


Figure 10 Understanding the plane of a section for (a) a blood vessel, (b) a sample of kidney tissue and (c) a neuron. Note that for the neuron, the thickness of the section is significant in relation to the thickness of the cell.

This exercise should make you appreciate that it is not just the orientation of the section, but also the position (depth) into the tissue or tissue block that affects the appearance of the structures. For example, the blood vessel in (a) can appear as one or two structures, depending on the level of the section within the block.

For this reason, it may be necessary to section a block at different levels to obtain suitable slides. (The initial cut-up of the tissue should have ensured that the area of interest is within the block so it should not be necessary to hunt through the block to find it.)

Size of sample

Another problem in interpreting sections is that some structures are too big to be seen in a thin section, because much of the cell and its overall architecture cannot be seen. The neuron (image (c)) in Figure 10 is a good example of this.

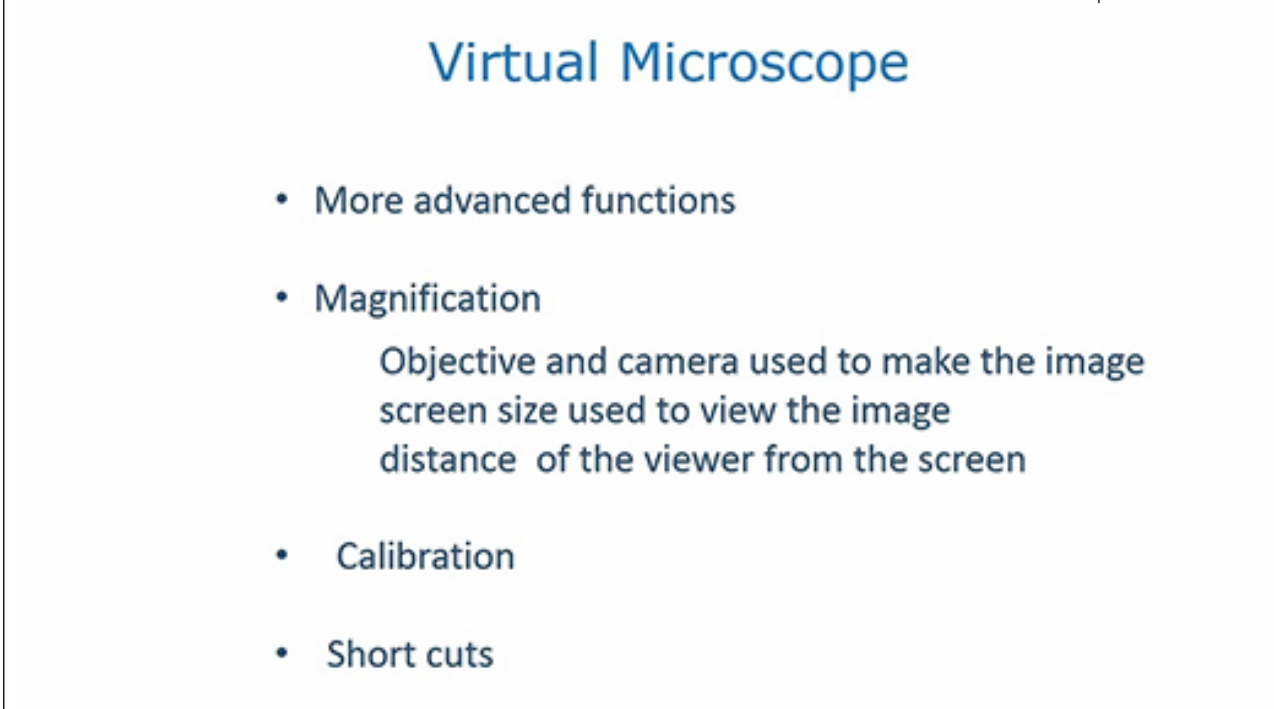
One solution is to cut thicker sections in order to capture more structures. However, as noted earlier, resolution is generally better in thin sections because in thick sections some cells will overlie others so that distinguishing details of individual cells or the tissue structure may be more difficult. Moreover staining thick sections requires much longer for the stains or antibodies to permeate the tissue, which slows the process of analysing and interpreting the material.

3 Measurements on the microscope

Sometimes it is important to measure the size of a cell, the distance between cells or the number of cells in a given area. Most light microscopes have the option to add a graticule

for determining distances or a grid into the light path, to allow such measurements to take place.

Video content is not available in this format.



These elements are usually added by exchanging one of the eyepieces for another with a graticule built into it. Therefore, the appearance of the graticule is fixed according to the eyepiece that is used; changing the objective does not change the appearance of the graticule.

A consequence is that the graticule has to be calibrated for each objective. This is most readily done by viewing a slide that has rulings etched into its surface, and observing how many units on the graticule correspond to, for example, 1 mm on the slide. Such a slide is shown below.

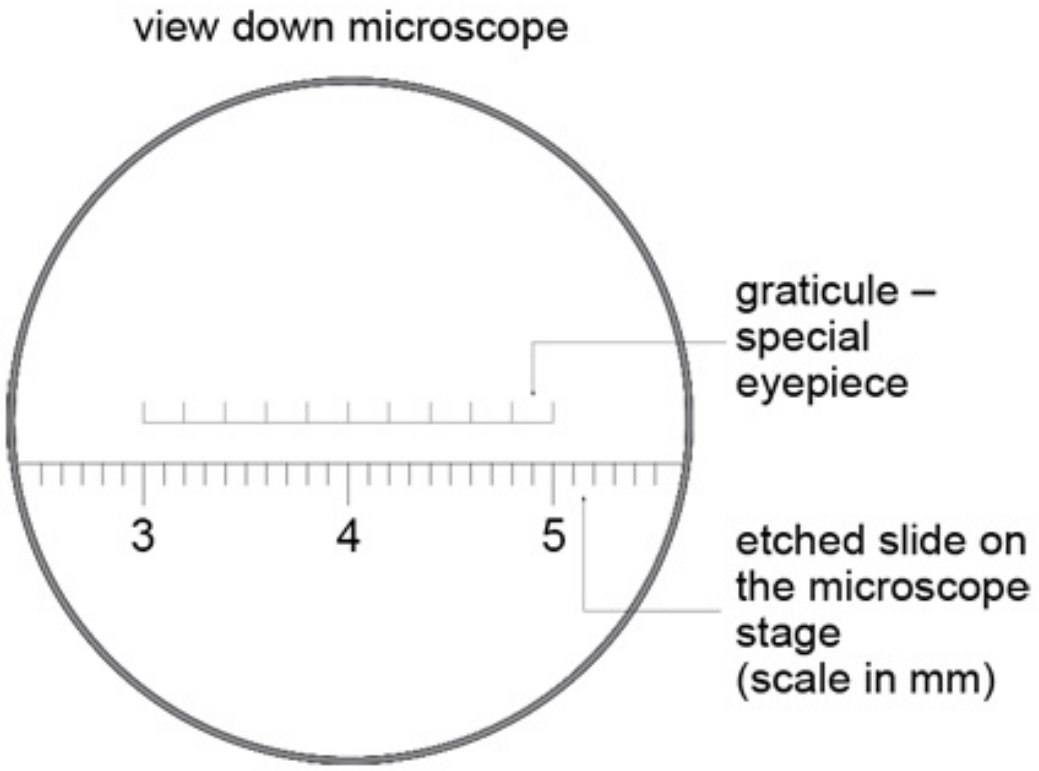


Figure 11 The appearance of an etched slide on a microscope stage when viewed with an eyepiece that includes a graticule.

The video above shows you how to calibrate an objective using a graticule and etched slide on the virtual microscope, as well as introducing a grid feature that you can apply. The grid can be used to measure the number of items within a particular area, for example, the density of blood vessels or the numbers of cells within a defined area of tissue. These are skills you will use in later parts of the course.

3.1 Observing different types of leukocyte

One of the most basic histological tests carried out is the differential white blood cell count in which the numbers of different types of leukocyte are counted. This process can help clinicians to diagnose diseases.

There are five main types of leukocyte, each with different functions.

1 *Neutrophils* are normally the predominant leukocyte in blood. They are involved in fighting infection by internalising (phagocytosing) foreign material, including many pathogens. Neutrophils may be increased in response to bacterial infection or inflammatory disease, although they may decrease in severe infection, or in response to cytotoxic drugs used for cancer therapy.

2 *Eosinophils* increase in response to some parasite infections and in allergies.

3 *Basophils* increase in some long-standing inflammatory conditions or in some hypersensitivity reactions.

4 *Lymphocytes* may increase in bacterial or viral infections. Decreased lymphocyte numbers are seen, for example, in the later stages of HIV infection.

5 *Monocytes* are scavengers that phagocytose pathogens and migrate into tissues to clear debris following infection, inflammation or cell damage. They may be increased in inflammatory conditions.

The cells differ in appearance when viewed in section under a microscope, as shown in the images below.

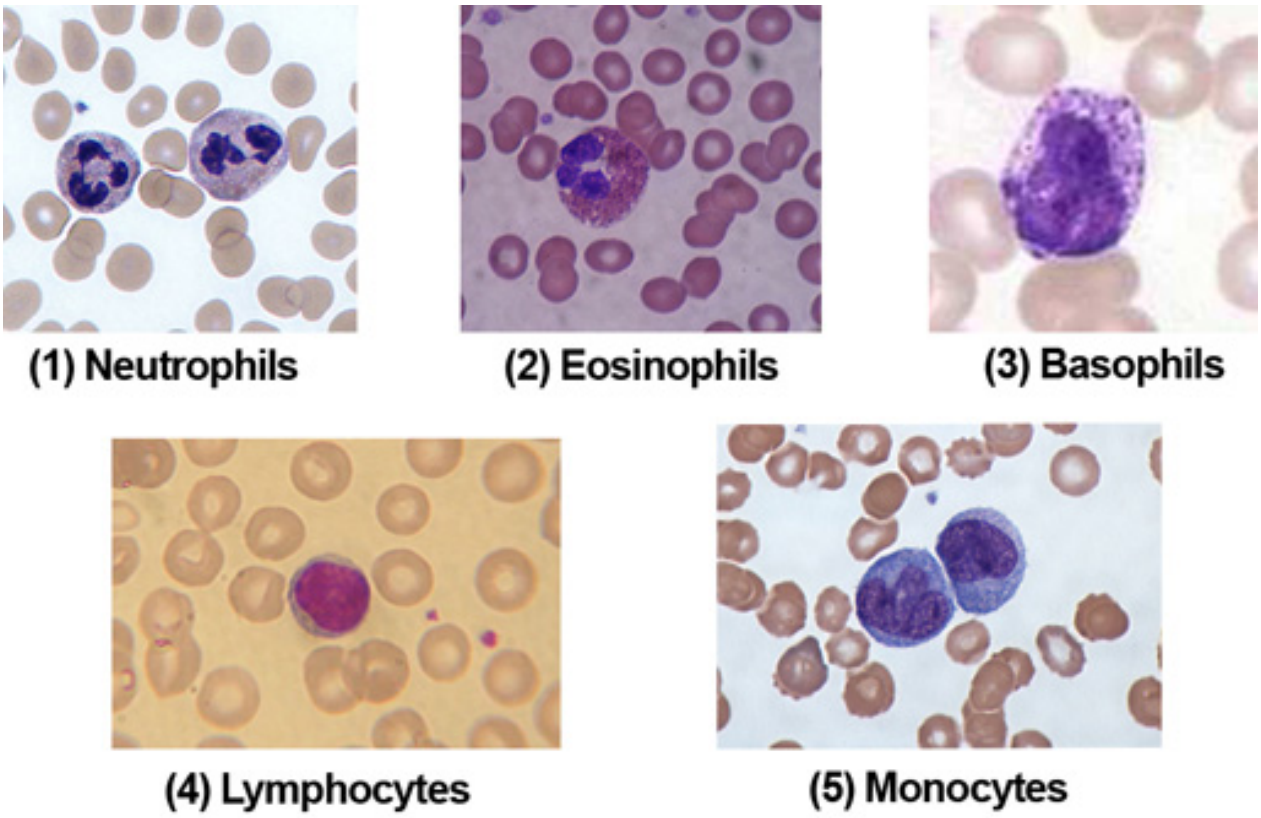


Figure 12 The appearance of the five main types of leukocyte.

The normal range for each of these cell types is quite variable, and is expressed as number of cells per litre of blood (see the table below). For example, if you counted 100 leukocytes, you would expect to find that 20–40 of them would be lymphocytes.

Table 1 Normal ranges for a differential blood leukocyte count

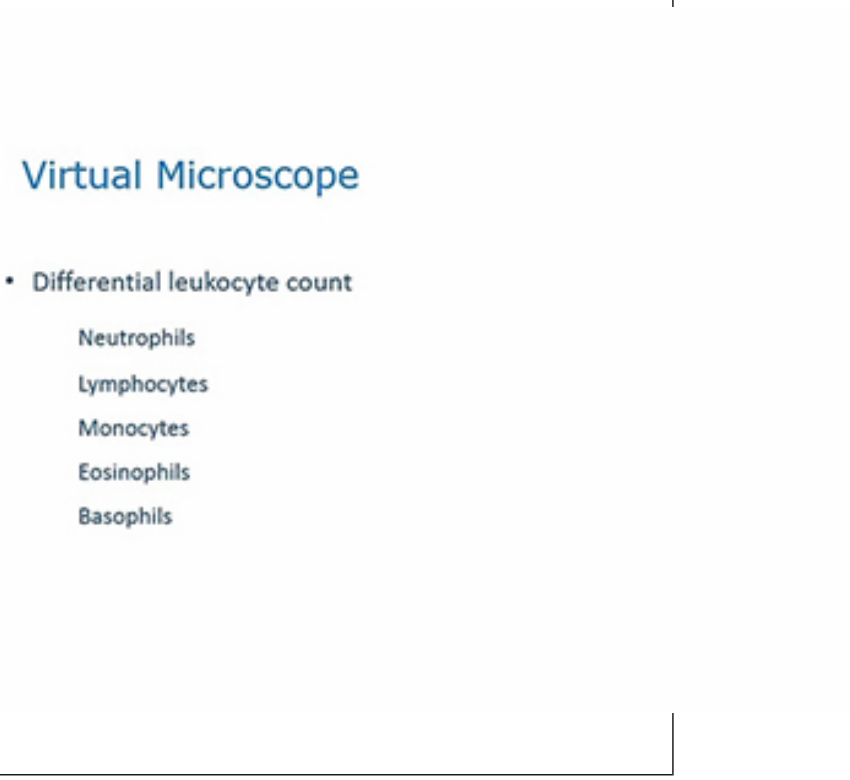
!Warning! Europa not supported**Leukocyte type**	!Warning! Europa not supported**Cells per litre**	!Warning! Europa not supported**Percentage**
!Warning! Europa not supportedNeutrophils	!Warning! Europa not supported2–7 x 10^{9}	!Warning! Europa not supported40–80%
!Warning! Europa not supportedEosinophils	!Warning! Europa not supported0.02–0.5 x 10^{9}	!Warning! Europa not supported1–6%
!Warning! Europa not supportedBasophils	!Warning! Europa not supported0.02–0.1 x 10^{9}	!Warning! Europa not supported<2%
!Warning! Europa not supportedLymphocytes	!Warning! Europa not supported1–3 x 10^{9}	!Warning! Europa not supported20–40%
!Warning! Europa not supportedMonocytes	!Warning! Europa not supported0.2–1.0 x 10^{9}	!Warning! Europa not supported2–10%

Values outside the normal range often indicate some kind of disease. High levels of any of them can indicate overproduction of that cell type, for instance, a leukaemia.

3.2 An example cell count

This video shows you how to use the virtual microscope to identify leukocytes in a blood smear and carry out a differential cell count.

Video content is not available in this format.



You will carry out a cell count of your own in the next activity.

3.3 Acute myeloid leukaemia

The blood smear on Slide 4 comes from a person who has acute myeloid leukaemia. Myeloid cells are the immature form of leukocytes and would not normally be found in the blood. Usually, they would develop in the bone marrow into neutrophils, basophils or eosinophils. However, in leukaemia there is overproduction and large numbers of myeloid cells are seen in the blood.

Activity 3

Open the virtual microscope in a new window or tab. Find Slide 4 in the ‘Week 1’ category.

Carry out a differential leukocyte count on the blood smear on Slide 4 from the ‘Week 1’ category within the virtual microscope. Aim to count at least 100 leukocytes.

3.4 Identify an abnormality – part I

In the next activity you will be using the virtual microscope again.

Activity 4

Open the virtual microscope in a new window or tab.

Carry out a differential leukocyte count on the blood smear shown on Slide 5 in the 'Week 1' category within the virtual microscope.

Consider these questions. You may find it helpful to refer to Figure 12 showing the appearance of the cells and Table 1 showing the relative abundance of different types of leukocyte.

- Is the differential blood count within the normal range?
- Do the lymphocytes appear normal?
- What is the diagnosis of this condition?

Provide your answer...

Discussion

The following conclusions can be drawn from analysis of Slide 5:

- The differential blood count is not within the normal range. There are very large numbers of lymphocytes in the smear.
- Additionally, the lymphocytes do not appear normal. The cells and their nuclei have an irregular shape.
- The diagnosis of this condition is a chronic lymphocytic leukaemia: the blood contains very high numbers of abnormal lymphocytes.

How did you get on? Did you manage to derive the same conclusions? Also, please don't worry if you were not able to derive all of these answers – it requires a certain amount of practice to count cells and draw conclusions from your observations. The next activity provides another opportunity for you to develop your skills in this area.

3.5 Identify an abnormality – part II

In the next activity you will be using the virtual microscope again.

Activity 5

Open the virtual microscope in a new window or tab.

Spend a few minutes comparing Slide 6 in the 'Week 1' category within the virtual microscope with the normal blood smear (Slide 1). Can you identify anything unusual in this smear?

Note down your thoughts.

Discussion

You may have noticed that some of the blood cells shown in Slide 6 look distinctly different from those found in the normal blood smear (Slide 1).

Specifically, many of the red blood cells in Slide 6 are elongated or have a sickle shape, compared to those in the normal blood smear. This is an example of sickle cell disease.

Sickle cell disease occurs as a result of a genetic variation in haemoglobin, which causes it to become less soluble. In people who have two genes for the condition (homozygous), the haemoglobin distorts the erythrocytes, producing the characteristic sickle cells, which are less able to flow through the blood capillaries.

4 This week's quiz

The following questions will help you understand and practise this week's material.

Complete the Week 1 quiz now.

Open the quiz in a new window or tab then come back here when you're done.

5 Summary of Week 1

Congratulations, you've now reached the end of Week 1.

You should have some insight into the role of histology in a hospital laboratory and in biomedical research. You should also be familiar with the capabilities of a simple light microscope, and be able to operate all the features of the virtual microscope.

You have been introduced to some of the processes involved in preparing material for microscopy, including fixation, sectioning and staining.

You have also been shown how to recognise different types of cells in blood and carry out a differential leukocyte count. Abnormalities in the appearance of the cells, or in their numbers, in the blood, can aid the diagnosis of genetic conditions, infection, inflammation and allergies. The differential cell count is a key guide in the diagnosis of different types of leukaemia.

In the next part of the course you will be introduced to a variety of different types of tissues, using the virtual microscope. The aim is for you to be able to recognise the normal appearance of each of these tissues, and be able to identify the cell types in them.

You can now go to Week 2.

Week 2: Tissues and cells

Introduction

Welcome to Week 2.

In the following video, David Male describes the subjects you will be covering this week, as you build on your ability to identify different types of cell.

Video content is not available in this format.

1 Identifying cell types within tissues

This is a short introduction to some of the types of cells that you will encounter when you start to examine tissues under the microscope.

Cells in the body differentiate into many types as they develop, and within a single organ there may be many different types of cell. For example, in the liver the major cell type is the hepatocyte, which carries out many metabolic functions, but there are also cells associated with blood vessels, bile ducts and immune defence, as well as various structural cells.

Despite the diversity of cells there are a number of patterns of cell organisation which occur in many different tissues. Some key examples are described in the text below.

Epithelial cells

Epithelial cells form tight cohesive sheets of cells which cover many of the body surfaces, such as the skin or the gut. They also form secretory glands, such as the breast, tear ducts, sweat glands and salivary glands. The ducts leading from the secretory cells in these tissues are lined with other types of epithelium.

A layer of epithelial cells may be just a single cell thick, or may be several cells deep. This difference affects their overall shape, which may be cuboidal, columnar or stratified (flattened). You can see some of this variation in the diagram below.

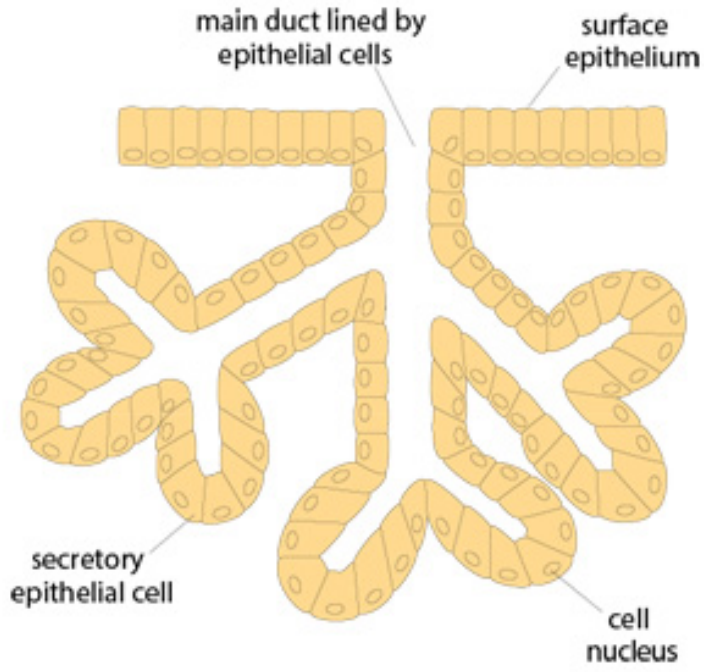


Figure 1 Arrangement of epithelial cells lining a secretory gland, its duct and the epithelial surface.

Endothelial cells

Another type of cell that is found in layers is the endothelial cell. These form a single layer that lines blood or lymphatic vessels.

Muscle cells

Muscle cells fall into three main types.

- Striated muscle fibres form the voluntary muscles of the body, i.e. the ones that you can choose to move.

- Smooth muscle is a contractile tissue found in many tissues which operates involuntarily. It is found in the gut and around blood vessels and many glands and ducts.
- Cardiac muscle is a specialised muscle found only in the heart.

Nerve cells (neurons)

Neurons have long processes (axons) which extend away from the nerve body. They form both the:

- central nervous system (CNS), namely the brain, spinal cord and retina of the eye
- the peripheral nervous system, which describes any nervous tissue outside the CNS.

Nerves run through virtually all tissues of the body, and there are large networks of nerves in tissues such as the gut.

Adipose tissue (adipocytes)

This type of cell stores fat, and is found in many tissues, not just in the main fat storage areas of the body. Adipocytes have a large central vacuole where lipids (fats) are stored.

Leukocytes (white blood cells)

As you read and observed in Week 1, leukocytes come in a number of different types. They are found in all tissues of the body (not just the blood), where they carry out the function of immune defence.

Lymphoid tissues, such as lymph nodes, tonsils, adenoids and Peyer's patches (a lymphoid tissue of the intestine), contain highly organised groupings of leukocytes that activate and coordinate the body's immune response to infection.

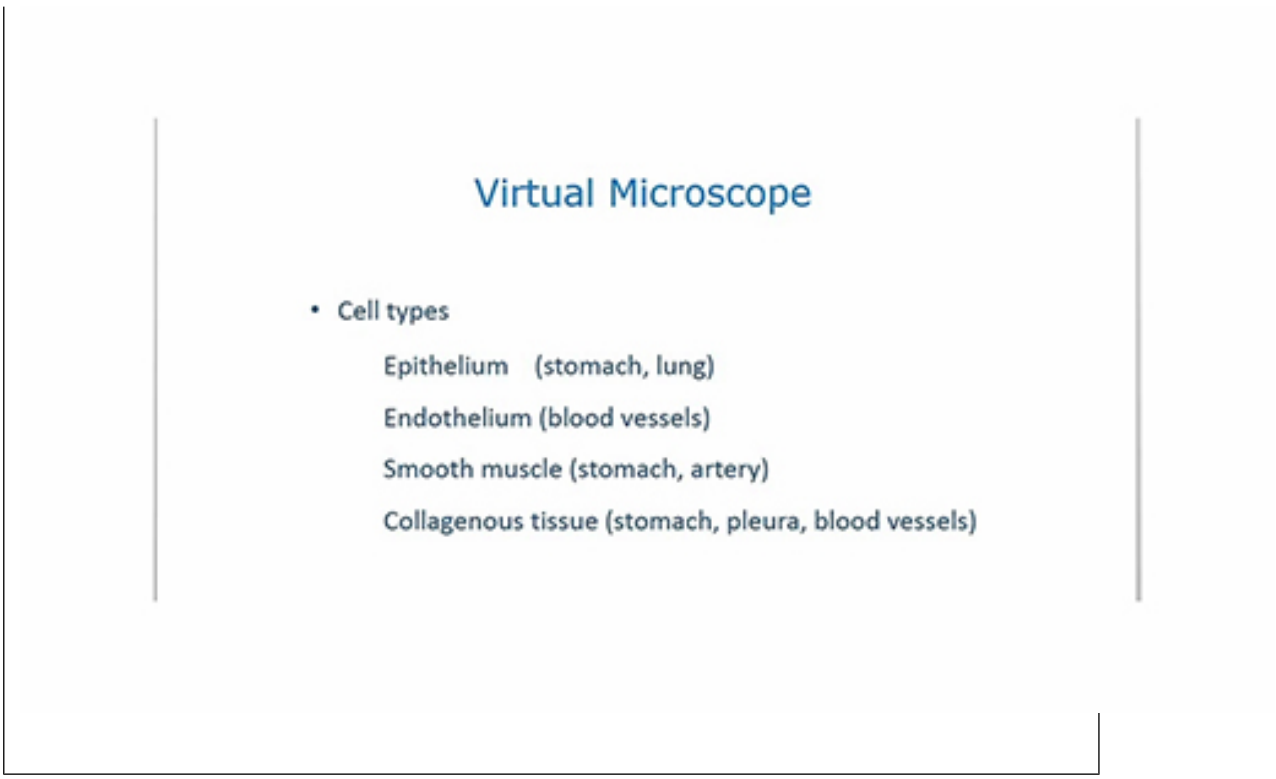
Extracellular matrix proteins

Bone, tendons and connective tissues are mostly formed of extracellular matrix proteins which are produced by specific cell types within the tissue. For example, collagen is a major extracellular matrix protein, and it is formed in several different types, depending on its function within the tissue.

1.1 Recognising common cell types

This video describes how to use the virtual microscope tool to recognise some of the different cell structures that you have just read about.

Video content is not available in this format.



You will explore some of these cell types in more detail as you progress through the rest of the course. However, what is important to recognise here is the fact that these distinct structures commonly recur in very different types of body tissue.

1.2 Histology of the skin

The skin is the first tissue we'll examine in more detail from a histological perspective.

The outermost layer of the skin, the epidermis, is formed by epithelial cells (see part (a) of the diagram below). In this case the epithelium is a multi-layered stratified structure, and its thickness depends on where on the body the skin is located.

Beneath the epidermis lies the dermis, which contains sweat glands, sebaceous glands that produce an oily secretion to lubricate the skin, and hair follicles. A number of cell structures are found in this layer of the skin, as illustrated in the figure. The nerves end in various sensory organs in the dermis, and they innervate the muscle which is attached to the hair.

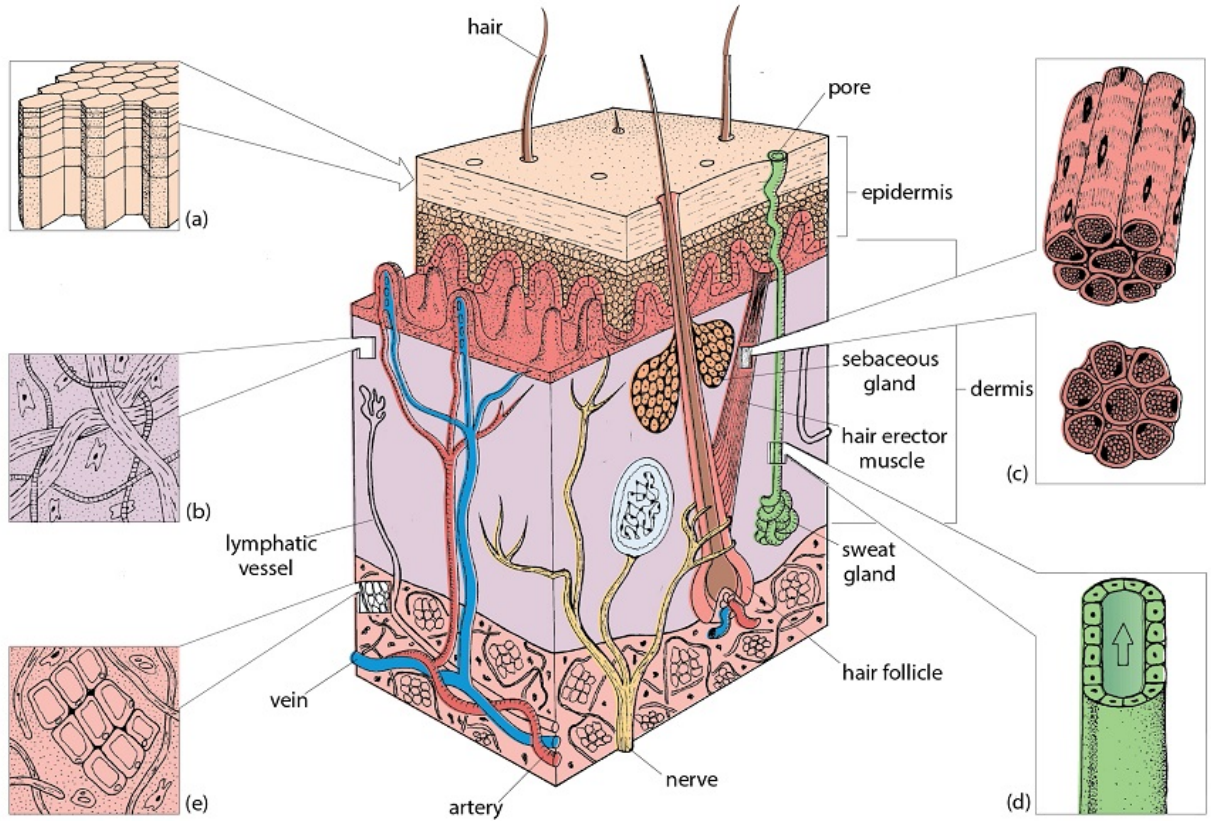


Figure 2 Structure of the skin showing the various types of cells and tissues: (a) epithelial cells in the epidermis; (b) connective tissue; (c) muscle; (d) epithelial cells lining the duct of a sweat gland; (e) adipose tissue below the dermis.

Take a few moments to familiarise yourself with the overall arrangement of the structures in the skin.

1.3 Skin sections

Next you'll examine structures in the skin in more detail via the virtual microscope.

Activity 1

Open the virtual microscope. (Remember, you may find it easier to open the virtual microscope in a new browser window or tab, to make it easier to refer back to these instructions.)

Load Slide 1 (from the 'Week 2' collection) onto the stage. This sample is a section through the skin of a fingertip. Spend a few moments looking at the slide, and making use of the legend provided.

You should have noticed that the epidermis is particularly thick and ridged towards the right-hand side of the sample. In the skin the epithelial cells are called keratinocytes because they form keratin, the thick impervious layer of the skin surface.

You may have also observed in this sample that the skin of the fingertips does not have hairs, but it does have sweat (eccrine) glands and a high density of sensory organs in the dermis. These sensory organs include structures such as the Pacinian corpuscles,

which specifically respond to touch and pressure, enabling us to feel the external environment.

Finally for this slide, spend a few moments trying to identify the connective tissue of the dermis and some blood vessels.

Now turn your attention to Slide 2 from the same collection. This is from pigmented skin.

Identify the cells in the epidermis which have high levels of the pigment melanin. Also, compare the thickness of the epidermis on this section with that on Slide 1. You should see that the contrast between their respective thicknesses is significant.

1.4 Histology of the gut

The next type of tissue that we'll examine more closely is that which forms the gut. Although the shape and dimensions of the gut vary along its length, the organisation of the different cell types that make up the gut wall is essentially similar throughout.

There are three main layers in the gut wall, as shown in Figure 1 below. Starting from the inside of the wall and working outwards these layers are the:

- mucosa
- submucosa
- muscularis externa.

Each of these layers, in turn, contains several cell types. Note that the adventitia (the name for the outer covering of any organ) does not form an integral part of the gut wall.

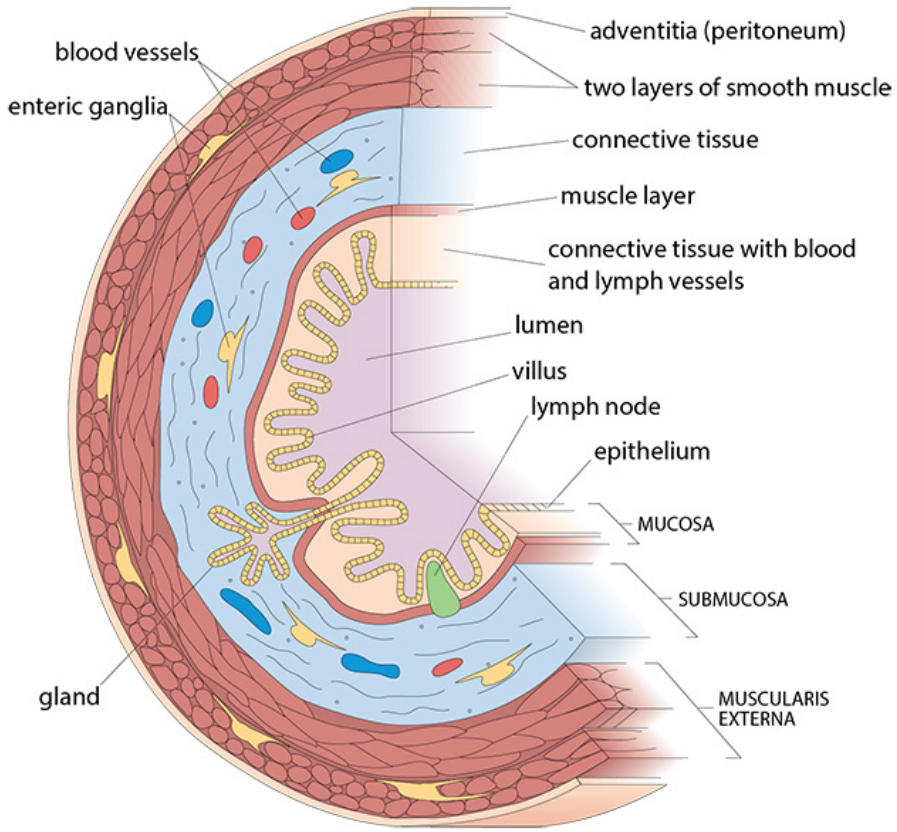


Figure 3 Cross-section of the gut showing the arrangement of the different tissue elements at the level of the ileum (small intestine).

Let's consider the composition of the three main layers of the gut wall in more detail.

Mucosa

In some areas such as the mouth, oesophagus and anus, where some physical protection is needed to prevent infection, the epithelium is several layers thick, and is something like that of the skin.

However, in most parts of the gut, the properties of the epithelium are rather different from those of the skin. Here the epithelium is only a single cell thick, with the apical membrane of these cells exposed to the lumen of the gut and the basal membrane facing the submucosal cells. All epithelial cells have two faces – the basal membrane lies on the connective tissue of the basal lamina and the apical membrane faces the surface of the tissue or duct.

These epithelial cells are specialised to perform digestive or absorptive roles. For example, the epithelial cells in the small intestine have small hair-like projections called microvilli on their apical surface.

Finally, in the stomach and intestines the epithelium is frequently invaginated (i.e. folded inwards) to form glands which consist of groups of secretory epithelial cells. Different types of gland are found in different regions of the gut.

Submucosa

The submucosa is a loose matrix of connective tissue in which blood and lymph vessels lie, and a large number of neuron cell bodies are grouped together as ganglia.

The enteric neurons (i.e. those associated with the gut) extend to the mucosa, where they influence both the production of digestive secretions by the epithelial cells and the state of dilation of the mucosal blood vessels.

Muscularis externa

The muscularis externa is a thick layer of smooth muscle. In almost all parts of the gut it consists of two separate muscle layers that lie at right angles to each other.

- The outer layer is longitudinal, that is it runs lengthways along the gut
- The inner muscle layer fibres encircle the wall of the tube.

Successive contraction and relaxation of these muscle layers results in peristalsis, which are waves of movement that propel the food through the gut.

Familiarise yourself with the overall anatomy of the gut before you move on to the next activity.

1.5 Epithelium in the gut

Look at some actual tissue samples for different parts of the gut.

Activity 2

Open the virtual microscope in a new window or tab.

Examine Slides 3–5 from the ‘Week 2’ category within the virtual microscope.

The slides depict sections taken from the stomach (fundus region), illeum and colon, respectively. Spend a few minutes looking at the samples and note the different types of epithelium lining the tissue.

In each case, aim to identify the typical structures that denote the three main layers of gut tissue:

- the mucosa
- the submucosa
- the smooth muscle of the muscularis externa.

1.6 How the virtual microscope images were produced

In this video, David Male discusses The Open University’s virtual microscope project.

Video content is not available in this format.

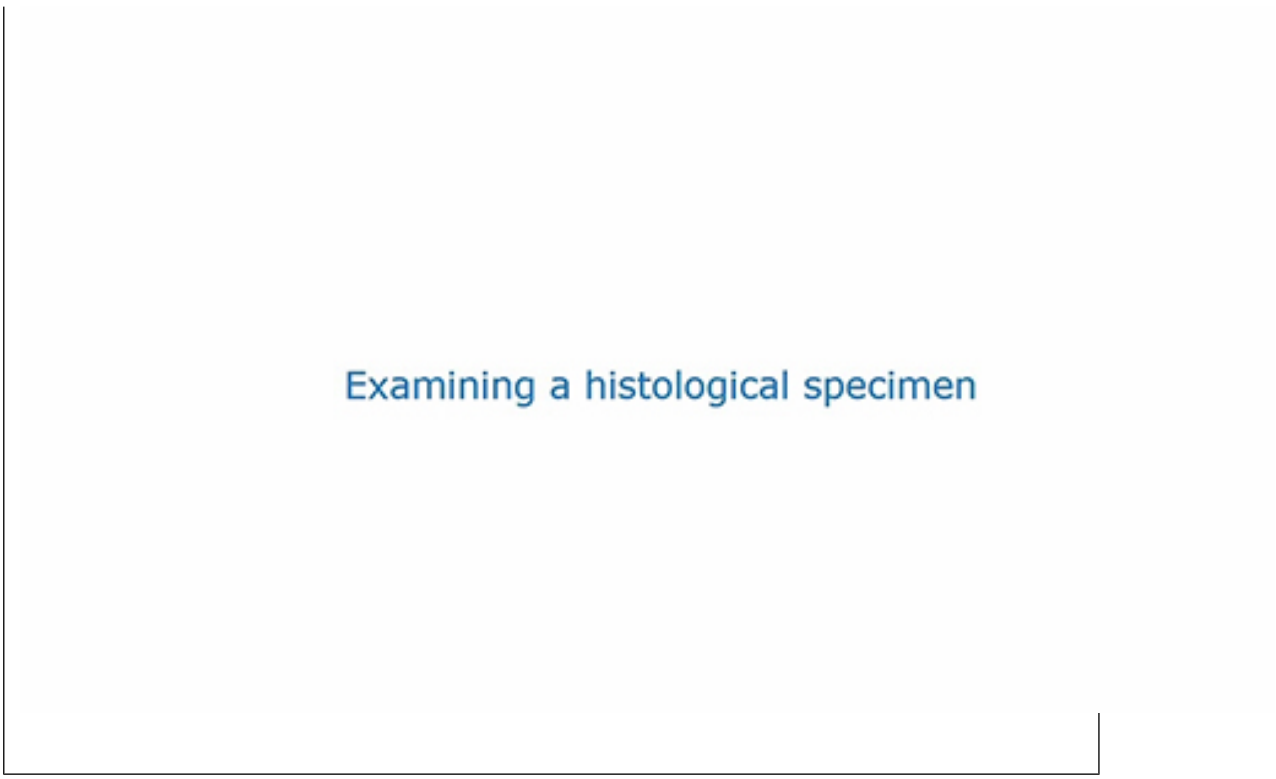
As the collection of slides available has been drawn from a number of laboratories, it gives you the opportunity to explore samples that you might not normally have access to.

2 Identifying tissues

Learn to identify different types of tissue using evidence provided by the virtual microscope.

In this video, David Male discusses how to use the virtual microscope to examine a histological specimen.

Video content is not available in this format.



David emphasises how important it is to make an overall scan of the sections before focusing on specific areas and selecting an objective that can identify the features of interest in those areas.

2.1 Introduction to different tissues

The rest of this week focuses on the task of identifying different types of tissue using evidence provided by the virtual microscope. Specifically, this involves working through Activity 3, looking at Slides 6–11 from the 'Week 2' collection.

Activity 3

Open the virtual microscope in a new browser window or tab and find the 'Week 2' collection. Slides 6–11 have been chosen to introduce a range of different tissues, so that you can recognise them again later on in the course. Use the instructions below, and the associated legends to navigate around the sections.

Lung (Slides 6 and 7)

Compare the normal lung (Slide 6) and the 'inflated' lung (Slide 7). You should be able to see that the alveoli are much larger on the inflated lung, which was injected to inflate it, before processing.

Both sections are from normal tissue. However, this pair of slides illustrates that the way in which a tissue is prepared can affect its appearance.

Blood vessels (Slide 8)

Arteries and veins often run together as pairs, although they appear quite different. The vein has a much thinner wall and, therefore, has collapsed and is less prominent than the artery in this slide.

The section also includes a number of smaller blood vessels. Arteries branch into arterioles and then into capillaries, the smallest blood vessels. Capillaries drain into venules, which feed into the veins.

Liver (Slide 9)

The liver has a complex organisation with an ‘open’ circulation. The circulation is described in this way because the blood within the organ is not confined within blood vessels.

Instead, the liver is supplied by the arterial blood (hepatic) artery and receives blood from the gut (hepatic portal vein). Blood flows through the liver along sinusoids, rather than capillaries.

Pancreas (Slide 10)

The pancreas contains two distinct types of tissue.

- The exocrine pancreas produces digestive enzymes.
- The Islets of Langerhans produce a number of hormones, including insulin, which control aspects of metabolism.

Notice that the Islets can most easily be identified using the lowest magnification objective.

Thymus (Slide 11)

The thymus is a lymphoid organ, in which a type of leukocyte known as T lymphocytes (or ‘T cells’) develop. (You have already encountered lymphocytes in the blood smears.) After their initial development in the thymus, T cells distribute to other lymphoid organs, including lymph nodes, the spleen and Peyer’s patches. The tissues where lymphocytes initially develop, thymus and bone marrow, are referred to as primary lymphoid organs, and the other organs, such as lymph nodes, are secondary lymphoid organs. T cells are involved in the recognition of infected cells and they also control and coordinate many other elements of immune responses.

T cell development occurs during the early years of life, and the thymus becomes gradually smaller with age. Notice the distinct areas within the tissue. T cell development starts in the cortex (outer layer) and the cells mature as they progress to the medulla (centre) of the organ.

2.2 Structure of a lymph node

Lymph nodes are encapsulated organs that are strategically placed along the lymphatic network. Here they can trap foreign material (antigens), which are presented to the lymphocytes by antigen-presenting cells, to initiate an immune response.

The lymphocytes are densely packed in the lymph node, and the tissue is organised both to facilitate the interactions needed to generate an immune response against the antigen and to promote rapid division of the responding lymphocytes.

Lymph nodes vary in size (from a few millimetres to 1–2 cm), and are distributed in different areas of the body. They are linked in chains by lymphatic ducts, so that fluid flowing out of one lymph node via the efferent lymphatic vessel becomes the inflow to the next in line, via the afferent lymphatics.

The fluid in question is called lymph, and is derived from the tissue that carries cells and foreign material to the lymph nodes. Eventually, lymph returns to the bloodstream via one of the body's two major lymphatic ducts.

Secondary lymphoid organs can be thought of as guard posts that are strategically placed to intercept any infectious agent that enters an area of the body. So, for example, the lymph nodes in the axilla of the arm (the armpit) will intercept infections which enter that part of the body.

Lymphocytes located in the local lymph nodes are responsible for the initial recognition of the infection and the development of the immune response. Once the immune response has developed, the cells will migrate out from the lymph node to the blood and, ultimately, cells will move to the site of infection to combat the pathogen there.

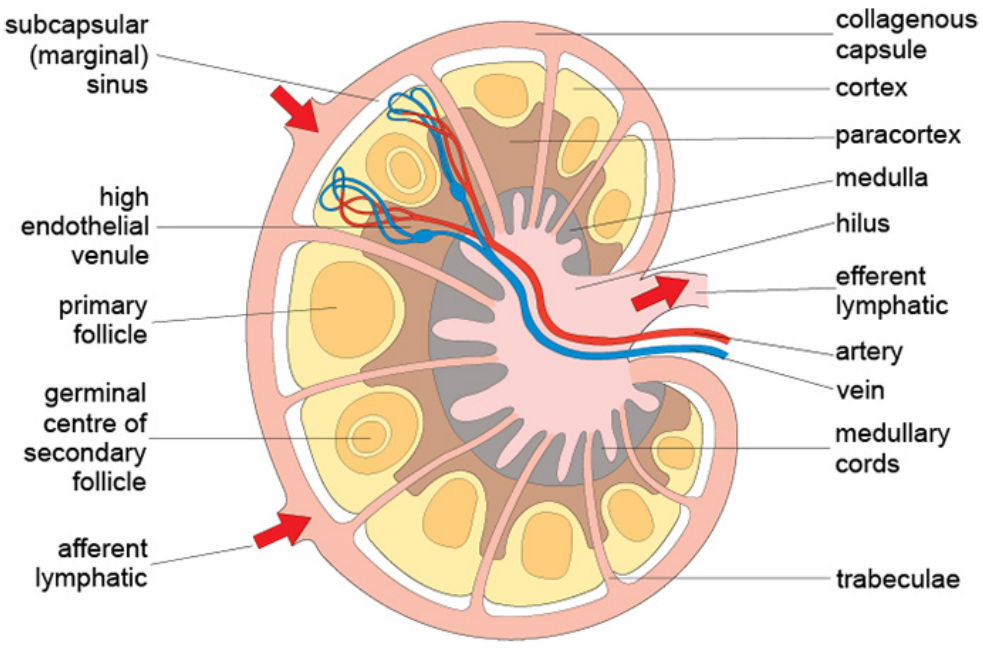


Figure 4 Structure of a lymph node.

Lymph nodes have a well defined structure with different sub-regions. Different types of leukocyte are localised within the regions so that they can interact with each other appropriately to initiate and develop the immune response. Antigens and cells enter the

node through afferent lymphatics, and cells and fluid leave through the efferent lymphatic. Cells (lymphocytes) can also enter the node from the blood by migrating across the specialised high endothelial venules. Within the node, cells distribute themselves to distinct zones. B cells proliferate and develop within the follicles of the cortex, while T cells are primarily located in the paracortex. The capsule, medullary cords and hilus are fixed structural elements of the tissue.

Activity 4

Open the virtual microscope in a new browser window or tab.

Look at Slide 12 in the 'Week 2' category within the virtual microscope. This slide is a section from a lymph node that you learned about above.

Now spend a few moments exploring Slide 12, and try to identify some of the structures described. (Don't forget to use the slide's legend to help guide your navigation of the sample.)

2.3 Immunohistochemistry

As you read in Week 1, the staining of cells and sections with antibodies has revolutionised histology as it allows the identification and localisation of individual molecules.

A molecule that binds to, and is recognised by, an antibody, is called an antigen, and, in the context of histology, such antigens are often referred to as markers, since they act as ways of recognising a particular cell. Many markers are designated by a CD number. Indeed, more than 250 markers have been assigned CD designations.

As you read previously, immunohistochemistry (IHC) is particularly valuable for distinguishing different cell types in the diagnosis of cancer. For example, different classes of lymphocyte appear virtually identical in size and shape (morphology), but they can be distinguished according to their surface markers. All T lymphocytes express CD3, and the two major subpopulations of helper T cells and cytotoxic T cells express CD4 and CD8, respectively. Therefore, a T cell lymphoma can be tracked in different tissues using these markers.

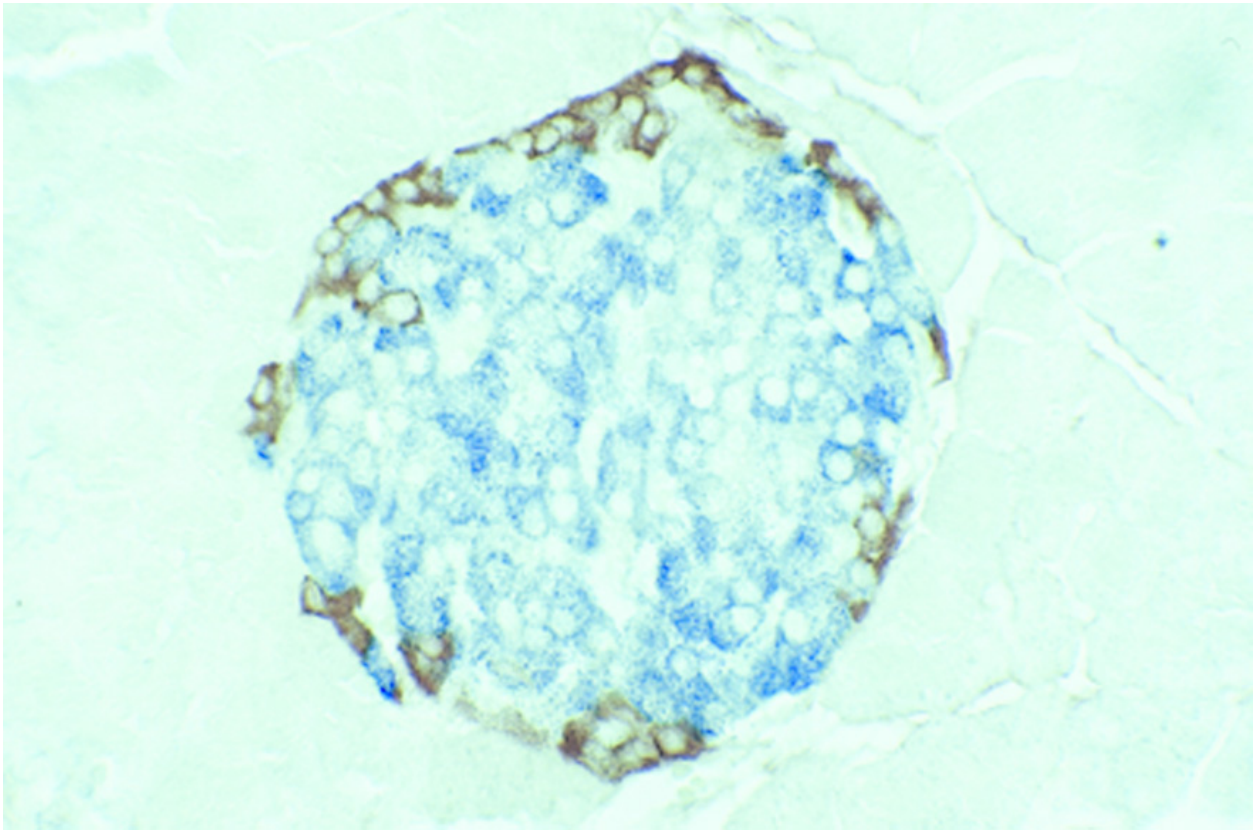


Figure 5 Immunohistochemistry. Double immunolabelling of an Islet of Langerhans in the pancreas identifies insulin-producing cells (blue) and glucagon-producing cells (brown).

Another use for IHC is in guiding treatment. For example, many breast cancers require oestrogen to divide, but a drug called tamoxifen can bind to the oestrogen receptor on the cancerous cells, blocking this proliferative effect. However, this only applies to some cancers, as others do not have an oestrogen receptor and are therefore not susceptible to tamoxifen therapy.

A researcher or clinician can use IHC to identify whether a breast cancer removed by surgery expresses the oestrogen receptor. The clinician wants to treat the patient so as to prevent any secondary tumours from growing and with this information they can decide whether or not tamoxifen therapy is appropriate.

2.4 Immunohistochemistry technique

A standard IHC protocol involves treatment of the section with an antibody that recognises the marker. This is referred to as the primary antibody.

The primary antibody is then recognised by a secondary antibody that is linked to an enzyme, or several copies of the enzymes. Such reagents are sometimes called conjugates, although this term can mean different things in other contexts.

Finally, the section is treated with a chromogen, a reagent that is acted upon by the enzyme, to deposit an insoluble coloured compound onto the cell, where the original primary antibody had bound.

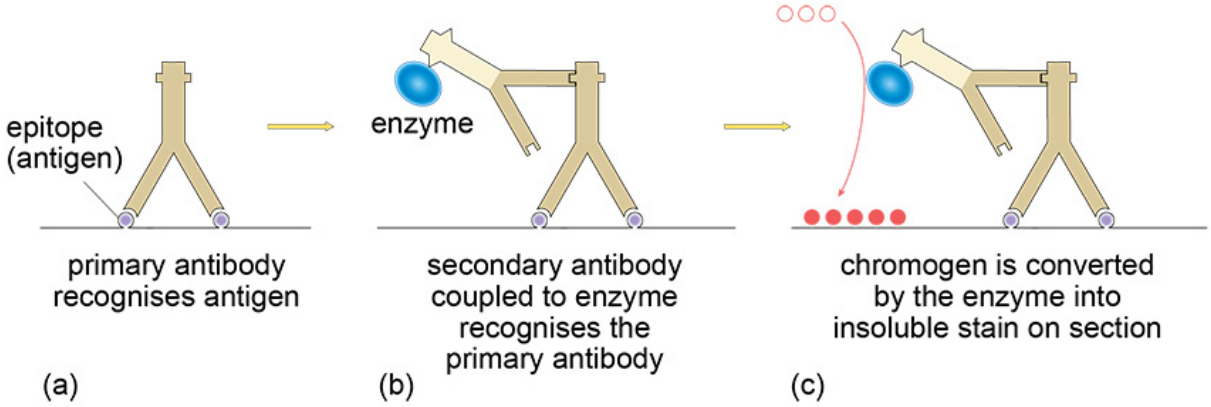


Figure 6 Principles of immunohistochemistry. (a) Unlabelled primary antibodies bind to the antigen on the section. (b) Labelled secondary antibodies bind to the primary antibodies. The secondary antibodies have an enzyme attached. (c) Chromogen is converted by the enzyme into an insoluble stain on the section.

2.5 Using IHC to identify B cells and T cells

T cells and B cells are the two major subtypes of lymphocyte. B cells may ultimately differentiate into antibody-producing cells, while T cells perform many functions in immune defence.

Within lymph nodes B cells tend to distribute to the follicles, and T cells to areas of the paracortex. The distribution can be readily seen by IHC staining of the cells with appropriate antibodies against their cell surface markers: CD3 for T cells or CD20 for B cells.

Activity 6

Open the virtual microscope in a new window or tab.

Look at Slides 13 and 14 from the ‘Week 2’ category.

Spend a few moments looking at where the T cells and B cells are distributed. Then compare these lymph node sections with the H&E stained section in Slide 12.

3 Putting it into practice

The final activity this week you will investigate three sample sections.

This is an opportunity for you to put into practice what you’ve learned this week about identifying types of tissue and will increase your familiarity with the virtual microscope.

Activity 6

Open the virtual microscope in a new window or tab. Find Slides 15–17 from the 'Week 2' slide set.

For each slide, answer the questions and then click to reveal the answer.

Slide 15

- What is the tissue in Slide 15?
- Does the tissue in Slide 15 appear normal?

Provide your answer...

Answer

This tissue is from a normal lung, and it was seen earlier this week. Notice the open structure of the alveoli where gas exchange takes place, which is a characteristic of a normal lung.

This section was included to see how well you can recognise a tissue from its histological appearance. Remember that even in a histopathology laboratory it is just as important to correctly identify normal tissues as it is to identify diseased tissue.

Slide 16

- What is the tissue in Slide 16?
- Does the tissue in Slide 16 appear normal?

Provide your answer...

Answer

This section of skin includes an area of wound healing. The area of a skin wound contains so-called granulation tissue (8615, 861), consisting of newly-formed capillaries (8615, 1257), leukocytes (8939, 1000) and proliferating fibroblasts (10466, 826). The collagen (11114, 1293) in the dermis is still rather disorganised.

This section was included to see whether you could spot these changes in the skin, even if you could not work out what had caused them. Now that you know what these changes are, navigate to the coordinates above in the Virtual Microscope to see them for yourself.

Slide 17

- What is the tissue in Slide 17?
- Does the tissue in Slide 17 appear normal?

Provide your answer...

Answer

This section has come from a person with cirrhosis of the liver. Cirrhosis of the liver is the result of chronic liver damage, which is caused by, for example, alcohol abuse.

The section shows broad bands of fibrosis (7450, 3650), which are areas of connective tissue with extracellular matrix proteins and fibroblasts. These broad bands of fibrosis

are separating the lobules (5385, 3650), in which the hepatocytes show fatty change (5550, 3360).

Nodules (8226, 4081) of regenerating liver cells may be present, but the fibrosis disrupts the normal blood flow through the liver from the portal vein to the hepatic vein.

4 Summary of Week 2

Having reached the end of the second week of the course you should now be able to identify a number of tissue types, including skin, lung, gut, liver, pancreas and lymph node.

In the process you have learned about tissue organisation, and should be able to recognise some of the cell types found within the organs mentioned above.

Finally, from a practical perspective, you have learned how to scan across a slide systematically, and you should now be starting to look for abnormalities in tissue structure based on your understanding of what healthy tissue looks like.

Next week you will be examining some more tissues and relating their structure to their functions.

Please note that there is no quiz this week.

You can now go to Week 3.

Week 3: Tissue structure and function

Introduction

Welcome to Week 3 of the course.

This week you will look at a selection of tissues that have different functions. You will continue your work with the virtual microscope tool to explore how the structure and function of these tissues are related.

As David Male explains, the relationship between tissue function and structure is a key consideration for histologists and other medical professionals, because understanding this association can support the diagnosis of disease.

Video content is not available in this format.

1 Functions of tissues

The structure of each tissue of the body is organised to carry out its own specific functions, and this is reflected in the arrangement of the cells and its histological appearance.

The week focuses on five functions of tissues, namely:

- secretion
- movement
- strength
- excretion
- communication.

As you should already be aware, different tissues are capable of different functions. However, these functions may be interrelated. For example, the cells of the endocrine system secrete hormones, which are involved in communication between different tissues.

Secretion is an example of a function that is particularly specialised. Many cell types release molecules into the extracellular environment, but secretion is a specific function carried out by epithelial cells. Some examples are given in Table 1.

Table 1 Tissues and secretion

Tissue	Secretion
Thyroid	thyroid hormones
Breast	milk
Salivary gland	saliva
Tear ducts	tears
Exocrine pancreas	digestive enzymes
Islets of Langerhans	insulin, glucagon
Stomach epithelium	acid, intrinsic factor

Cells that carry out secretion generally store the secreted proteins either inside the cell, in secretory vesicles, or in extracellular depots (e.g. the thyroid gland). The secreted material is released when the cell or tissue receives an appropriate signal, as seen in the Figure 1 below.

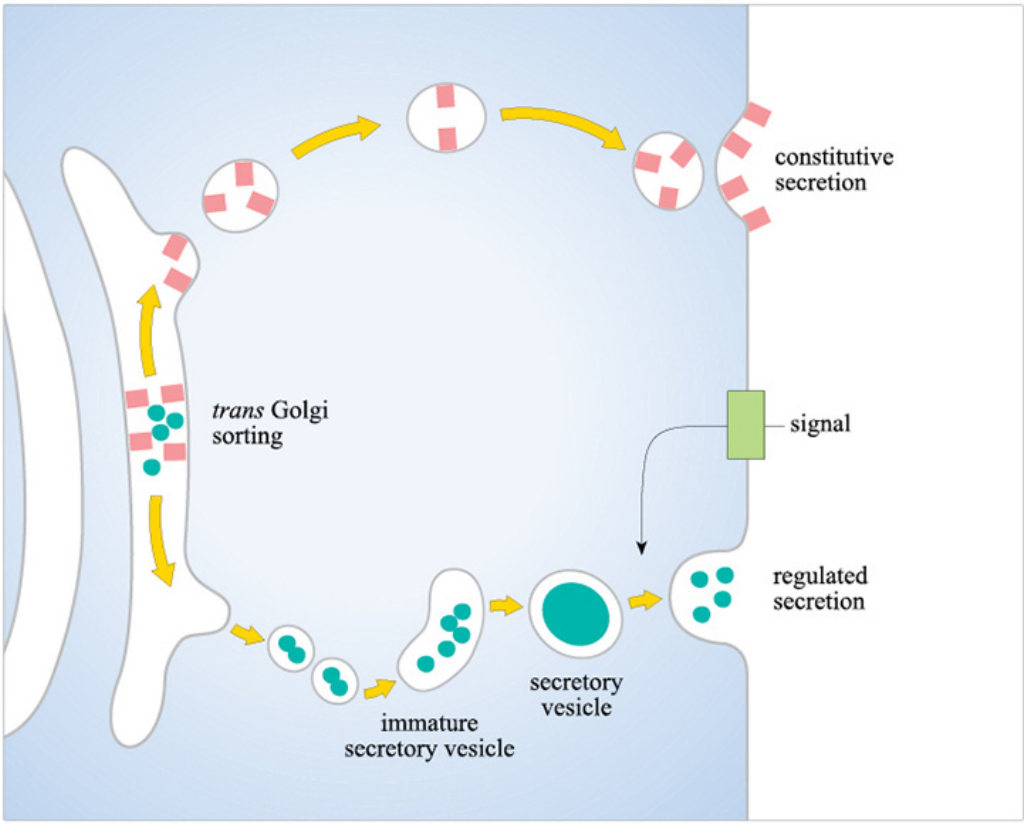


Figure 1 Secretion of proteins produced in the Golgi apparatus. Secretion may either be constitutive (continuous) or the secreted molecules may be stored in vesicles and then released when a signal is received.

Tissues respond to a variety of stimuli. For example, the thyroid responds to thyroid-stimulating hormone (TSH) released by the pituitary gland, which promotes the division of thyroid epithelial cells and the release of thyroid hormones.

It is important when viewing a tissue to consider how it may have adapted to physiological changes or signals from other cells. For example, what factors can you think of that might cause the expansion (hypertrophy) of muscle cells in the heart (cardiac myocytes)?

There are a number of factors you could identify to explain this observation, including: a programme of heavy exercise or training; high blood pressure, requiring greater effort from the heart to pump blood; and heart valve regurgitation, causing inefficient pumping of blood into the circulation. Understanding the likely cause of things that you observe in tissue sections is key, as it allows decisions to be made as to what medical intervention (if any) is required.

1.1 Structure–function relationships

The cells within tissues are generally arranged to carry out the function of the organ most effectively. Consider the lung as an example.

The lung's primary function is gas exchange. Red blood cells carry oxygen from the lungs to the other tissues and return carbon dioxide to the lungs. The physiology of the blood transport systems need not concern us here, but the relationship of the cells in the lung is optimised for gas exchange as shown in the diagram.

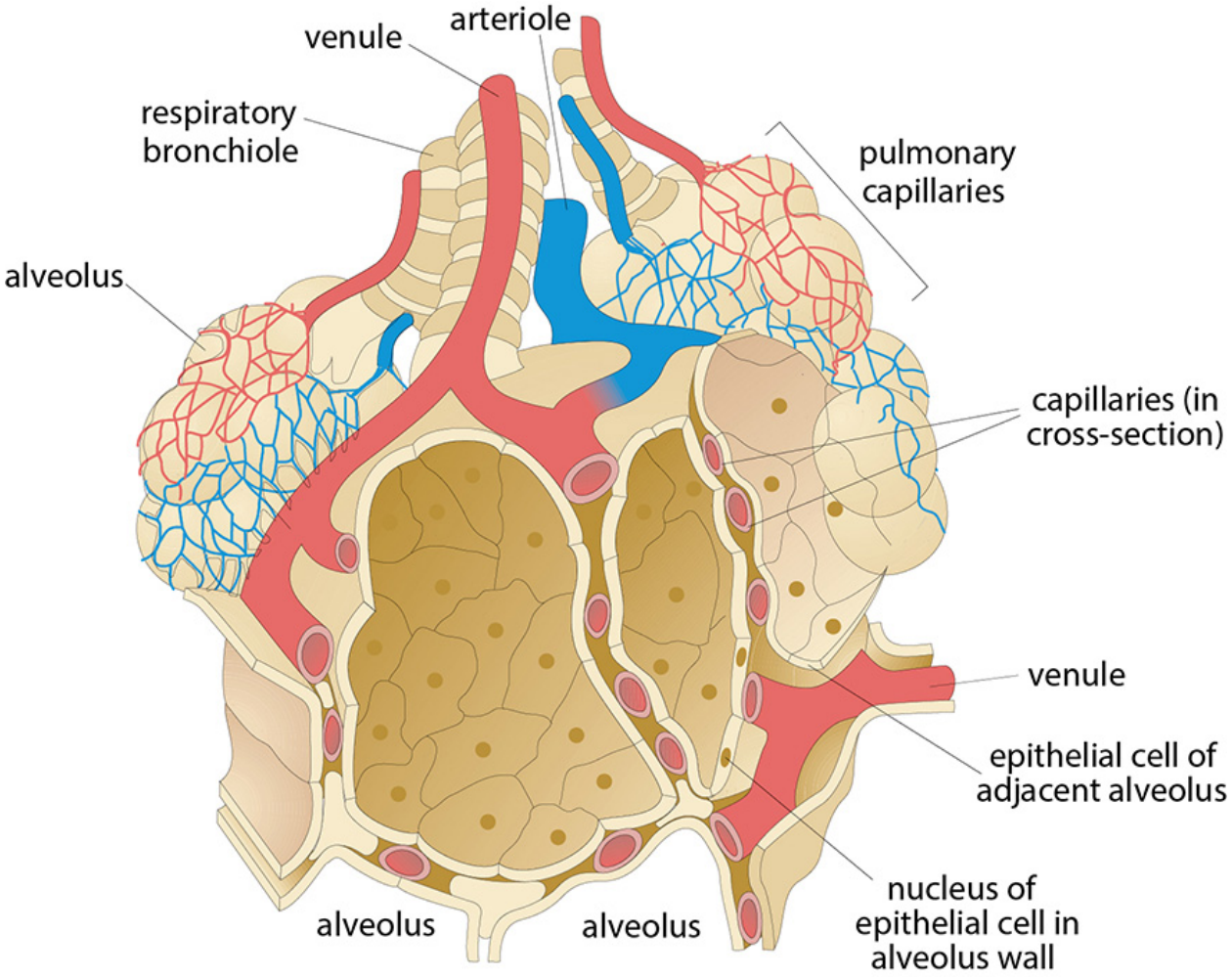


Figure 2 Schematic diagram of an alveolus, in contact with pulmonary capillaries (Villee, 1989).

Another tissue that you have already seen is the gut. (Recall that the appearance of the gut varies greatly along its length, although the overall arrangement of the layers of cells is basically the same throughout.)

The main site for absorption of nutrients is in the intestine, which has a very large surface area, with numerous villi. The villi have a rich blood supply and lymphatic drainage; therefore, this region of the gut is particularly well suited for their function.

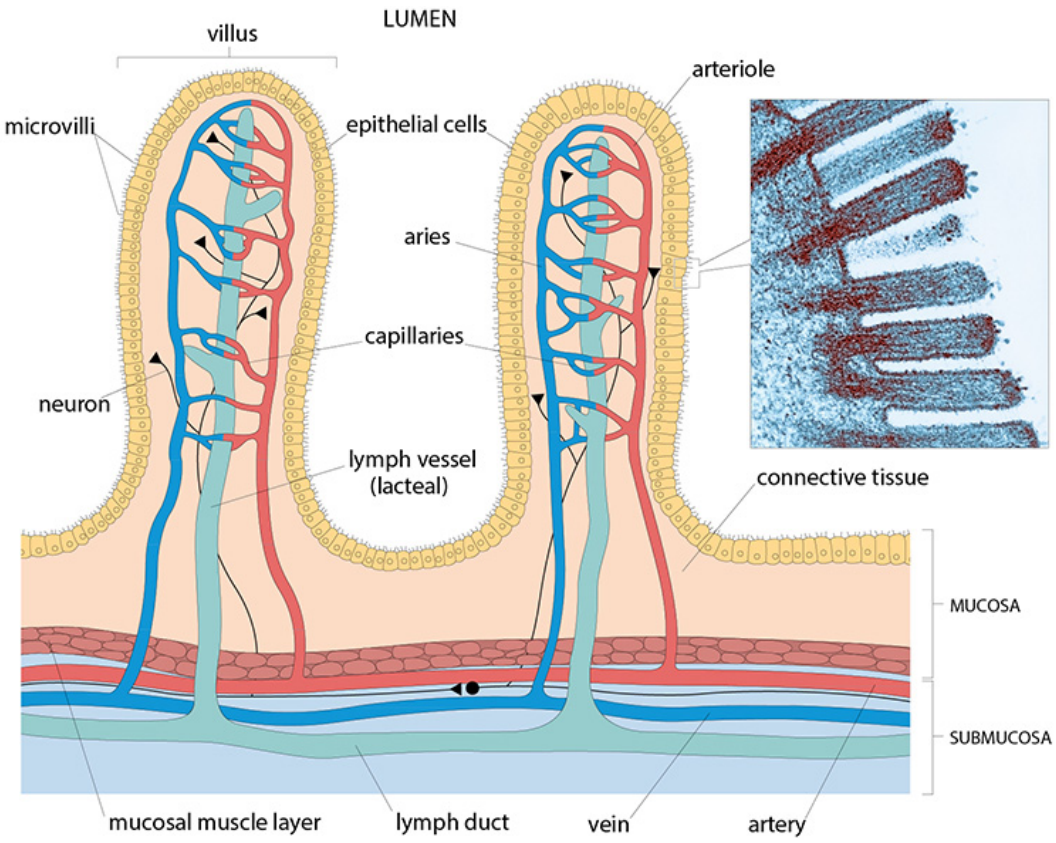


Figure 3 Schematic diagram of villi in the small intestine.

The rest of this week guides you through studying the structure–function relationships of a range of tissues, as grouped by the main functionalities we identified earlier. You will start by looking at secretion, before progressing on to movement, strength, excretion and communication.

As you learn to recognise each tissue, try to relate the histological appearance to the 3D structure of the organ and its underlying function.

2 Tissue structure and functions

In the next sections you'll learn about specialist tissues for secretion, movement, strength, excretion and communication and examine them under the virtual microscope.

Secretion: Functions of the thyroid

The thyroid is a two-lobed endocrine (hormone-secreting) gland located in the neck, as can be seen in Figure 4 below.

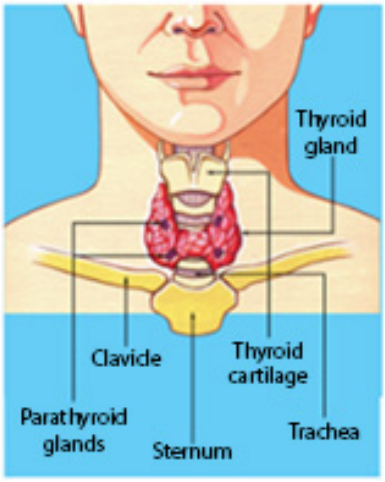


Figure 4 Location of the thyroid gland (adapted from BTF, 2016).

The gland produces two related hormones: thyroxine (T4) and tri-iodothyronine (T3), which are involved in the control of metabolic rate. T4 contains four molecules of iodine and T3 contains three molecules of iodine. Deficiency of iodine leads to proliferation of the cells in the thyroid, in response to the unmet demand for the hormones.

Histologically, normal thyroid tissue consists of many follicles lined with a single layer of epithelial cells called thyrocytes. These cells synthesise a protein (thyroglobulin), which acts as a precursor and storage site for the thyroid hormones. The thyroglobulin is secreted and stored inside the thyroid follicles.

When stimulated by thyroid-stimulating hormone (TSH) from the pituitary, thyroglobulin is internalised (phagocytosed) by the thyrocytes and broken down to release the hormones, which are then secreted into the blood.

Thyroid histology is altered in conditions such as Graves' disease (as a result of an overactive thyroid) and in Hashimoto's thyroiditis, an autoimmune disease, where the body's own immune system attacks and destroys the thyroid epithelium.

Activity 1

Having read a little about the structure and function of the thyroid you will now examine a sample of tissue using the virtual microscope.

Open the virtual microscope in a new window or tab. Find Slide 1 in the 'Week 3' category. Slide 1 shows a thyroid lobule containing about 50 follicles lined with thyroid epithelium, which produce the hormones thyroxine (T4) and tri-iodothyronine (T3).

Spend a few moments familiarising yourself with the structure of this sample, as guided by the slide's interactive legend.

2.1 Secretion: functions of the breast

The breast is another example of a secretory tissue.

Structurally, the breast consists of a number of lobes connected by lactiferous (milk-carrying) ducts to the nipple. Each lobe is a compound gland branching into lobules and

alveoli lined with epithelial cells, which are supported in a loose matrix of connective tissue. The connective tissue includes accumulations of adipocytes (fat cells).

During pregnancy the duct system and alveoli expand considerably as the epithelial cells lining the alveoli are responsible for milk secretion. At this time the connective tissue is correspondingly reduced. The alveoli are surrounded by contractile cells (myocytes), which put pressure onto the alveoli to promote milk secretion.

After weaning, when breast milk is no longer required, the alveoli regress, leaving just the duct system in place.

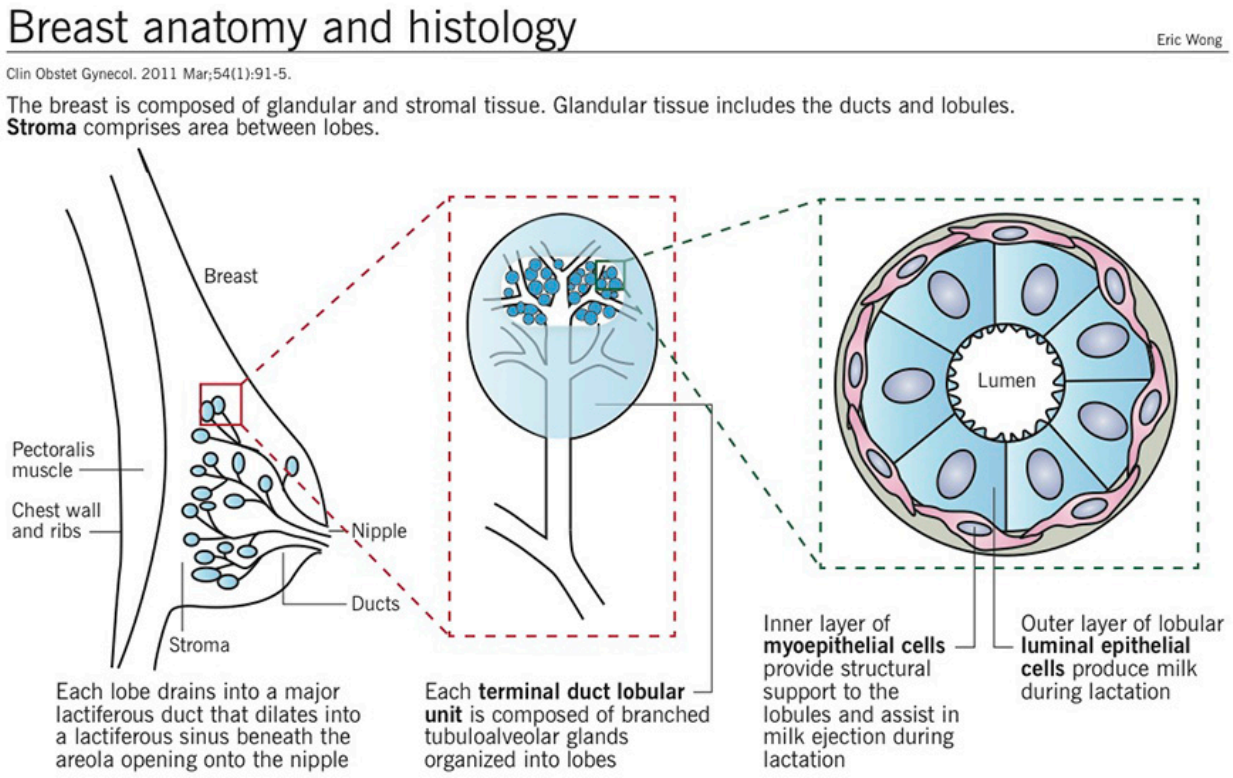


Figure 5 A diagram showing breast anatomy and histology.

Activity 2

Open the virtual microscope in a new window or tab. Find Slides 2 and 3 in the 'Week 3' category.

Spend a few minutes comparing the histology of a normal breast (Slide 2) and a lactating breast (Slide 3). Notice the expansion of the alveoli and the secretory epithelium in the lactating breast. These sections demonstrate how a tissue can vary greatly in its histological appearance as a result of normal physiological changes.

2.2 Movement: functions of muscle

Focus now shifts to the function of movement, and the muscle tissue that facilitates it.

All muscles are contractile and excitable and they contain the proteins myosin and actin, which are responsible for contraction. However, the functions of the muscle types and the

arrangement of cells within them vary. As you read earlier in the course, from a histological perspective there are three different types of muscle:

- striated (or skeletal) muscle
- smooth muscle
- cardiac muscle.

Skeletal muscles are the voluntary muscles, attached to bones via tendons, which allow voluntary movement of the body. Muscle tissue is made from a collection of highly specialised cells, known as muscle fibres. These are formed by the fusion of individual muscle cells (myocytes).

The muscle tissue is surrounded by supporting and protecting bands of fibrous connective tissue called fascia. The plentiful nerve and blood supplies which an active muscle requires are located within fascia.

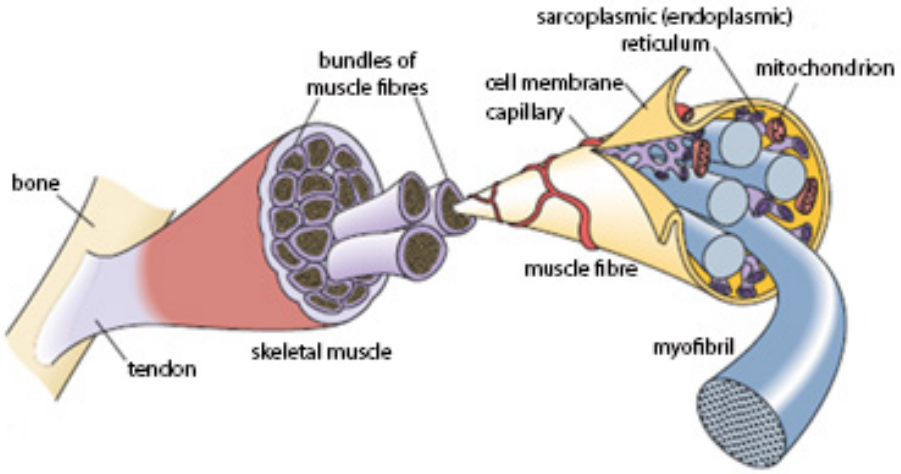


Figure 6 The internal structure of a skeletal muscle fibre (Gottfried, 1994).

Within a myofibril, actin and myosin are interleaved, producing the characteristic striated (striped) appearance, which can be seen under the microscope and is shown schematically in the figure below. When a muscle contracts the actin and myosin slide across each other, causing the striations to bunch up.

Energy is required to relax the muscle (surprisingly the default condition is contraction). When a muscle runs out of energy it contracts, as occurs in cramp and rigor mortis.

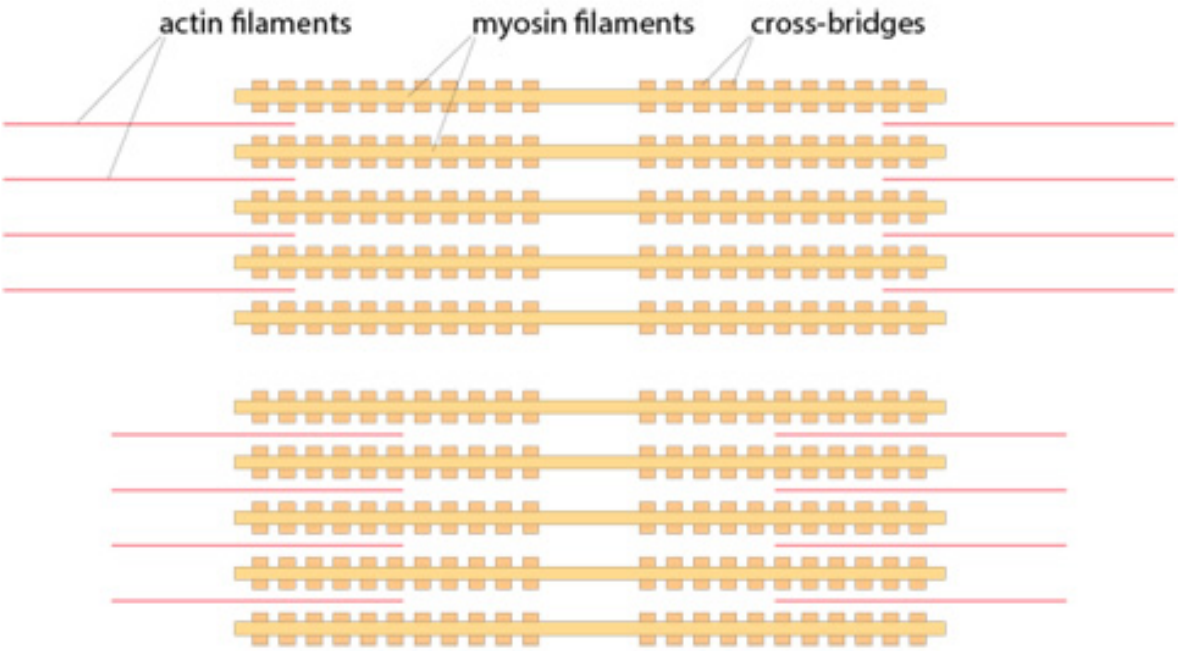


Figure 7 Arrangement of actin and myosin in a myofibre produce striations. The upper diagram shows the relaxed state and the lower diagram the contracted state.

In contrast to skeletal muscle, both cardiac and smooth muscle are involuntary muscles, meaning that we cannot voluntarily control their contraction. In cardiac muscle the myocytes form a network, with crosslinks and intercalated discs between the cells. They have striations comparable to those in skeletal muscle.

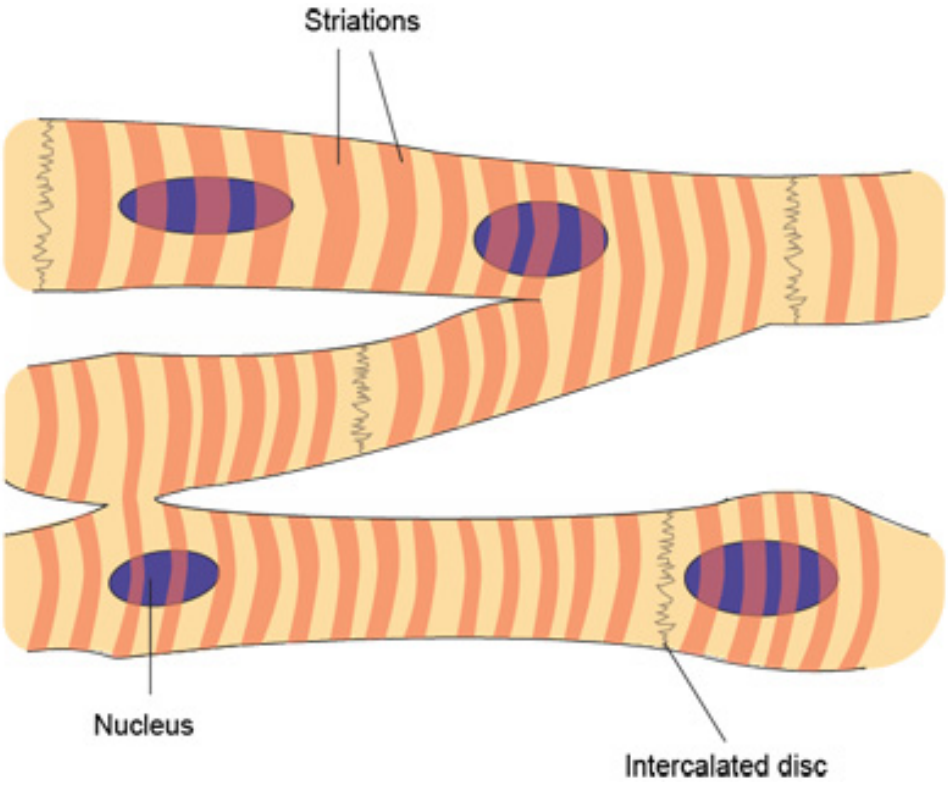


Figure 8 Arrangement of myocytes in cardiac muscle.

Smooth muscle is found in the wall of blood vessels and many hollow organs, including the uterus, the bladder and along all parts of the gastrointestinal tract.

Activity 3

Open the virtual microscope in a new window or tab. Find Slides 4–6 in the 'Week 3' category.

See if you can identify the characteristics of the three different types of muscle: striated muscle (Slide 4), cardiac muscle (Slide 5) and smooth muscle (Slide 6).

Note that smooth muscle is illustrated in Slide 6 in the wall of an artery. However, it can also be seen in different areas of the gut (see Slides 3–5 in the 'Week 2' category).

2.3 Structure and strength: functions of bone and cartilage

Tissues such as bone and cartilage give our bodies structure and strength. The examples used are bone and cartilage.

There are many different types of cartilage, including articular cartilage, which covers the joints at the end of the bones. However, the example provided in the slide set that you will examine shortly is from the trachea (windpipe). This structure has bands of hyaline cartilage, which strengthen the trachea, to resist collapse or closure as the head moves.

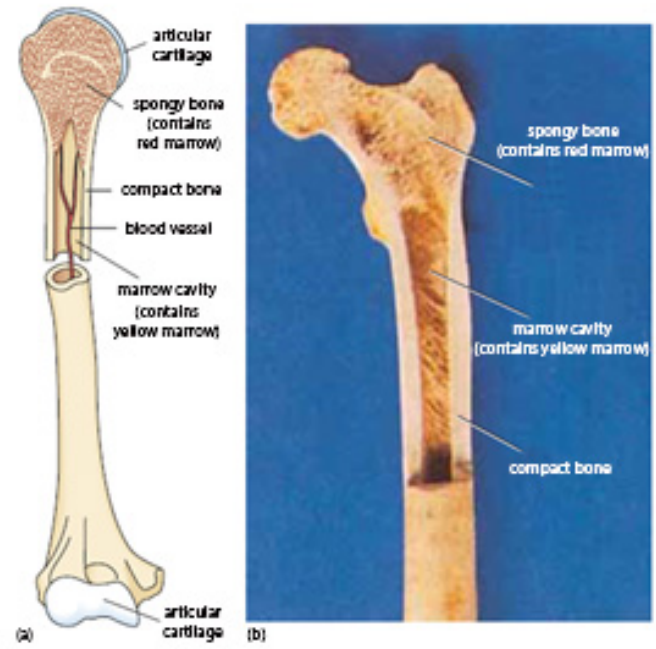


Figure 9 (a) Diagram and (b) photograph of a partially sectioned long bone (femur), showing its internal structure. Erythrocytes and leukocytes are produced from stem cells located primarily in the red marrow. Yellow marrow produces factors (cytokines) that promote the development of the blood cells (Tortora and Grabowski, 1993).

Notice in these strong tissues how the cells form only a small proportion of the total tissue mass. They are responsible for forming, remodelling and repairing the tissue, but the strength is provided by the extracellular matrix (and mineralisation), including proteins such as collagen and elastin in tendons.

Mineralisation produces highly ordered structures around the cells, and is arranged to resist the stresses placed on the tissue. The hardest tissues of the body – bones and teeth – are mineralised with calcium hydroxyl apatite.

Activity 4

Open the virtual microscope in a new window or tab. Find Slides 7–8 in the 'Week 3' category.

First, take a look at the section of bone shown in Slide 7. Identify the cells, osteoblasts (which produce the bone) and osteoclasts (which remodel it), and familiarise yourself with their appearance and structure.

Next, in the section of trachea shown in Slide 8, identify the chondrocytes that form the connective tissues, and in each case relate the position of the cells to the extracellular structures.

2.4 Excretion: functions of the kidney

The kidneys are excretory organs that perform three main functions to produce urine, filtration, reabsorption and secretion. They are situated at the back of the peritoneal cavity.

A cross-sectional slice through the kidney shows an outer layer (renal cortex), a middle layer (renal medulla), and an inner area (renal pelvis), where the ureter widens to join the kidney. These three structures are shown in Figure 10.

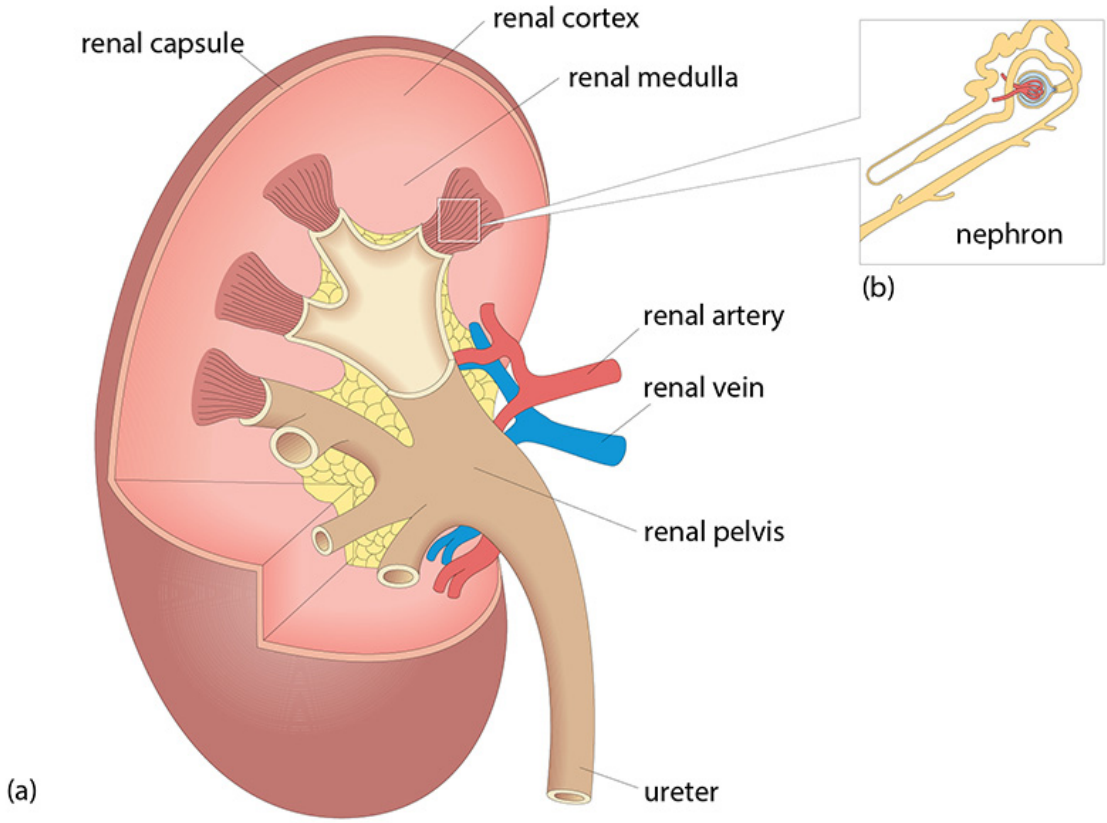


Figure 10 Diagram of the kidney structure with an enlarged diagram of a nephron. The ureter carries urine from the kidney to the bladder.

Within each kidney there are approximately one million structures called nephrons, each of which acts as an independent filter and urine-processing unit. A nephron consists of a renal corpuscle, which lies in the cortex, and a long tube which collects and processes the filtered fluid, called a renal tubule.

At the renal corpuscle, a network of very small-diameter blood capillaries, known as the glomerulus, comes into close contact with the closed end of the tubule, which is composed of a single layer of epithelial cells – the Bowman's capsule.

In this specialised region, fluid is filtered out of the blood capillaries, across the epithelial cells and into the lumen of the tubule. The filtrate then passes along the tubule, which is convoluted (in some cases looping down into the medulla), before finally joining with the renal pelvis, where the urine is emptied into the ureters.

It is during its passage along the tubule that the contents of the filtrate are processed, and urine is formed. Most of the filtered water, glucose, amino acids, sodium and other ions are reabsorbed by the epithelial cells of the tubules. Waste substances are either not reabsorbed at all, or only partially reabsorbed.

Some molecules and ions are also secreted into the tubule by the epithelial cells and, together with waste products which remain in the filtrate, are excreted in the urine.

Cells of the kidney thus perform three main functions to produce urine:

- **filtration** occurs in the glomerulus/Bowman's capsule

- **reabsorption** and **secretion** occur in the tubules.

The following diagram illustrates the blood supply to different parts of the nephron. High pressure within the glomerulus promotes the initial filtration of the blood plasma, and the network of vessels associated with the loop of Henle allows reabsorption of ions and water.

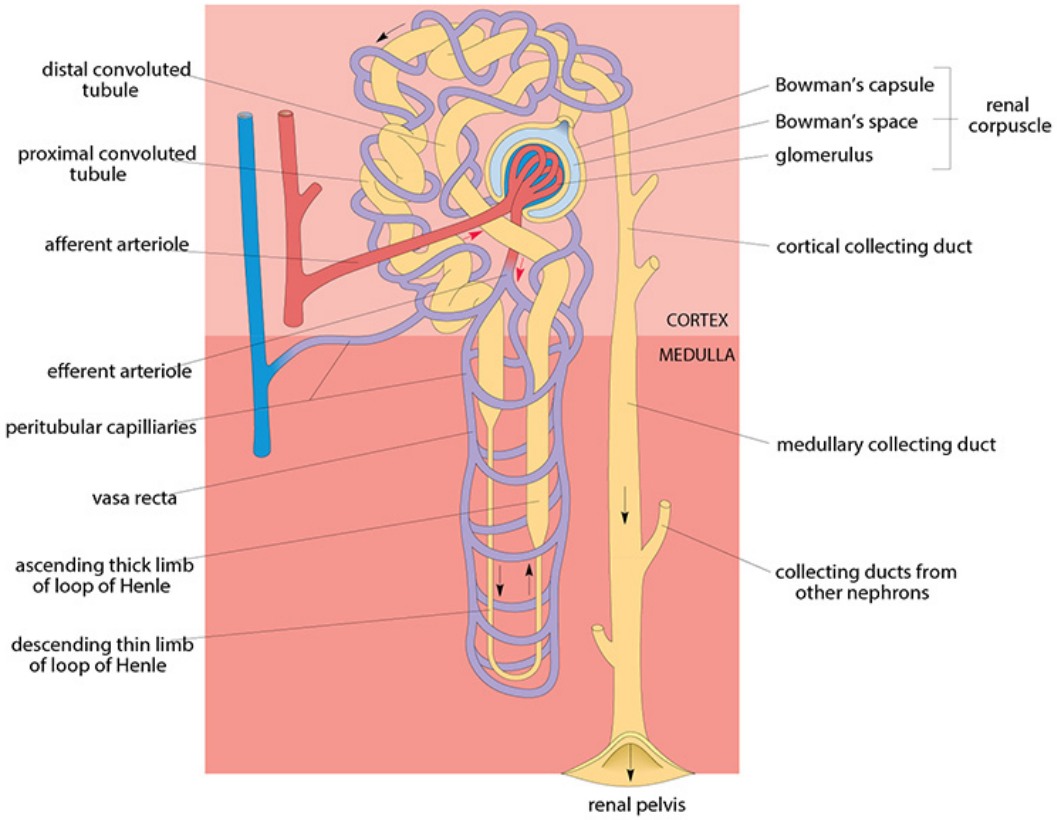


Figure 11 The blood supply to different parts of the nephron.

2.5 Histology of the kidney

As the anatomy and histology of the kidney is quite complex, this video gives you a short tour of the section, before you look at it yourself in the virtual microscope.

Video content is not available in this format.

Activity 5

Open the virtual microscope in a new window or tab. Find Slide 9 in the 'Week 3' category.

Now it's your turn to identify the main areas that exist within the kidney sample shown in Slide 9. Try to locate each of the following:

- the glomerulus
- Bowman's capsule
- convoluted tubules
- the loop of Henle
- collecting tubules.

2.6 Communication: functions of the nervous system

The function of the nervous system, and the cells that comprise it, is to communicate signals that originate both within the body and in the external environment.

As you read earlier in the course, the nervous system is divided into two main parts.

- the central nervous system (CNS) includes the brain, spinal cord and retina of the eye
- the peripheral nervous system (PNS) is the network of nerves outside the CNS that runs through virtually all other tissues.

Both parts are illustrated in Figure 12.

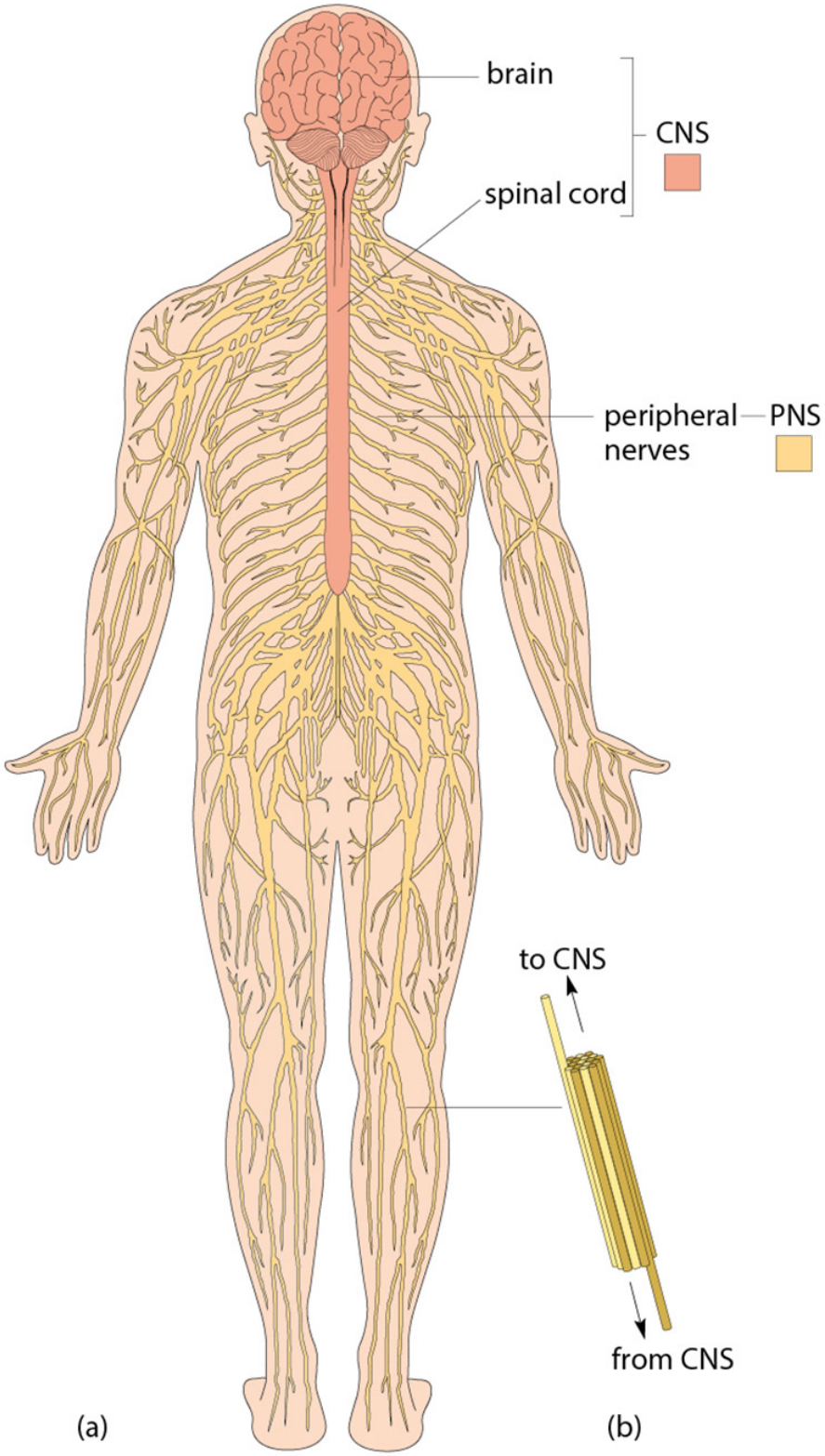


Figure 12 a) The human nervous system showing the CNS and PNS. (b) A diagram of a small peripheral nerve showing the mixture of nerve fibres travelling from the CNS (efferent neurons) which control muscle movement, and nerves travelling to the CNS (afferent neurons) which convey sensory information.

There are a greater abundance of nerve cells in some parts of the body than others. For example, there is a dense plexus of nerves associated with the gut, which is know as the enteric nervous system. This network of cells controls peristalsis – the coordinated contraction of the muscles along the gut wall that moves matter through the digestive system.

The roles and structures of neurons

Around half of the cells that comprise the nervous system are called neurons.

Neurons typically consist of a cell body, which contains the nucleus of the cell. Dendrites extend from the cell body, and this is where information (signals) are received. Each cell also has a single axon, which relays signals onwards to other neurons or to other cells such as muscle. A number of possible arrangements of neural structures are depicted below in Figure 13.

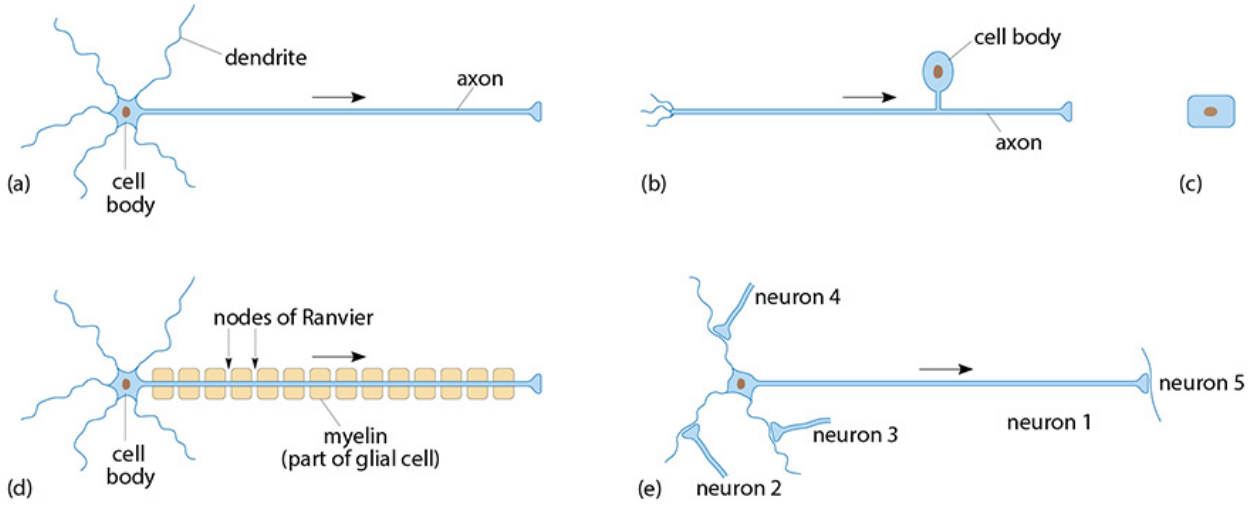


Figure 13 Different types of neuron. (a) With the cell body at the end of the axon. (b) With the cell body to the side of the axon. (c) With no processes. (d) With an insulating sheath of myelin around the axon, which speeds nerve conduction. (e) A small nerve network showing inputs (neurons 2, 3 and 4) to neuron 1, and its output to neuron 5.

The roles and structures of other cell types

The other cells that comprise the nervous system perform supporting functions for the neurons, and are collectively known as glia. Examples of glia are astrocytes, oligodendrocytes, Schwann cells and microglia, and these are described in more detail below.

Astrocytes control many metabolic functions within the nervous system and they signal between neurons and the vascular system to control blood flow.

Many nerves have a sheath of insulating material (myelin) around their axons, which allows faster conduction of the signal (see part (d) of the previous figure). Such nerves are said to be myelinated, to distinguish them from unmyelinated nerves. In the CNS the

myelin sheath is produced by oligodendrocytes, and in the PNS it is produced by Schwann cells.

About 10% of the cells in the CNS are microglia; they are the representative of the immune system within the nervous system, and they respond to infection, damage and inflammation.

The anatomy and connectivity of neurons in the brain is complex and cannot be discerned in regular histological sections. Moreover, the area that is seen in a single section is quite limited. Nevertheless, it is important to be able to recognise the appearance of CNS tissue and some peripheral nerves.

Activity 6

Open the virtual microscope in a new window or tab. Find Slides 10–13 in the 'Week 3' category.

The slide collection includes two examples of a peripheral nerve (Slides 10 and 11). Study these sections and identify the axons and the elements of the accompanying blood vessel.

There are also sections from two different areas of the brain: the cerebral cortex (Slide 12) and the cerebellum (Slide 13). Notice the completely different arrangement of the neurons in these two areas.

2.7 Normal tissues

You have nearly reached the end of this week, and this is a good opportunity to try to apply some of the things you've learned about observation and the structure–function relationship that exists in tissues.

Activity 7

Open the virtual microscope in a new window or tab. Use the slides from the course so far to consider the following questions and make notes.

1. What differences can you see between the two sections of breast tissue in Slides 2 and 3 from the 'Week 3' collection? How do these differences relate to the function of the tissue?

Provide your answer...

2. What differences are there between the types of skin shown in Slides 1 and 2 from the 'Week 2' collection? How might this relate to the functions of different areas of the skin?

Provide your answer...

3. What other types of skin are seen on the body? If you look at an area of skin on your forearm, does it all appear uniform? Would you consider a mole (nevus) to be a normal area of skin?

Provide your answer...

3 This week's quiz

Now test your ability to recognise tissues, and specific features within them.

The questions that follow require you to observe Slides 14, 15 and 16 in the virtual microscope. We recommend that you open the tool in a new browser window or tab, so you can easily alternate between the slides and the questions.

Complete the Week 3 quiz now.

Open the quiz in a new window or tab then come back here when you're done.

4 Summary of Week 3

By this stage in the course you should be able to identify some additional tissue types, including thyroid, breast, different types of muscle, kidney, bone, some types of cartilage and some areas of the CNS.

In this week specifically we have highlighted the relationship between the structure of a tissue and its function. We have also alerted you to the possibility that many tissues vary in their appearance, depending on normal physiological changes. A section that appears 'atypical' may not appear as such because of a pathological change.

In Week 4 you will be examining some tissues which do have pathological changes, and you will gain some insight into how histology is used for the diagnosis of disease.

You can now go to Week 4.

Week 4: Recognising disease

Introduction

Welcome to the final week of the course.

Infection can affect any tissue of the body, producing cell damage and inflammatory reactions.

As David Male explains in the video below, the focus of this week is on studying pathological sections, namely those that show histological evidence of disease.

Video content is not available in this format.

The sections that you will look at have been organised around three main types of pathology:

- infection and inflammation
- degeneration and cell death
- tumours (specifically hyperplasia, dysplasia and neoplasia).

You will learn more about these topics as you progress through the week.

In the end-of-course quiz you'll look at a number of different pathological sections to identify the abnormalities, to try to deduce the underlying causes and, finally, to venture a diagnosis of what the disease might be.

1 Infection and inflammation

Histological examination of tissues can help diagnose disease, because each condition produces a characteristic set of changes in the tissue structure.

There are such a wide variety of diseases that histology alone usually cannot produce a diagnosis, although in some cases the histological appearance is definitive.

For example, a pathologist might see signs of a viral infection in the brain because of tissue damage and inflammation, but they would be unable to tell what virus is responsible for these changes. To identify the virus might require immunohistochemistry (IHC) for a specific viral protein or, more likely, the diagnosis would be confirmed by other symptoms and/or examination of a blood sample (serology).

In some cases the histological appearance is characteristic of a specific disease. For example, the appearance of 'owl-eye' cells in the brain, as shown in the micrograph below, is diagnostic of a particular type of measles infection. However, normally histopathology reports only form one part of the picture of a disease that the clinician is trying to assemble.

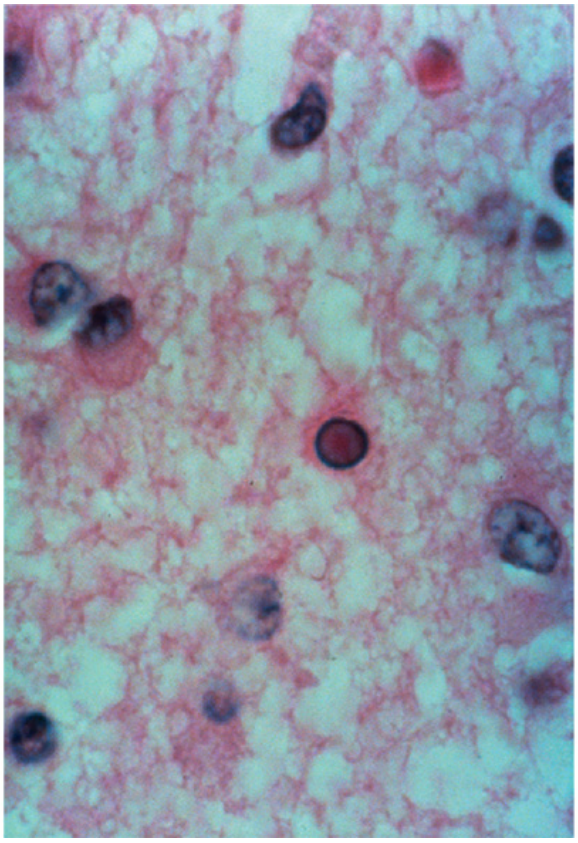


Figure 1 Owl-eye bodies in infected neurons of a patient with subacute sclerosing panencephalitis (SSPE).

Although diseases are very diverse, the responses made by the body are more limited, and fall into specific categories. For example, inflammation may be seen in response to an infection, as a result of physical damage or as part of an autoimmune disease, where the immune system attacks components of the body.

You'll start looking at pathology in more detail by looking at two common causes of infection.

1.1 Infection

Infection can affect any tissue of the body, producing cell damage and inflammatory reactions. There are many microbiological agents that cause infection, but two of the most common are viruses and bacteria.

Viruses

Viruses are generally too small to be seen with the light microscope. However, their presence can often be inferred by the changes they produce in tissue, even if their identity requires confirmation by IHC, serology or molecular biology.

Bacteria

Bacteria can be seen with the light microscope using high magnification objective lenses, but the numbers of bacteria that are present in a tissue can be highly variable – even in one disease. A classic example of this variability is leprosy, where there may be very large numbers of bacteria in the skin (lepromatous leprosy), or very few (tuberculoid leprosy), depending on the precise nature of the infection.

Distinguishing the type of bacteria in a thin section of a lesion generally requires specialised histological stains, although the morphology (shape) of the bacteria may also be informative, as can be seen in the micrograph below.

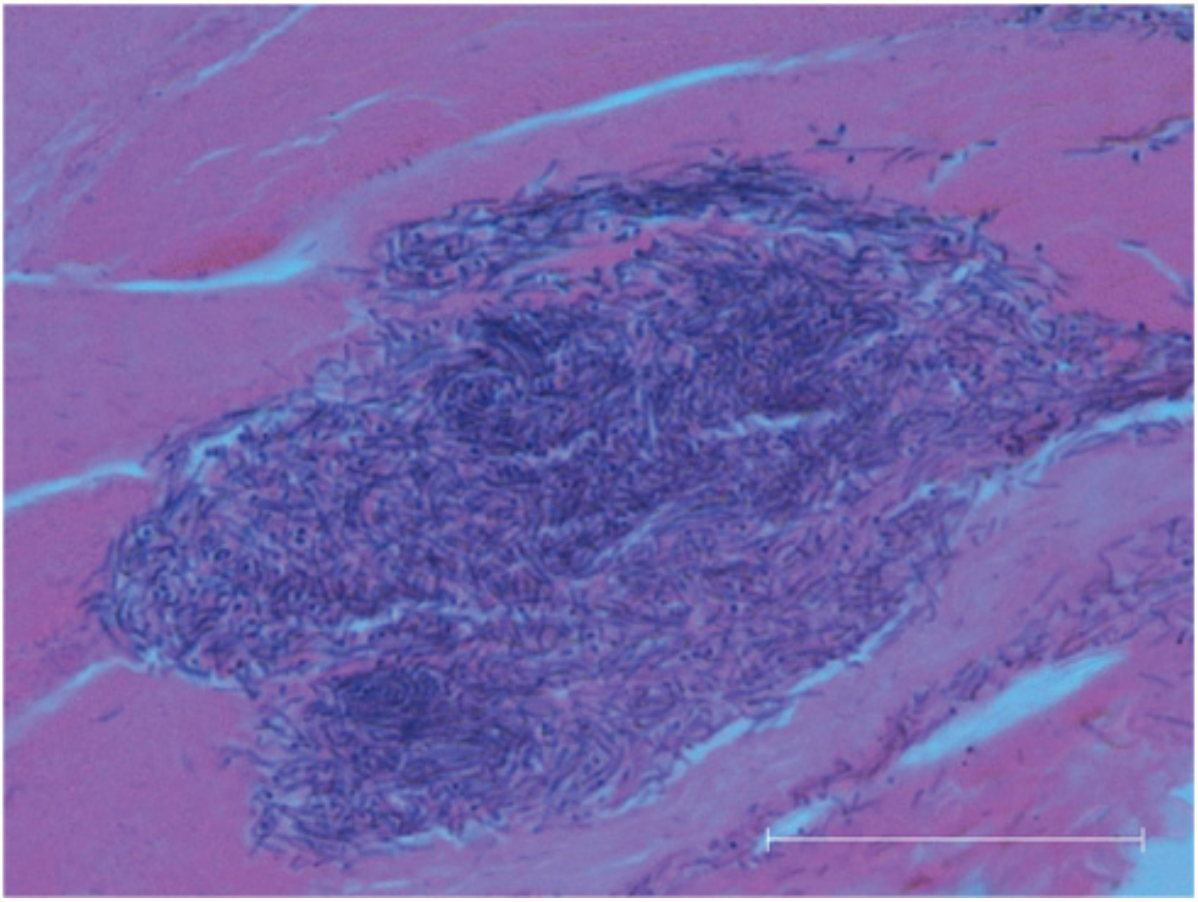


Figure 2 Gas gangrene in a muscle. The micrograph shows a colony of bacteria, stained with haematoxylin. The bacteria have the characteristic shape and growth pattern of clostridia. Scale bar = 40 μm.

As with viral infection, the histological findings are an adjunct to serology and microbiology in producing a diagnosis, rather than a stand-alone explanation.

Activity 1

Based on what you learned earlier in the course, can you identify what type of infection can be detected in the blood smear?

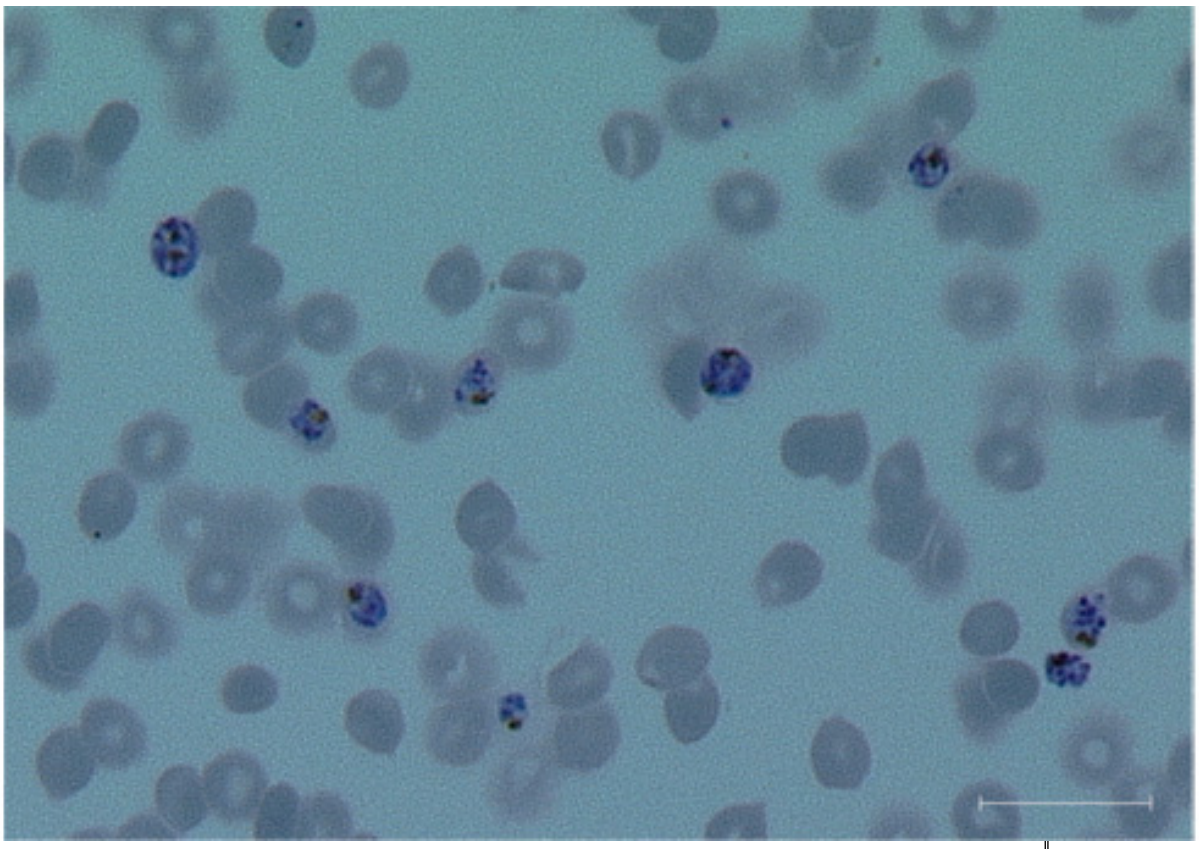


Figure 3 (Scale bar = 20 μm)

Answer

This is a blood smear from a patient with a malarial infection. Several of the erythrocytes are infected. Malarial infection of blood was shown in the 'Week 1' slide set in the virtual microscope.

1.2 Inflammation

Inflammation is a common response to tissue injury or infection, and occurs when the body's defence systems are brought to the site of the damage.

There are three main components of inflammation as illustrated in the diagram below (Figure 4).

1 An increase in the blood supply to the affected area, caused by dilation of arterioles supplying the area.

2 An increase in the permeability of capillaries, which allows larger serum molecules, such as antibodies, to enter the tissue.

3 The migration of leukocytes from the blood into the tissues: the leukocytes cross the endothelial cells, which line the venules, and then move out into the tissue.

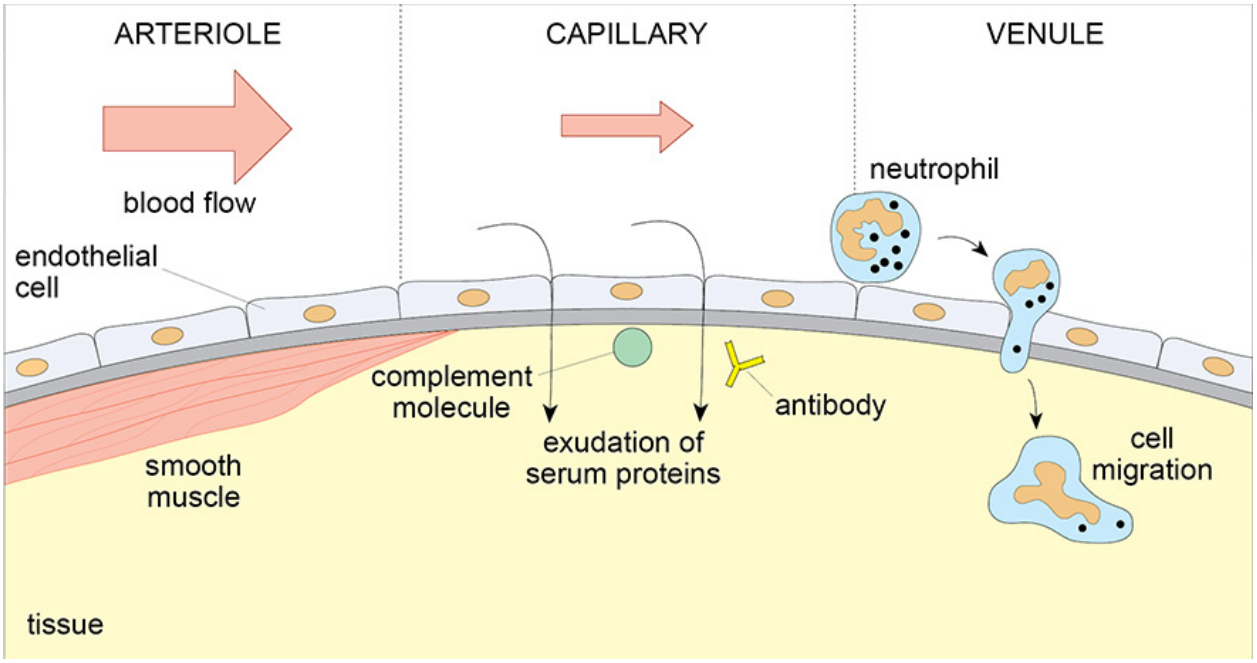


Figure 4 The three main features of inflammation.

The blood that is brought to the site of the injury contains a number of proteins that stop bleeding, help clear infection and induce repair or regeneration of the tissues. It also contains different types of leukocyte (white blood cells) each of which have evolved to deal with different types of infection.

Inflammation can be separated into two main types.

- Acute inflammation develops quickly and resolves within days.
- Chronic inflammation can last for months or years, usually because of the persistence of the initiating factor.

More details about both types of inflammatory response, and how they can be recognised in tissue samples, is provided below.

Acute inflammation

The histological appearance of acute inflammation is quite different from that of chronic inflammation and the distinctive features can point to the initiating agent. For example, an infection of the skin with *Staphylococcus aureus* usually produces an acute inflammatory response, whereas infection with *Mycobacterium leprae* (leprosy) typically produces persistent infection and chronic inflammation.

One of the key histological differences between acute and chronic inflammation is seen in the sets of leukocytes that are present in the tissues. In acute inflammation polymorphonuclear neutrophils usually predominate, whereas macrophages and lymphocytes predominate in chronic inflammation. Eosinophils are often prevalent in sites of helminth infections (parasitic worms). Hence, the characteristics of inflammation are determined both by the tissue in which it occurs, and by the initiating agent and its persistence.

Chronic inflammation

Chronic inflammation is seen in diseases where there is persistent infection, usually because the pathogen can resist the body's immune defences. If the infection is cleared, chronic inflammation resolves, but residual damage may still be evident in the tissues.

Chronic inflammation also occurs in many autoimmune diseases. The immune system normally recognises and tolerates all of the body's own tissues. However, in some conditions the immune system reacts against a tissue resulting in autoimmune disease.

In autoimmunity the target of the immune response is one of the body's own proteins or cellular components, and consequently the stimulus for inflammation cannot be cleared, although the condition may improve if the normal controls that prevent autoimmune reactions are restored. The targets may be individual molecules found in a specific tissue, or antigens present in many tissues or in the extracellular matrix.

Table 1 gives some examples of such diseases and the target antigens.

Table 1 Autoimmune diseases affecting specific tissues

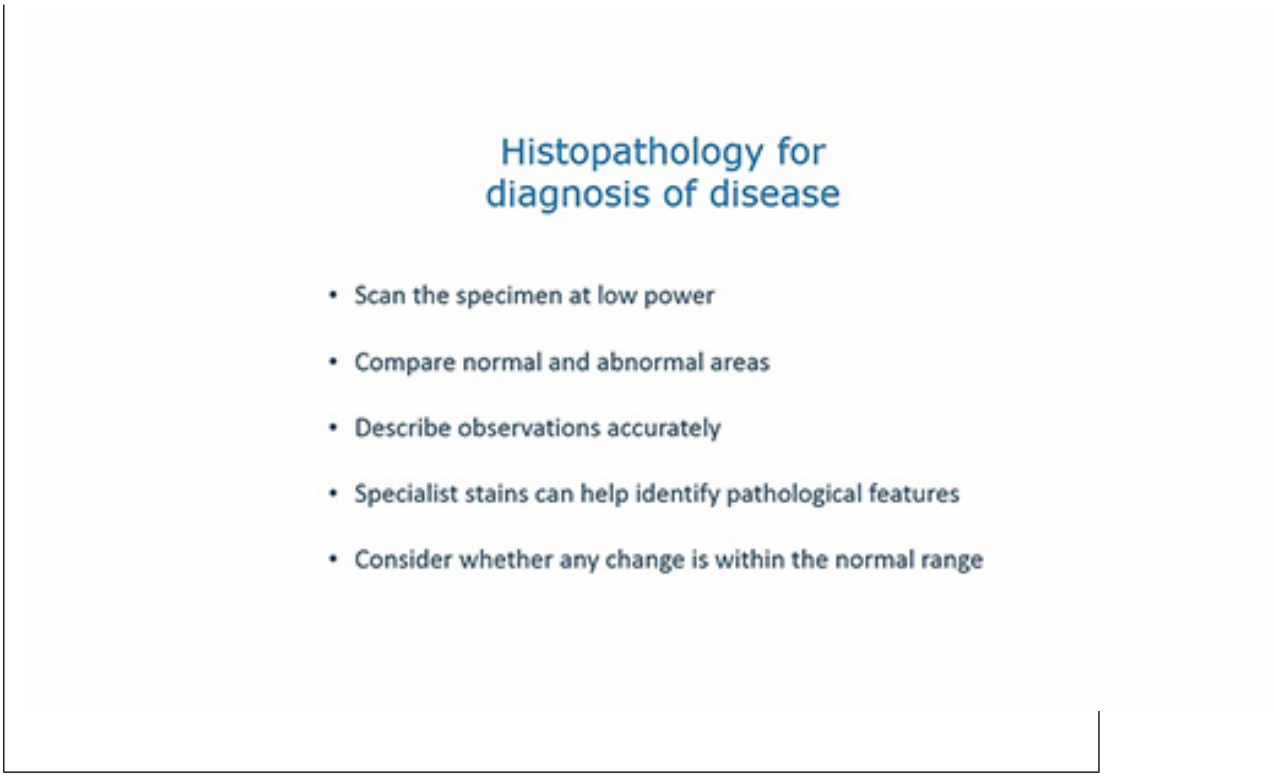
Disease	Organ(s)	Target antigens	Histological appearance
Hashimoto's thyroiditis	Thyroid	Thyroglobulin, Thyroid peroxisomes	Destruction of thyroid follicles with severe inflammation
Goodpasture's syndrome	Kidney Lung	Basement membranes	Damage to kidney glomerulus and/or lung alveoli
Myasthenia gravis	Skeletal muscle	Acetyl choline receptor	Degeneration of the motor endplate at nerve/muscle junction
Pemphigus	Skin Mucosa	Desmosome proteins in keratinocytes	Separation of layers of epithelium
Type 1 diabetes	Islets of Langerhans	Pancreatic beta cells, insulin, GAD (enzyme)	Selective damage and loss of cells of pancreatic islets with inflammation
Multiple sclerosis	Brain Spinal cord	Components of myelin	Focal areas of inflammation and loss of myelin in the central nervous system

Inflammation is associated with many types of tissue damage, although the appearance will vary depending on the tissue and the initiating factor. The slide collection that you will examine shortly includes some examples of inflammation as a consequence of infection.

1.3 Histopathology for the diagnosis of disease

In this video, David provides some guidance for the systematic examination of slides, based on an understanding of what the normal tissue looks like.

Video content is not available in this format.



When viewing a section, it is important to note and report any changes objectively. Interpretation of what has caused the changes takes considerable experience. Even if you have a good idea of the underlying cause, observations and interpretation should be clearly distinguished.

1.4 Infection

This activity will show you examples of diseases that affect tissues with which you are familiar. In each case the normal tissue section is included (Slides 2 and 4), so that you can compare normal and diseased tissue more easily. You should use the legends to navigate around the sections.

Activity 2

Open the virtual microscope in a new window or tab. Find Slides 1–4 in the 'Week 4' category.

Look at Slides 1 and 2. They are sections of lung. Slide 1 is from a patient with miliary tuberculosis, which resulted from an infection with the bacterium mycobacterium tuberculosis. Notice how the bacteria have produced cell death (necrosis) in the centre of the tubercle, and the distinctive appearance of the chronic inflammatory reaction surrounding it.

Look at Slides 3 and 4. They are sections of kidney. Slide 3 is from a patient who had chronic inflammation caused by a bacterial infection (*Staphylococcus aureus*). The bacteria are not visible with the H&E stain, but notice the infiltration of the kidney cortex by large numbers of leukocytes (macrophages, neutrophils and lymphocytes).

1.5 Identify an infection

In this activity you'll identify the tissue and try to identify any abnormality.

Activity 3

Open the virtual microscope in a new window or tab. Find Slide 5 in the 'Week 4' category.

Look at Slide 5 and identify the tissue. Try to identify any abnormality in it. You may find it helpful to look back and compare this section with normal tissue. for example Slide 1 in the 'Week 1' collection.

When you have noted the appearance of the tissue, think about the pathological changes that can be seen and what is causing them.

Provide your answer...

Answer these questions about your findings:

1. What tissue was on Slide 5?

- ○ Lung
- ○ Liver
- ○ Cerebral cortex
- ○ Thyroid
- ○ Lymph node

2. What abnormality did you identify on Slide 5?

- ○ the liver cells (hepatocytes) contain many pale staining vesicles
- ○ there is evidence of haemorrhage (bleeding) into the tissue
- ○ the capsule of the tissue is ruptured
- ○ there are cysts containing a multicellular organism

Answer

This section of liver is from a person infected with a parasitic worm (schistosomiasis). An immature fluke can be seen at [2948, 2145].

3. What pathological processes did you see on Slide 5? Select all that apply.

- ☐ acute inflammation with many neutrophils
- ☐ chronic inflammation with macrophages
- ☐ cell death (necrosis)
- ☐ fibrosis – the deposition of fibrous connective tissue

Answer

Around the parasites are many leukocytes [2706, 2387] including lymphocytes, macrophages and eosinophils. The parasites have been walled off by fibrous tissue [2759, 2211].

A scientific approach to these slides involves:

- observation and description of the tissue and any changes
- interpretation of the findings and possible inferences.

These two stages correspond to the ‘Results’ and ‘Discussion’ sections in scientific papers. The observations and description should be clear, accurate and unequivocal. The interpretation may or may not be correct. A histopathologist will give their best opinion, perhaps with a differential diagnosis (probability) for the underlying cause of any changes in the tissue.

Remember that the finding ‘no unusual features’ is just as important in reaching an accurate diagnosis of disease, as the identification of any histopathological change.

2 Cell death and degeneration

Cell loss occurs in many tissues with age but the effects are particularly notable in tissues that have a limited capacity for regeneration, such as nerves in the CNS, the retina of the eye and the sensory cells of the inner ear.

When cells die they do so in two main ways, by apoptosis or necrosis. This is illustrated in Figure 5.

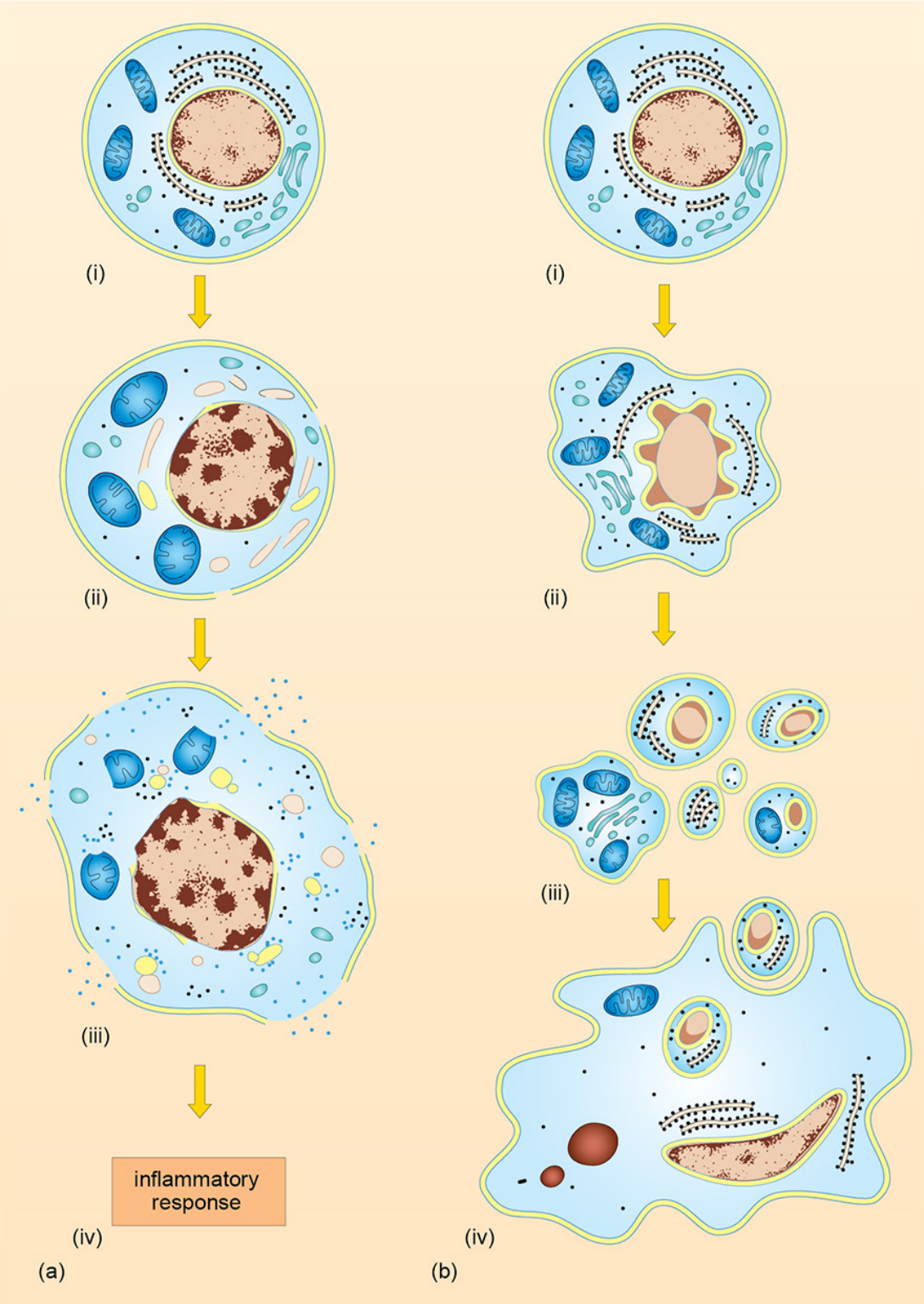


Figure 5 Schematic diagram comparing (a) necrosis and (b) apoptosis. The first events in necrosis are irregular condensation of the nucleus, swelling of the mitochondria and breakdown of membranes and ribosomes. The cell is eventually disrupted, releasing its contents and inducing an inflammatory reaction. In contrast, a cell undergoing apoptosis shows condensation of the nucleus into fragments and shrinkage of the cell. The nucleus and cytoplasm break up into fragments called apoptotic bodies, which are phagocytosed by mononuclear phagocytes.

Apoptosis

Apoptosis is programmed cell death; the cell dies as part of its normal programme of development, it may be lacking in growth factors or it may be instructed to die by cells of the immune system because it has become infected. Even pre-cancerous cells may be propelled into apoptosis by the normal cellular controls that check the development of tumours.

In all cases, apoptosis is a highly ordered process. If it occurs as part of a developmental process, it does not induce inflammation – the dead cells are quietly removed by phagocytes within the tissue. Therefore, it is often very difficult to identify apoptotic cells within tissues, since they are usually individual cells with small condensed nuclei and little cytoplasm.

Cell death in degenerative conditions (e.g. Alzheimer's disease) appears to occur by apoptosis. Although the loss of individual cells is histologically undramatic, the cumulative loss of cells in such degenerative conditions can cause major loss of function in the affected tissue. Moreover, cell loss may be accompanied by the accumulation of products of tissue breakdown, which are histologically evident.

Necrosis

In contrast, necrosis is wholesale unregulated cell death, caused by lack of nutrients or infection.

For example, the failure of the blood supply to an organ (ischaemia) due to thrombosis will cause massive cell death due to lack of oxygen. A large area of cell death caused by ischaemia is called an infarction. Another example of cell necrosis is seen in severe viral infections with cytopathic viruses (e.g. polio).

Necrosis is an uncontrolled process and the dying cells release their contents. Areas of necrosis are characterised by infiltration with inflammatory cells; macrophages and neutrophils enter the area over a number of days and weeks and clear the dead cells and associated cellular debris. Such large areas of cell loss and inflammation are frequently easily seen in pathological specimens, even without microscopic examination, as can be seen in the micrograph below.

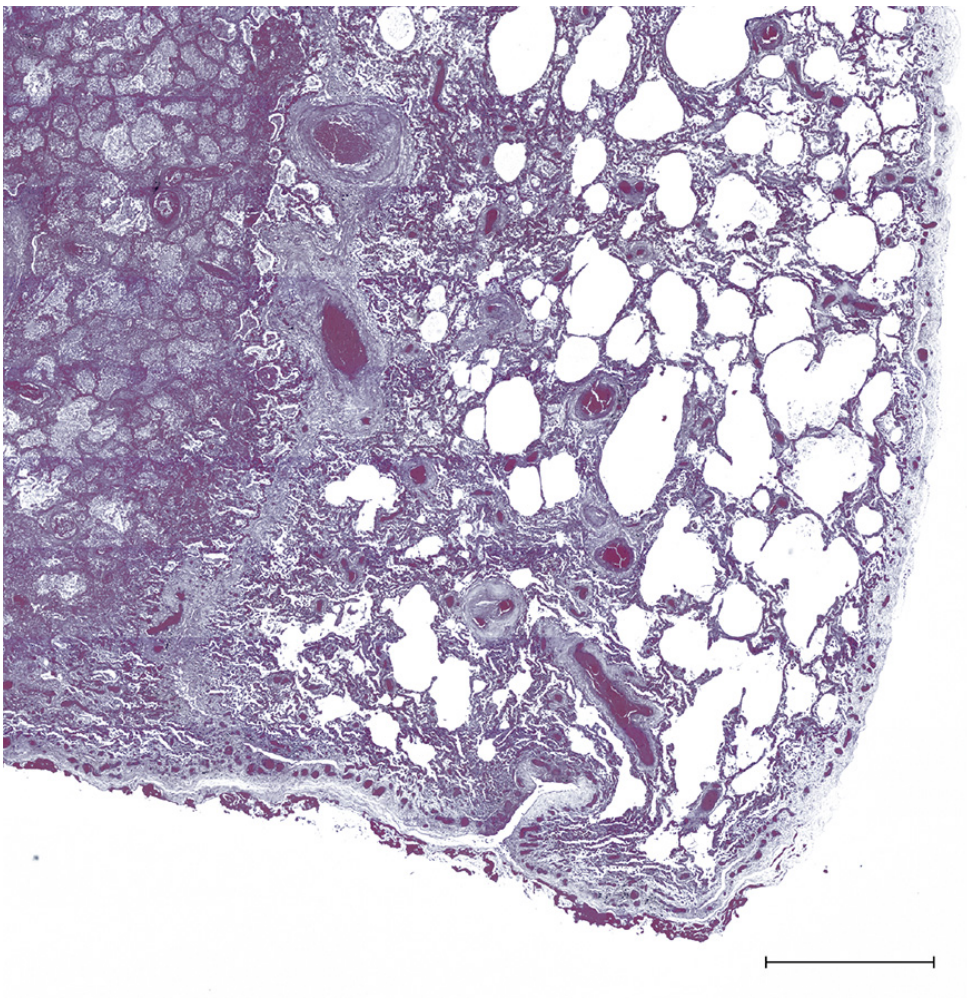


Figure 6 An area of necrosis in the lung caused by blockage of the blood supply is visible on the left of this section. The area on the right includes surviving lung tissue, which is thickened and has areas of fibrosis. Scale bar = 2 mm.

2.1 Cell death in the CNS

Cell death and degeneration within the nervous system is referred to as neurodegeneration. These processes occur in a number of genetic conditions as well as conditions associated with aging including Alzheimer's disease and Parkinson's disease.

Cell loss occurs in many tissues with age but the effects are particularly notable in tissues that have a limited capacity for regeneration, such as nerves in the CNS, the retina of the eye and the sensory cells of the inner ear. Histologically, it is more difficult to identify something that is not there than a change in the structure of the tissues.

In diseases such as Alzheimer's disease there is progressive loss of neurons and shrinkage of the brain, which may be more evident in the gross pathology, although counting the relative numbers of cells within an area can also give some histological indication of the cell loss. More evident are characteristic accumulations of proteins. Degenerating neurons leave tangles of fibres (neurofibrillary tangles) produced by degenerating components of the cytoskeleton. In addition, there are extracellular accumulations of amyloid within the brain.

Amyloid

Amyloid is an extracellular insoluble deposit of protein, and amyloidosis refers to the diseases in which amyloid occurs. In some cases production of amyloid is a primary event, and in others it is secondary to infection or a tumour. The actual protein type varies, depending on the cause of the condition. In some cases it affects individual organs, such as the brain in Alzheimer's disease, but in the so-called 'systemic amyloidoses' many organs may be affected, including the lungs, kidney, heart and spleen.

There are a number of hereditary conditions in which the person lacks enzymes that break down particular macromolecules; they are collectively called storage diseases because the components that cannot be degraded within lysosomes accumulate and form insoluble deposits. This is particularly noticeable in the brain where they are often associated with neurodegenerative diseases; examples of this are shown in the micrographs below.

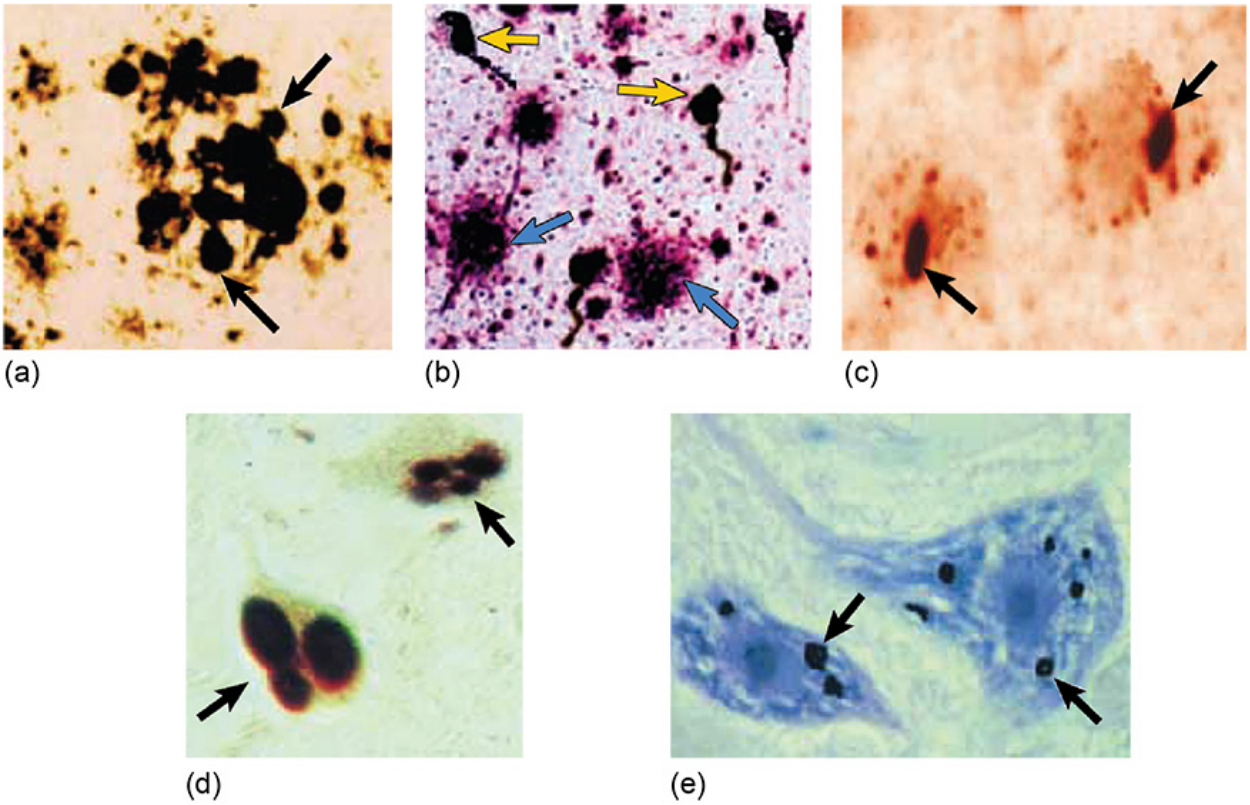


Figure 7 Protein aggregates in brain cells associated with neurodegenerative disease. Arrows highlight (a) extracellular plaques in prion disease, (b) extracellular plaques (blue) and neurofibrillary tangles (yellow) in Alzheimer's disease, (c) nuclear aggregates in Huntington's disease, (d) Lewy bodies in Parkinson's disease, (e) nuclear aggregates in amyotrophic lateral sclerosis.

2.2 Histopathology of the nervous system

In the activity below you will examine sections from the nervous system in conditions in which neurons die. These are Alzheimer's disease, dementia with symptoms of Parkinson's disease, frontotemporal lobe dementia and cerebrovascular disease.

Activity 4

Open the virtual microscope in a new window or tab. Find Slides 6–14 in the 'Week 4' category.

Start with Slide 6. Slide 6 is from a normal cortex for comparison. The outer layer of the brain, the cortex, contains large numbers of neuronal cell bodies and appears as 'grey matter'. Beneath the cortex are areas with a high proportion of axons and myelin-producing oligodendrocytes, which appear as 'white matter'.

Next look at Slides 7 and 8. They are from the cortex of a person with Alzheimer's disease. Slide 7 shows the loss of neurons from the outer layers of the cerebral cortex. Slide 8 is stained to show accumulation of the protein β-amyloid; the accumulation of the protein in discrete plaques is very evident.

Next look at Slides 9 and 10. They are from the midbrain of a person with dementia with Lewy bodies (DLB) and symptoms of Parkinson's disease. In this condition neurons from particular regions of the brain, controlling movement, are selectively lost. In addition, intracellular accumulations of protein, termed Lewy bodies, are detected by an H&E stain (Slide 9). The Lewy bodies can be identified by immunohistochemical staining with antibodies that recognise the protein α-synuclein (Slide 10).

Next look at Slides 11 and 12. They show frontotemporal lobe dementia (FTLD). In this condition there is severe neuronal loss from the cortex (Slide 11) and neurons often have protein inclusions. However, unlike Alzheimer's disease, amyloid plaques and tangles are absent. Slide 12 is a section from the spinal cord, which shows motor neuron loss.

Finally, look at Slides 13 and 14. They are from a patient who had cerebrovascular disease. Slide 13 shows areas of cell death caused by lack of an adequate blood supply (infarcts). In this case the condition is associated with thickening of the walls of the blood vessels, narrowing the vessel lumen and deposition of β-amyloid, a condition termed amyloid angiopathy (Slide 14).

Slides 7–14 were kindly loaned for imaging by Dr Andrew King and Dr Safa Al-Saraj from King's College Hospital, London.

3 Tumours

Cell division is normally a highly regulated process. The number of cells in any tissue is usually fairly constant, although some tissues can respond to physiological demand by an increase in cell number. Tumours disrupt this regulation.

Hyperplasia

What process occurs as mountaineers acclimatise to high altitude? The number of erythrocytes in their blood increases. But why does this happen? The fall in the level of oxygen in the air at altitude means that the capacity of the blood to carry oxygen increases, to compensate. There is a progressive increase in the number of erythrocytes over a period of weeks as the bone marrow responds by increasing production.

Other types of cell may increase in number in response to appropriate stimuli. For example, in a guitar player, the basal cells of the epidermis in the fingertips can proliferate to produce hard pads of keratin (calluses), caused by repeated contact with the strings. Cell proliferation and the consequent increase in cell numbers, seen in these two examples, is called hyperplasia. It is a normal physiological response to demand placed on a tissue.

Dysplasia

If cell division becomes poorly regulated, cells may lose some of their morphological characteristics and/or functions. The tissue becomes disordered in appearance, often with an increase in the number of immature cells, and greater variability between cells. This appearance is called dysplasia.

It should be emphasised that dysplasia does not necessarily show that the cells have become cancerous; however, it does suggest underlying changes in the cells, which may predispose them to cancer. In this sense, dysplasia may be a stage on the way to cancer development. For example, when histologists screen cervical smears, they are particularly looking for changes in the normal morphology of the cells which indicate pre-cancerous changes.

Neoplasia

Neoplasia is the term used to describe the development of tumours or cancerous tissue.

The development of a tumour requires a series of changes in the biology of the cell, with progressive loss of the controls that limit cell division. Even a cell that is undergoing uncontrolled proliferation will not necessarily be malignant. Malignancy typically arises when the dividing cells invade the normal tissue and move away from their site of origin. Because of the great variety of different tumours, it is impossible to generalise. Nevertheless, it is very important for a pathologist to be able to distinguish between a benign tumour and a malignant cancer, since the treatment required will usually be radically different. Consequently, pathologists often grade tumours according to how malignant/invasive they are.

Histologists can get some impression of the rate of cell division within a tissue, according to the number of mitotic figures – the number of cells showing the characteristic pattern of separating chromosomes, seen as the cell divides. Invasion of tumour cells within the tissue can be estimated, by observing where the cells are in relation to their normal position and in relation to other cells in that tissue, and this forms an important element in the pathological report on a tumour.

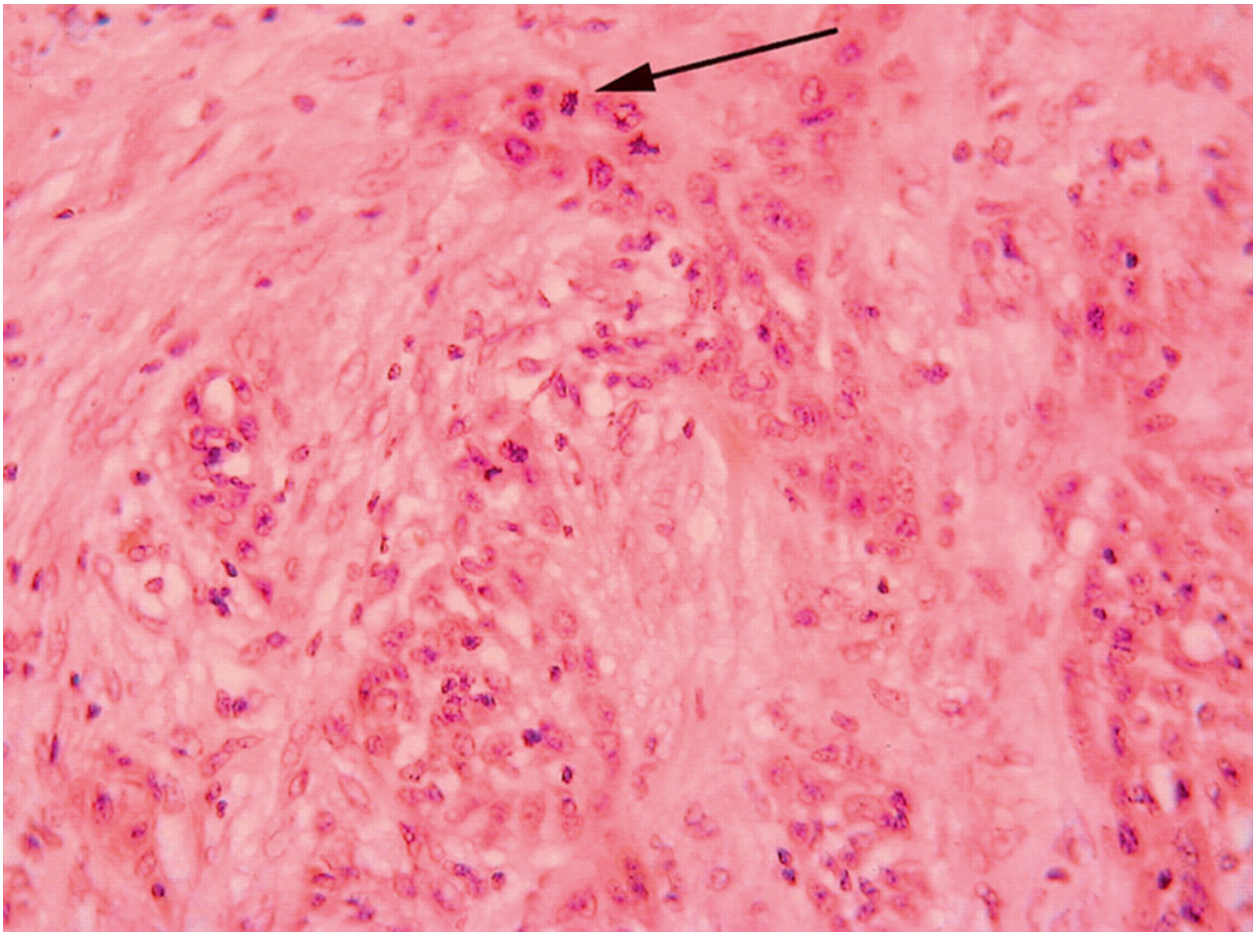


Figure 8 A mitotic figure in a carcinoma of the breast (arrowed) indicates cell division.

3.1 Metastasis

Tumours can also move away from their original tissue by invading blood vessels or lymphatic ducts and being carried to distant sites. This process is called metastasis.

Tumour cells that are carried through lymphatics will usually metastasise to local lymph nodes; this is the reason that surgeons may remove lymph nodes as well as the original tumour, to treat a cancer.

Tumours that metastasise via the bloodstream must first invade a blood vessel at the initial tumour site, and then exit the blood vessels in a different organ to establish a new tumour site. Such an event is relatively rare for any individual tumour cell; nevertheless, metastasis accounts for 90 per cent of cancer-related deaths, so identification of metastatic tumours is important both for prognosis and treatment.

Pathologists recognise metastatic tumours, because the affected organ contains clumps of cells that are completely uncharacteristic. In some cases, the primary tumour type can be recognised because it has retained some distinctive characteristics of the original cell type. However, the original identity of tumour cells is not always self-evident and this is particularly true of metastatic tumours.

Therefore, it may be possible to observe a metastatic tumour in a tissue, but be impossible to identify the primary cell type and the original site of the tumour, at least by H&E staining. In this case additional staining, particularly immunohistochemistry, is

valuable to identify the original cell type, because it can provide an important guide for patient scanning, further surgery, radiotherapy and drug treatment.

3.2 Neoplasia and hyperplasia

For this activity, refer to the ‘Week 4’ category within the virtual microscope, Slides 15–18.

Activity 5

Open the virtual microscope in a new window or tab. Find Slides 15–17 in the Week 4 category.

Start with Slide 15. It shows a primary liver carcinoma, a cancer which has arisen from a liver cell. Such primary tumours are relatively uncommon in the liver. Notice the irregular cell morphology, the lack of normal liver anatomy and the invasion of the normal tissue by the cancer. (For comparison look back at the normal liver on Slide 9 in Week 2.)

Next look at Slide 16 which is also from a liver. In this instance the tissue contains a secondary tumour, an adenocarcinoma, which has arisen from epithelial cells. This tumour is a metastasis from the primary site, which has been carried to the liver through the blood. However, it is not possible to tell from the H&E staining where the original tumour was located.

Look at Slide 17. It shows uncontrolled cell growth (hyperplasia) in the skin caused by a viral infection (*Molluscum contagiosum*). In some cases viral infection can lead to neoplasia; however, viral infections are generally controlled by the immune system. (Look at Slide 18 to compare this with normal skin.)

3.3 Put it into practice

In the next activity, you are invited to investigate, identify and comment on two slides drawing on what you’ve learned.

When reporting on sections it is important to clearly distinguish observations and interpretations. You might want to make a short written report on these two sections, before responding to the questions. Then compare your observations and conclusions with the answers.

Activity 6

Slide 19

Open the virtual microscope in a new window or tab. Find Slides 19 and 20 in the ‘Week 4’ category.

Look at Slide 19. Identify:

- the tissue shown in Slide 19
- whether the section is normal or abnormal.

Provide your answer...

Discussion

The tissue shown in Slide 19 is from a lung and it is not normal. You can compare this section with Slide 6 from Week 2, which is from a normal lung. In particular, the area near the upper right of the section (8000, 4000), does not look like the normal tissue. Navigate to these coordinates in the Virtual Microscope to see them for yourself.

Now try to identify what kind of pathological reaction can be seen in the tissue on this slide. What might cause this type of change?

Provide your answer...

Answer

The section of lung shows areas of low grade inflammation (8480, 2904) with some thickening of the walls of the alveoli (7285, 2903).

This biopsy came from a patient with cytomegalovirus infection. Some macrophages (6637, 1215) in the alveoli have dark-staining intra-nuclear inclusions (6475, 802). Although the virus itself cannot be seen with the light microscope, its appearance on infected cells is characteristic. Navigate to these coordinates to make sure that you can identify these features.

You would probably not be able to identify the type of infection from the histology alone. However, you might have worked out that an infectious agent is one likely cause of inflammation in the lung.

Slide 20

Look at Slide 20. Identify:

- the tissue shown in Slide 20
- whether the section is normal or abnormal.

Provide your answer...

Discussion

The tissue shown in Slide 20 is from a thyroid and it is not normal. You can compare this section with Slide 1 from Week 3, which comes from a normal thyroid. Slide 20 comes from a patient with a goitre, i.e. an enlarged thyroid. The changes are seen throughout the section.

Compare Slide 20 with Slide 1 from Week 3. Make notes about:

- what type of reaction you can see in the tissue on this slide
- what might have caused this type of change, which has resulted in enlargement of the thyroid.

Provide your answer...

Answer

The tissue shows hyperplasia of the thyroid, which is typically produced by a lack of iodine in the diet and/or abnormally high production of thyroid-stimulating hormone (TSH) by the pituitary.

The thyroid epithelial cells are dense and form clumps within the acini (2421, 3842), but the overall anatomy appears normal, so there is no evidence that the cells are neoplastic. Moreover, hyperplasia has occurred throughout the gland.

Neoplasia generally arises in one area of a tissue (due to gene mutation in one cell or group of cells), and there is usually some relatively normal tissue still present, which may have been included in the histological section. Notice also that the acini in Slide 20 have scalloped edges in some places, caused by the active removal of stored colloid to release the thyroid hormones.

All of these observations indicate hyperplasia, rather than neoplasia. This section illustrates how understanding the normal function of a tissue can shed light on the histological changes that have occurred.

4 End-of-course quiz

The final quiz of this course pulls in material from each of the weeks and presents you with some problems, where histology can help in understanding biology or in the diagnosis of disease.

Some of the questions are straightforward and directly related to material presented in the course. Others are intended to make you think about the implications of what you have learned. Do not worry if you cannot immediately see or deduce the correct answer, histology is usually only one part of the picture when it comes to making a diagnosis of disease.

Complete the end-of-course quiz now.

Open the quiz in a new window or tab then come back here when you're done.

5 End-of-course summary

You have now reached the end of the course and, in this final week, we have introduced you to the concept of pathological changes in tissues, including infection, inflammation, cell death and degeneration, neoplasia and hyperplasia.

These biological processes occur individually and sometimes concurrently in normal tissues and in different diseases. Understanding the normal appearance and function of a tissue allows for the identification of potential abnormalities, which inform the diagnosis of disease.

This course has focused on several important tissues of the body and provided an overview of their structure and function. It has also introduced you to light microscopy and histology within the context of specific diseases.

In the video below David gives a summary of the course and suggests where to take your study of histology and histopathology next.

Video content is not available in this format.

David mentions The Open Science Laboratory where you can explore a collection of 320 annotated histological and histopathological slides.

Tell us what you think

Now you've completed the course we would again appreciate a few minutes of your time to tell us a bit about your experience of studying it and what you plan to do next. We will use this information to provide better online experiences for our learners and to share our findings with others. If you'd like to help, please fill in this optional survey

Where next?

If you've enjoyed this course you can find more free resources and courses on OpenLearn.

Making the decision to study can be a big step and The Open University has over 40 years of experience supporting its students through their chosen learning paths. You can find out more about studying with us by visiting our online prospectus.

You may be particularly interested in our courses and qualifications in Science.

References

Holman, D. W., Klein, R. S. and Ransohoff, R. M. (2011) 'The Blood-Brain Barrier, Chemokines and Multiple Sclerosis', *Biochimica et Biophysica Acta*, vol. 1812, no. 2, pp. 220–30 [Online]. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3005102/ (Accessed 19 April 2018).

British Thyroid Foundation (BTF) (2016) *Your Thyroid Gland* [Online]. Available at http://btf-thyroid.org/information/your-thyroid-gland (Accessed 19 April 2018).

Gottfried, S. (1994) *Human Biology*, St Louis and London, Mosby.

Tortora, G. J. and Grabowski, S. R. (1993) *Principles of Anatomy and Physiology*, 7th edn, New York, HarperCollins.

Villee, C. A. (1989) *Biology*, 2nd edn, Philadelphia, Saunders College Publishing.

Acknowledgements

This free course was written by David Male.

Week 1

Images

Course image: © Likoper / Shutterstock.com

Figure 6: with kind permission of Dr Dimitri P. Agamanolis, M.D.

Figure 7: courtesy of Dr Eva Del Valle Suarez.

Figure 10: © David Male.

Figure 12: © Nicolas Grandjean, Dr Graham Beards/These files are licensed under the Creative Commons Attribution-Share Alike Licence (http://creativecommons.org/licenses/by-sa/3.0/), Bobjgalindo/This file is licensed under the Creative Commons Attribution-ShareAlike 4.0 International Licence (https://creativecommons.org/licenses/by-sa/4.0/), CS99

Tables

Table 1: Image assets: Nicolas Grandjean, Dr Graham Beards/These files are licensed under the Creative Commons Attribution-Share Alike Licence (http://creativecommons.org/licenses/by-sa/3.0/), Bobjgalindo/This file is licensed upder the Creative Commons Attribution-ShareAlike 4.0 International Licence (https://creativecommons.org/licenses/by-sa/4.0/), CS99

Audio-visual

We are especially grateful to Peter Mooney for his tour around the histopathology department at Milton Keynes Hospital.

Week 2

Images

Audio-visual

Week 3

Images

Week 4

Images

Interactive

For providing slides to examine in the Virtual Microscope, special thanks goes to:

Cambridge University, Department of Pathology

University of Leicester

King's College Hospital, London

University College Hospital, London

Milton Keynes Hospital, Department of Histopathology

All other organisations who have donated to the OpenScience Laboratory.

Every effort has been made to contact copyright owners. If any have been inadvertently overlooked, the publishers will be pleased to make the necessary arrangements at the first opportunity.

Don't miss out

If reading this text has inspired you to learn more, you may be interested in joining the millions of people who discover our free learning resources and qualifications by visiting The Open University – www.open.edu/openlearn/free-courses.

978 1 4730 2554 7 (.kdl)978 1 4730 2556 1 (.epub)