Chinese Medical Students **Monday 24 July 2018**
Corpus Christi College **10:00 – 12:00 noon**
Dr A Whyte

Lymphoid cells: Cells of the adaptive immune system

1.0. Aims

To develop an understanding of the locations and arrangements of lymphoid tissue around the body and to relate this to the function of the individual cells within the adaptive immune system.

2. To recognise the individual cell types found within lymphoid tissue.
3. To explore the development and maturation of B and T lymphocytes.

2.0. Anatomy of the lymphoid system

Lymphoid tissue normally occurs as lymph nodes, as nodules in the spleen and as focal collections along mucosal surfaces, such as the upper respiratory and intestinal tracts, where they are called mucosal associated lymphoid tissue (MALT). ANTIGENS enter lymph nodes via the afferent lymph. Antigens reach the spleen from the blood and they reach MALT from the adjacent mucosal surface. Most lymphocytes enter lymph nodes, MALT or spleen from the circulating blood.

Lymphocytes originate from the bone marrow but they can proliferate after leaving it. Different populations home to particular sites in the body.

In **mammals**, **B lymphocytes** undergo some maturation in the **bone marrow,** but in birds maturation occurs in a specialised diverticulum of the cloaca known as the **Bursa of Fabricius**.

The **thymus** is important for the development and maturation of **T lymphocytes.**

Museum Specimens

Thymus, cervical lymph nodes and tonsils - P84.078
From a nine year old boy who died of a cerebral haemorrhage and bronchopneumonia. The pericardial sac has not been opened.

Bursa of fabricus (from a hen) - R84.1003A & R84.1003B

3.0. Entry of antigen to the lymphoid system

The distribution of lymphoid tissue described above ensures rapid contact between **lymphocytes** and their **accessory cells** with potentially harmful agents. Lymphoid tissue is not static; normally abundant in early life, it decreases with

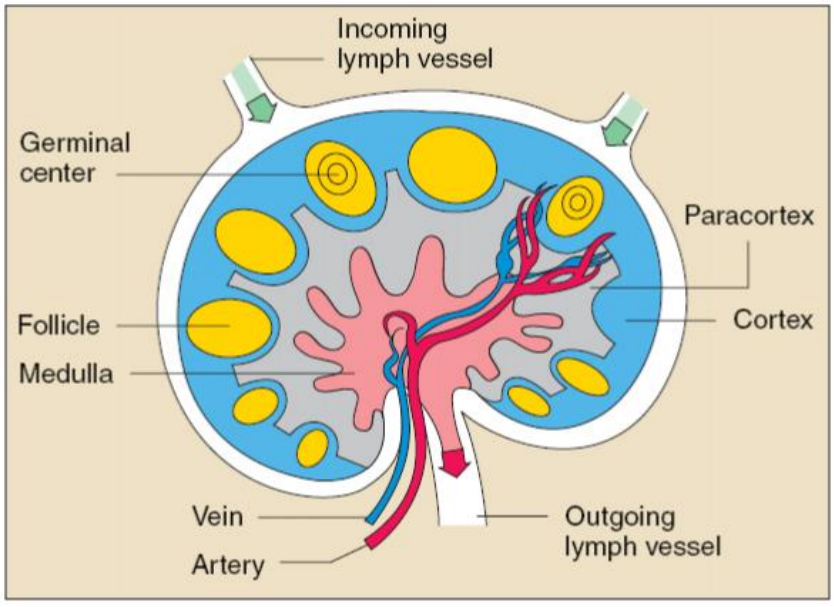
old age. In response to stimulation it can **re-form** and may develop at most sites.

Antigens can be transported free, or phagocytosed and carried by **macrophages,** or minute quantities can be endocytosed by specialized **antigen presenting cells (APCs).** These antigen-carrying cells use the lymph flow to enter nodes. Within lymph nodes they become closely associated with **T lymphocytes**, thus promoting antigen presentation and recognition.

Lymphocytes mainly enter lymph nodes from the blood by means of specialized venules known as **high endothelial vessels,** but they occasionally enter via the **afferent lymph**. High endothelial vessels are lined by enlarged endothelial cells with an increased level of activation. The cells express cell adhesion molecules.

Afferent lymph arrives from nodes upstream, as well as from the tissues. Lymph carries lymphocytes, albeit a smaller number than those that enter via high endothelial vessels, and also carries free antigens. Most importantly, it carries dendritic cells (a type of leucocyte specialized for antigen presentation). Cells destined to recirculate exit via the medulla and efferent lymphatic vessel and return to the bloodstream.

4.0. Histology of the lymphoid system



General structure of lymph nodes: Lymph nodes can be regarded as having a 'cortex' (outer part) and a 'medulla' (inner part). B lymphocytes **aggregate around** follicular dendritic cells to form distinct **FOLLICLES**, which are found in the **cortex**. Within the follicles, some T cells are also present.

Germinal Centre Formation: If any of the B cells within the follicles are activated by antigen in an appropriate environment which includes T cell help, they enlarge and proliferate. The antigen is recognized by their immunoglobulin receptors (i.e. cell surface antibody). Many of the B cells undergo **somatic hypermutation**. Eventually, plasma cells (and also some memory cells) are produced. When this B cell activation occurs, the centre of the follicle appears paler and is now called a **germinal centre**. Here, enlarged B lymphocytes show considerable mitotic activity. B lymphocytes which are *not* selected undergo cell death by apoptosis, and macrophages phagocytose their remnants.

Follicular dendritic cells: The follicular dendritic cells (FDCs) hold **antigen/antibody complexes** distributed along their dendritic (branching) processes. Antigen in this form can only be accessed by B cells with highly

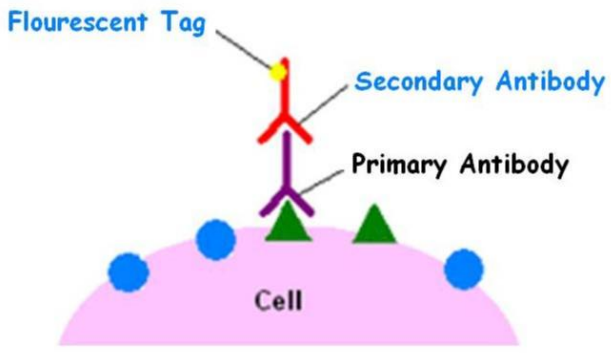
specific immunoglobulin receptors on their cell surfaces. Increased affinity for antigen is achieved by the process of hypermutation; appropriate contact with the FDCs' complementary receptors and stimulation by T cells all contribute to **selection** of B cells with increased affinity for antigen. **(N.B. You are not able to see the follicular dendritic cells in H&E sections.)**

B lymphocytes: Besides the B lymphocytes (described above) found in follicles, small B lymphocytes surround the germinal centres as a dark rim which is referred to as a '**mantle**'. These B-cells are described as "resting" and are not thought to be taking part in the current response. During a humoral (antibody) response, the B lymphocytes become **plasma cells** (plasma cells may occasionally be seen in the medulla of the node, but the medullary cords and sinuses are very difficult to identify in the sections). Their antibodies pass into the efferent lymph and reach the bloodstream. Plasma cells themselves do not circulate in significant numbers and most die after a few days, but memory cells do circulate and a significant number migrate to the bone marrow, where longer lived plasma cells develop. B lymphocytes can also circulate in the blood to reach a site of infection and mature into plasma cells at that site.

T lymphocytes: A small but important number of T lymphocytes (T helpers) are found in the B cell follicles. On the whole, however, T cells predominate in the **interfollicular areas**, sometimes called the **paracortex**. This region is where the antigen presenting cells settle and become interdigitating cells.

4.1. Lymph nodes – NDP Images: H5: 76.661
5.1: 01.229 & 02.153
Glass Slides: H5: 76.661
5.1: 01.229 & 02.153
Image Maps: N_HL_LN2_26; N_HL_LN2_02
(Remember to look first with the naked eye)

You are provided with some sections of normal lymph node stained with H&E, and also **immunostained** with several different antibodies. In order to immunostain a tissue section, the section is first incubated with an antibody (usually from a mouse) against the protein of interest. Then, this antibody is detected by a second antibody (e.g., a goat antibody raised against mouse antibodies). Thirdly, an enzyme can be coupled to this second antibody. This enzyme, in turn, converts a substrate into visible brown insoluble crystals. These brown crystals are deposited specifically in the areas where the (initial) antibody is bound on the tissue section.



Look carefully at the sections, first with the naked eye and then under the microscope, moving from low power (x4 objective) up to the x10 and then the x40 objective. Using the diagram on page 3 and the annotated photosheet available (P1) on your bench, identify the different areas in the lymph node. Draw a labelled sketch of the slide in your notes, ensuring that you include the cortex, the medulla, the sinuses, the paracortex and several lymphoid follicles/ germinal centres. (N.B. The immunostained sections may not be from the same lymph node, so your sketch is to remind you of the principles rather than the exact features of your specific lymph node.)

4.1.1. Lymph nodes - CD3 Immunostaining - T lymphocyte marker

NDP Images: 5.2: 01.230 & 02.154
Glass Slides: 5.2: 01.230 & 02.154
Image Map: N_HL_LN2_08

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD3+ (brown) on your labelled sketch.

Q.1. Which areas of the lymph node have most CD3 staining?

Q.2. Are there any CD3+ cells in the germinal centres? If so, why are these CD3+ cells present here?

Q.3. At high power, which parts of the cells seem to express CD3 (nucleus or cytoplasm/ membrane)?

N.B. CD3 is a protein complex expressed on T cells, which binds to the T cell receptor at the cell surface and plays an essential role in T cell receptor signal transduction.

4.1.2. Lymph node - CD20 immunostaining - B lymphocyte marker

NDP Images: 5.3: 01.231 & 02.155
Glass Slides: 5.3: 01.231 & 02.155
Image Map: N_HL_LN2_13

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD20+ (brown) on your labelled sketch.

N.B. CD20 is a protein expressed on the surface of B cells. Its function is unknown.

4.1.3. Lymph nodes - CD68 Immunostaining - Macrophage/dendritic cell marker

NDP Images: 5.4: 01.232 & 02.156
Glass Slides: 5.4: 01.232 & 02.156
Image Map: N_HL_LN2_22

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD68+ (brown) on your labelled sketch.

Q.5. Where do you see CD68 positive cells in the lymph node?

Q.6. Associated with B cell areas, specialised macrophages are present. Where can you see these?

Q.7. What is the main function of these specialised macrophages?

Q.8. In T cell areas, CD68+ cells are present. What are these likely to be? What is their main function?

Q.9. At high power, can you see any differences in morphology (shape) between the CD68+ cells in T cell areas and those elsewhere in the lymph node? Explain any differences you see.

Q.10. Some CD68+ cells are present in the sinuses of the lymph node. Where have these come from and what is their function?

N.B. CD68 is a protein expressed on the surface of macrophages. It is not, however, entirely specific for this cell type. It is also present on monocytes and dendritic cells. The function of CD68 is unknown.

4.1.4. Lymph node Photo sheet (P5.1)

4.2. The Spleen

Functions: The **spleen filters the blood and can mount an immune response to blood-borne antigens**. It removes particulate debris (such as bacteria) from the blood and effete ("worn out") red blood cells are phagocytosed by its **reticulo-endothelial system** (see below).

Structure: The spleen has a rich blood supply. Two major areas are recognized within it. These are known as **red pulp** and **white pulp** (appears as small white spots to the naked eye).

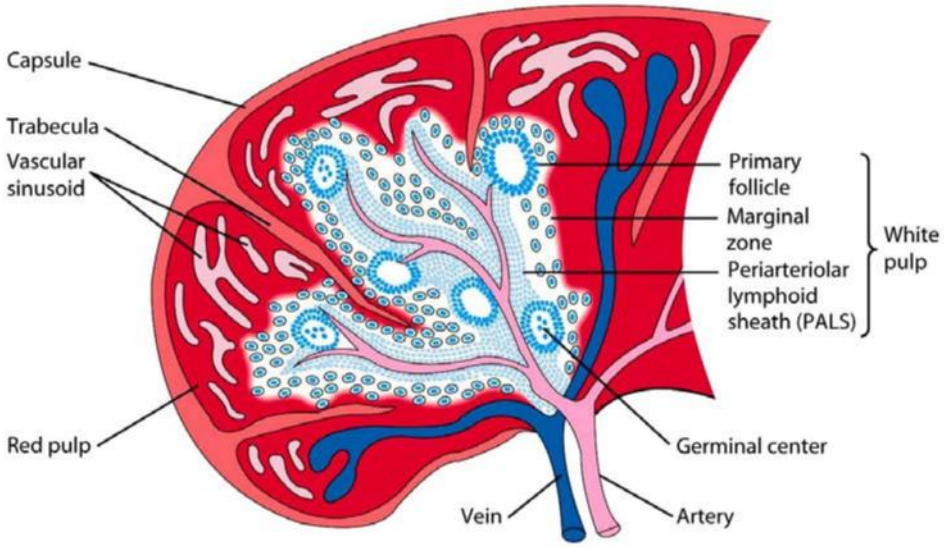
Red Pulp: This is composed of sinusoids (passageways) through which the blood flows. The sinusoids are lined by a type of macrophage and they are said to be part of the "Reticulo-Endothelial System".

Reticulo-Endothelial System: When **monocytes** from the blood enter the tissues, they are called **macrophages**. Macrophages have a wide range of important roles in acute and chronic inflammation, the process of healing and the immune system. Some monocyte/ macrophage populations move about freely, while others (**sessile macrophages**) are attached to the lining of wide blood vessels (**sinuses or sinusoids)** within organs and in lymph vessels in which the

flow is slow. Such sinuses or sinusoids are found in the **liver** and **spleen** and are known as the **reticulo-endothelial system**. Since the cells of the monocyte-

macrophage system capture antigen, it is unsurprising that areas of **lymphoid tissue** exist adjacent to them. Phagocytosis of particulate debris and effete red blood cells by macrophages occurs in the sinusoids (sometimes called the cords of Billroth) in the red pulp of the spleen**.** Macrophages are also resident in many other tissues, for instance the alveolar macrophages in lung and the microglia in the brain.

White Pulp: The white pulp consists of cells of the immune system: lymphocytes and macrophages. Both T and B lymphocyte areas are present within the white pulp. Immune responses can be generated here to antigens taken up from the blood.



4.2.1. Spleen: normal - refer to diagram above and to the annotated image of the spleen on P5.2;

NDP Images: H7: 76.659 & 72.1199
Glass Slides: H7: 76.659; 72.1199
Image Map: N_HL_SP_23

In this section of spleen, try to identify the separate areas of red pulp, in which large numbers of red blood cells are visible within the sinusoids, and white pulp in which numerous small deeply haematoxyphilic (blue staining) lymphocyte nuclei are seen.

Museum Specimens

Spleen: normal - P84.470
From a boy of ten years who died following a brain haemorrhage.

Spleen: normal - P87.224
Unknown
Spleen: normal - P84.078

From a nine year old boy who died of a cerebral haemorrhage and bronchopneumonia.

5.0. Thymus

Function of the thymus: T lymphocytes become activated following the recognition of an antigenic peptide, which is presented to the T lymphocyte on a major histocompatability complex (MHC) molecule by an antigen presenting cell (APC). The antigen presenting cell is usually a dendritic cell, although it is sometimes a B lymphocyte or macrophage. The antigenic peptide is derived from a protein, which has been endocytosed and broken down by the antigen presenting cell into small fragments (peptides). If this protein was a host protein, it is important that T lymphocytes do not exist which could recognize such a self-peptide.
The thymus is responsible for the selection of T lymphocytes that have the ability to recognize host MHC (antigen presenting molecules) with adequate affinity to be useful, but which do not have a *very high* affinity for host MHC and/or self-peptides from host proteins.

Structure of the thymus: A cortex and medulla can be distinguished in the thymus. Like all lymphoid organs, there is a rich vascular supply to allow movement of lymphocytes to and from the thymus. Besides precursors of lymphocytes, specialized thymic epithelium, dendritic cells and macrophages are also present. The thymus is large in the foetus and child, but involutes during adolescence, becoming difficult to identify in the majority of adults.

Processes in the thymus: Precursors of T lymphocytes develop in the bone marrow and make their way to the thymus via the blood. Once in the thymus, they are known as **thymocytes**. These cells are not yet committed to being CD4+ or CD8+ T lymphocytes, but start to express both CD4 and CD8 in the thymic cortex.

Positive selection occurs mainly in the thymic cortex on **thymic epithelial cells** and some thymic dendritic cells. T lymphocyte precursors (thymocytes) are "tested" for their ability to bind to host MHC (regardless of the peptide) to some extent. Those that bind class I MHC retain only CD8 expression, while those that bind class II MHC retain only CD4 expression. Thymic epithelium may be seen in both the cortex and medulla. However, it is believed mainly to play a role in the cortex.

Negative selection occurs mainly in the medulla on **dendritic cells**. Here thymocytes with dangerously high affinity for self peptides are deleted. They receive signals from the APC that cause them to undergo apoptosis.

5.1. Thymus - Photosheet: P5.2,
NDP Images: 56.0323 & 81.0858

Photosheet showing H&E, CD3, CD68 and cytokeratin immunostaining in the thymus.

Q.11. What process takes place in the cortex? Which immunostain supports this?

Q.12. What process takes place in the medulla? Which immunostain supports this?

Q.13. What are the different functions of mature CD4+ T cells and CD8+ T cells? How could you distinguish between them in tissues?

Museum Specimens

Thymus: normal - P84.470
From the same boy as above. The thymus atrophies in later life. (What is its role?)

6.0. Process identification and report writing

6.1. Appendix: normal for comparison

NDP Images: A13 or A15: 76.554 & 84.50
Glass Slides: A13 or A15: 76.554 & 84.50

Note the plasma cells in the lamina propria, **normal lymphoid tissue** in the submucosa forming lymphoid follicles with germinal centres. The appendix may be regarded as a significant collection of lymphoid tissue for the intestinal tract.

6.2. Appendix: abnormal

NDP Images: 5.5: 79.558 & 55.229
Glass Slides: 5.5: 79.558 & 55.229

This is a transverse section of a vermiform appendix from a young person with abdominal pain. Can you make any comments on this? (ask one of us for help)

7.0. Origins of words used to describe the lymphoid system

PREFIX / SUFFIX	ETYMOLOGY
lympho-	Latin: "*lympha*" – water
macro-	Greek: "*makro*" – large
-phage	Greek: "*phagein*" – eat
pino- Greek: "*pinein*" – drink	

Monday 24 July 2018 **Dr A Whyte 14:00 – 16:00**

Acute and Chronic Inflammation

1.0. Introduction

Nature has evolved a variety of sophisticated and subtle processes by which danger to the survival of an organism is recognised, challenged and, hopefully, overcome. These processes constitute two overlapping sets of reactions and their effects and are called innate and adaptive immunity (immunity means protection). Innate immunity is an immediate, albeit relatively non-specific response to danger and is elicited by tissue damage, e.g. at a site of injury or at a focus of infection. The reaction is termed inflammation.

1.1. Acute Inflammation

Acute inflammation is the local response elicited by tissue damage. A characteristic feature is leakage of blood proteins into the tissue (predominantly **fibrinogen**, which is quickly converted to **fibrin**) and the recruitment of leukocytes from the blood. Together these constitute an inflammatory exudate. The first leukocytes to be recruited are **neutrophils** (NPLs), also called polymorpho-nuclear leukocytes (PMNLs) and these are characteristic cellular markers of acute inflammation.

The central aim of this practical is to recognise the characteristic histological features (footprint) of acute inflammation by the identification of neutrophils and fibrinous exudate in pneumonia (acute inflammation in the lung).

As time passes following acute injury other leukocytes are recruited into the tissue, e.g. lymphocytes and monocytes and these give rise to the characteristic pattern of chronic inflammation. These features, along with the processes of organisation of dead tissue and of repair will be dealt with later.

It should be remembered that all of the leukocytes that enter tissues in any form of inflammation are derived from the pool of circulating cells in the blood. The predominant leukocyte in normal blood is the neutrophil. It is readily recognisable by a **poly-lobated nucleus**. When it enters the tissue this characteristic morphology is still recognisable, but, as the cells begin to die, the poly-lobated structure of the nucleus is less obvious (why might this be?).

Neutrophils in Tissue: Image Map: N_CS_NU_09

2.0. Cells of the haemopoietic system:

Normal blood film – H1: NDP Images: 86.547; 96.354
Glass Slide: 86.547; 96.354
Image Maps: N_HL_BF_09

Although most of the classes that deal with the appearance in disease are based around tissue sections, cells can also be visualised in films or smears of biological fluids. Seen this way, whole cells rather than a section through them are represented and it is often easier to recognise details of cellular structures such as the nucleus. Before studying neutrophils in a tissue section, therefore, it is useful to identify them in a blood film (also look at the demonstration boards which detail the origins of these cells and their development).

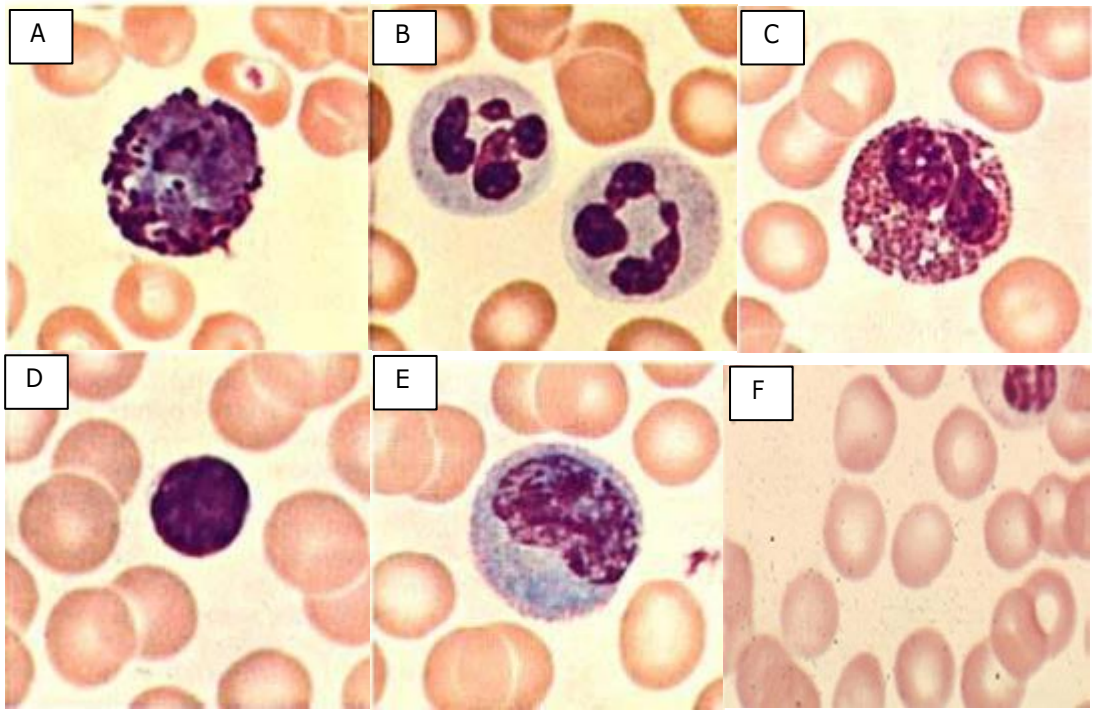
Look at the thin end of the film where the cells are well spread out.

Try to identify:

(1) Red blood cells (RBCs; erythrocytes) which are round, anucleate, and have a pale biconcave centre

(2) Leukocytes, the predominant cell being the neutrophil.

Q14 What are the other cell types present in the blood?



3.0. Acute Inflammation of the lung: Pneumonia

You are provided with sections of normal lung for reference:

NDP Image: Normal Lung - CR8: 70.16A
Glass slide: CR8: 70.16A
Image Map: N_CR_LU_02

and two cases of acute bronchopneumonia. Look for evidence of an acute inflammatory exudate, noting the large number of **neutrophils** and also the presence of **fibrin**.

3.1. Bronchopneumonia – NDP Image: 2.1: 80.226

Glass Slide: 2.1: 80.226
Image Map: A_AI_BP_LU_01

This tissue section was taken from a 30 year-old woman who died after an attack of acute bronchopneumonia.

Note the following features (in comparison to the healthy lung):

- In the centre of this section, viewed with the naked eye, there is a dark, Y-shaped structure - it is a longitudinal section through a bronchus at a branch-point. Is this the normal appearance of a bronchus? Which cells fill the bronchus?

- Note the **patchy** appearance of the alveolar spaces. Which cells fill these spaces? What are their key features?

- Large areas look **red** because of **vasodilatation** and **haemorrhage**. Are the red blood cells contained within vessels (thin walls)? What causes haemorrhage?

- The **bronchial epithelium** has become detached in places. Why?

- Note the pale pink homogeneous material that is **fluid exudate** containing protein (particularly fibrin) in some alveoli. Why might this impair breathing?

The **patchy pattern** of inflammation involves the **bronchi** and the adjacent lung parenchyma; it is therefore called '**Bronchopneumonia'**

[Note: black granules of inhaled carbon, often around the bronchi and within the lung macrophages which is normal. These deposits are increased greatly in individuals who smoke.]

3.2. Lobar pneumonia – NDP Image: 2.2: 65.140; 62.38

Glass Slide: 2.2: 65.140; 62.38

This tissue section was taken from a 53 year old homeless person who died, having been breathless for a few days, and who had not sought any medical attention.

Note the features in comparison to the case of bronchopneumonia (4.1):

-

- Fibrin within the alveoli appears as delicate threads

Diffuse infiltration contrasting with the patchiness of bronchopneumonia (notice that although all the alveoli contain an inflammatory exudate, the alveolar walls are intact)

- Fibrin over the pleura becomes compacted as a result of respiratory movements (**fibrinous pleurisy**)

Lobar pneumonia refers to a rapidly spreading inflammation which can develop as a result of infections by bacteria, particularly those which have thick capsules, e.g. pneumococci, which have polysaccharide capsules. The lung parenchyma quickly fills with proteinaceous fluid and leukocytes and when the infection reaches the pleura, this in turn becomes inflamed and fibrinogen leaks from blood vessels onto the surface.

Museum Specimens
Lung: Lobar Pneumonia - 21.53
The lower and middle lobes are affected with a fibrinous membrane coating the pleura. No clinical details available.

Lung: Lobar Pneumonia - 28.205
The upper and part of the lower lobe are affected and there is extensive pleurisy. A case of pneumococcal pneumonia and septicaemia which developed in a 34 year old woman following childbirth.

3.2. Lobar pneumonia – special stain (Trichrome)

NDP Image: 2.3: 62.38 & 56.36
Glass Image: 2.3: 62.38

This special stain is a mixture of three dyes and is used to stain **fibrin scarlet**. (Look for the red network in the air spaces). **Connective tissue** and other elements are **blue**; red blood cells vary in colour, from orange to yellow.

4.0. Questions

Think about the following questions:

Q15 Where in the body are neutrophils formed? What route did the neutrophils take to reach the lumen of the bronchus?

Q16 How long do leukocytes remain in the blood? What happens to them eventually?

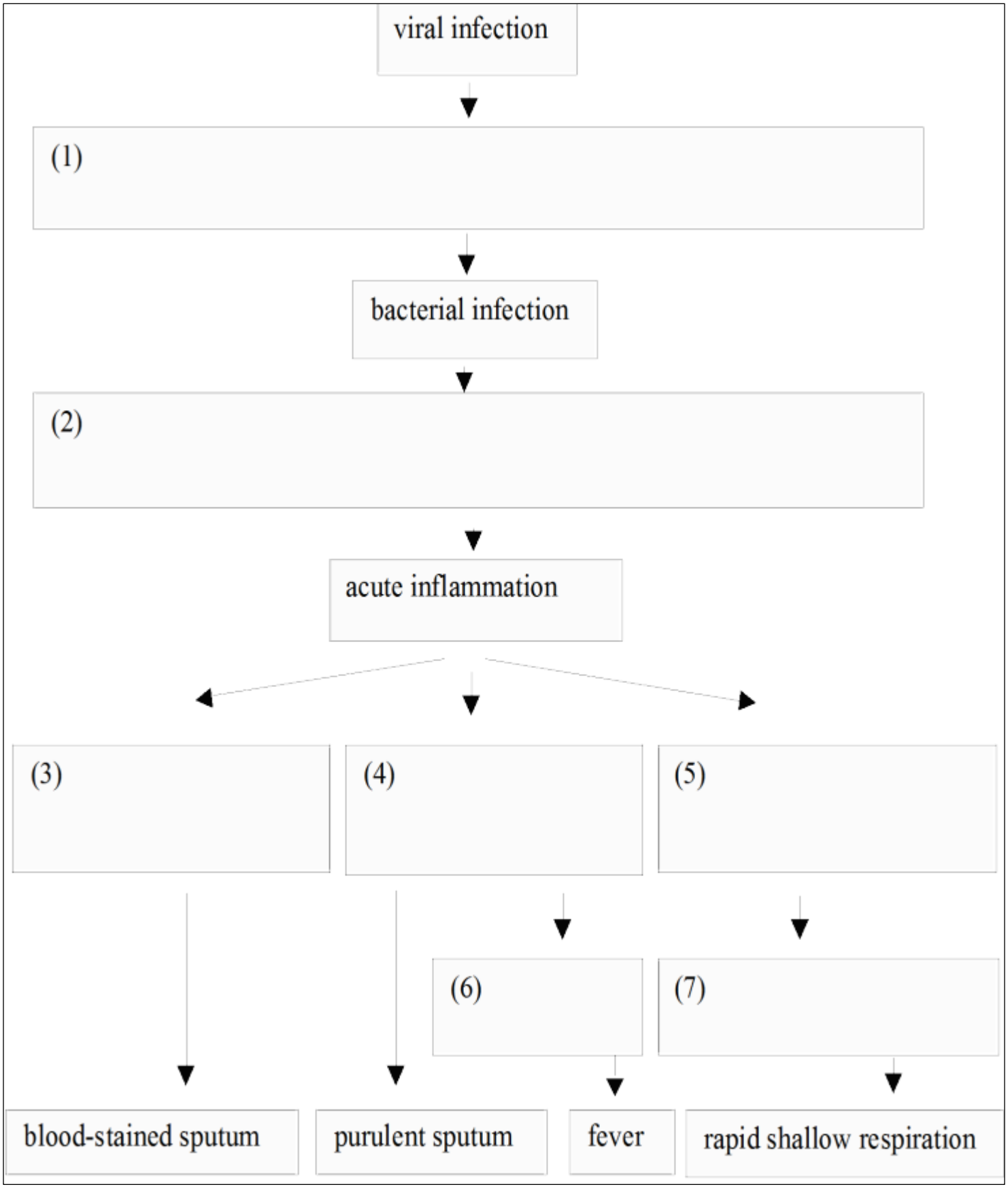
Q17 What would their future have been, had the tissue not been harvested?

Q18 Are there deleterious as well as beneficial consequences of this degree of neutrophil extravasation and activation?

Q19 How do you suppose a viral infection (such as influenza) of the bronchial epithelium may increase susceptibility to bacterial bronchopneumonia?

Now build up a picture of how tissue reactions to injury are initiated and how they produce effects that are recognised as symptoms (i.e. the things that the patient identifies) and the signs (i.e. the things that can be demonstrated objectively by clinical examination or various imaging methods). Fill in the empty

boxes in the following flow chart, indicating the factors responsible for the progression from one stage to the next:


viral infection
(1)
bacterial infection
(2)
acute inflammation
(3)
(4)
(5)
(6)
(7)
blood-stained sputum
purulent sputum
fever
rapid shallow respiration

STOP HERE...

Chronic inflammation

2.0. Introduction

Chronic inflammation describes a process of prolonged duration with **tissue destruction, inflammation and healing** all proceeding simultaneously.

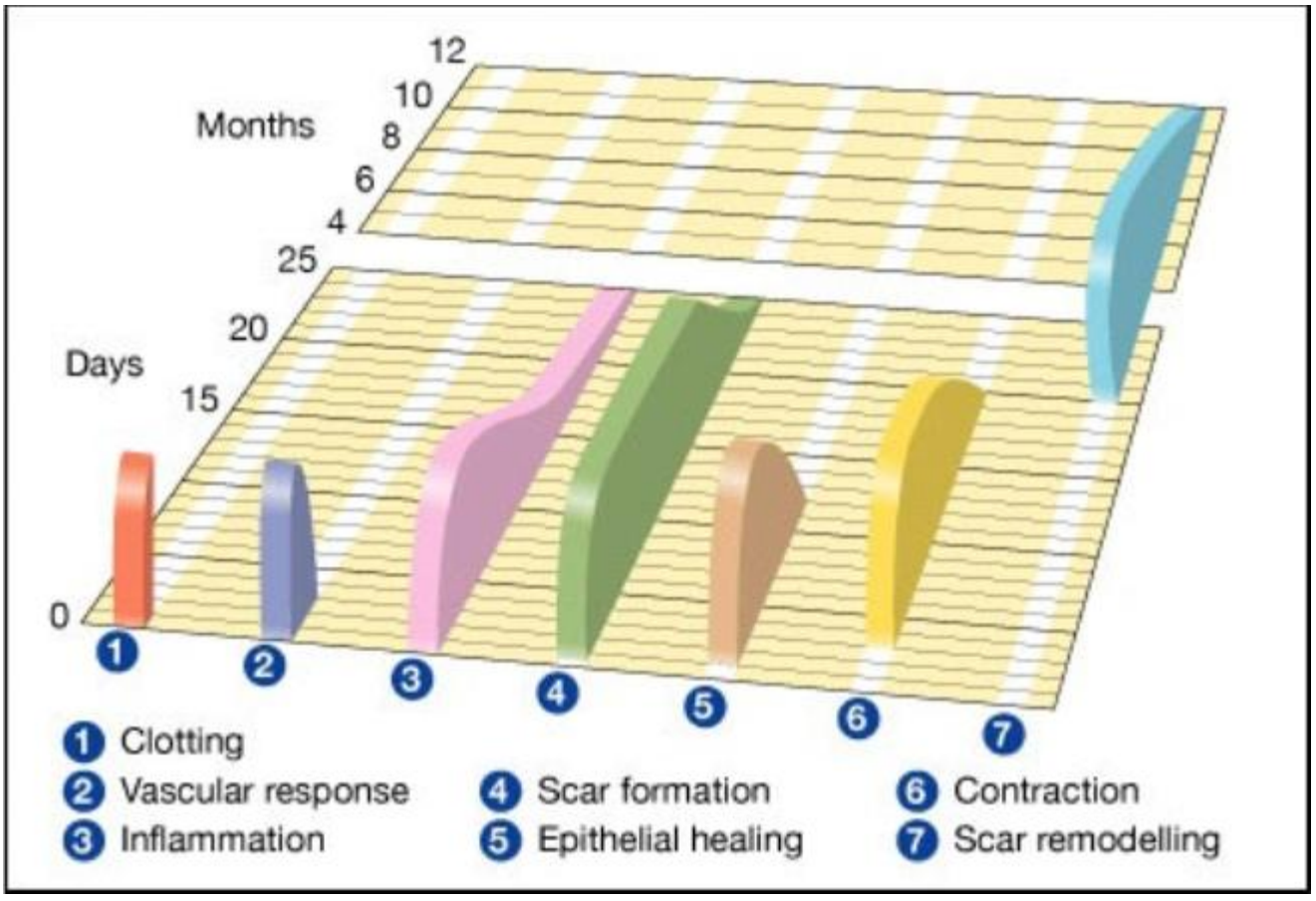
Chronic inflammation may:

1. follow acute inflammation
2. result from repeated bouts of acute inflammation
3. be a smouldering, low-grade response to persistent infections, sustained chemical injury, or long-term exposure to non-degradable, toxic substances
4. be associated with harmful immune responses, such as hypersensitivity (e.g. allergic reactions) and/or autoimmunity.

The key features and cellular components of chronic inflammation include:

A. tissue destruction;
B. infiltration by macrophages, lymphocytes and plasma cells (occasional eosinophils);
C. repair by granulation tissue (capillaries & fibroblasts) laying down fibrous tissue (mostly collagen), thus producing a scar – this process is also called organisation.

In ideal situations, dead cells and inflammatory exudate would be completely cleared by macrophages, a process called **Resolution**. Sometimes this is not possible and other responses are set in motion. This leads to **Organisation**, which is the gradual conversion of the unwanted non-viable tissue into fibrous tissue – in lay terms a scar. The entire process is often described by the generic term **Repair**. Accompanying this process there may also be replacement of damaged epithelium by **Regeneration** to form new epithelium to cover a surface defect. Thus, the process of **Healing** involves a combination of regeneration and repair.



In chronic inflammation, **monocytes** in the blood are recruited to the site, where they mature in the tissue to form **macrophages**. **Lymphocytes** also enter the tissue. The macrophages secrete cytokines that are chemoattractant for **endothelial cells** and **fibroblasts**. The endothelial cells participate in the formation of new blood vessels (**angiogenesis**) that provide oxygen and nutrients to the area, whilst the fibroblasts synthesise the collagenous fibrous tissue. The lymphocytes respond to persistent foreign antigens and the B cells differentiate into **plasma cells** that synthesise immunoglobulin (antibody). This combination of capillaries and fibroblasts forming collagenous fibrous tissue is referred to as **granulation tissue**.

Thinking in 3-dimensions you can picture granulation tissue as the new formation of a complex maze of tiny capillary vessels made by the endothelial cells (angiogenesis). Macrophages clear debris, but at the same time macrophages secrete mediators that promote the growth of new capillaries and recruit and stimulate fibroblasts, forming granulation tissue. The capillaries establish a temporary circulation to the area, with beneficial effects, which include bringing oxygen and nutrients. The migrating fibroblasts synthesize collagen, forming the fibrous tissue to fill in the space left by clearance of damaged tissue. You could think of the granulation tissue as providing a 'bridge' between unwanted necrotic tissue and inflammatory exudate on the one hand and the final repair on the other. Usually the fibrous tissue forms gradually, the early scar grows to fill the space to be repaired, then eventually there is remodelling of the fibrous tissue and it shrinks to form a mature scar.

Macrophages are the central figures in chronic inflammation, as they can interact with lymphocytes, secrete factors involved in tissue injury (reactive oxygen species, proteases, inflammatory mediators), clear debris, as well as co-ordinate the granulation tissue response by inducing angiogenesis (via secretion of FGF & VEGF) and recruiting fibroblasts for synthesis of fibrous tissue (by secretion of FGF & TGF-beta).

3.0. Organisation of imflammatory exudate

3.1 Organising pericarditis of the heart (H&E and trichrome stains to show fibrin) – NDP Images: 3.1: 83.204; 3.2: 83.204 (Trichrome)

Glass Slides: 83.204; 3.2: 83.204 (Trichrome)
Photos: P3.1A & P3.1B

This heart was examined at post mortem (photos P3.1A and P3.1B). You can see the major vessels clearly. Note on the surface of the heart there is a thin layer of **fibrinous exudate**.

The pericardium refers to the thin layer of cells forming a sac around the heart, the cardiac equivalent of the peritoneum. Inflammatory exudate has formed over the heart. The section shows the thin atrial wall covered by a layer of eosinophilic, **compacted fibrin**. Growing into it from the base are new capillaries springing from the pre-existing blood vessels. Between them are many macrophages with a few lymphocytes, plasma cells and fibroblasts.

This pericarditis was caused by metabolic injury (an example of sustained chemical injury) resulting from renal failure. (Why retained waste products in the blood commonly injure the pericardium specifically is a mystery).

Q20. What is the special name given to this combination of fibroblast cells and new blood vessels?

Museum Specimens

Fibrinous pericarditis of the heart due to uraemia (renal failure) - P77.770

From a woman, who died of renal failure at the age of 60. Certain metabolic products (e.g. urea) in excess can cause tissue damage. Although it is not clear why, the pericardium is unusually susceptible. The presence of damaged or dead cells causes the release of inflammatory mediators, which affect blood vessels causing dilatation, increased permeability and the other processes of acute inflammation.

Fibrinogen escaped with other plasma proteins and was converted to fibrin, forming this yellowish film over the surface of the heart.

Fibrino - purulent pericarditis - 21.60

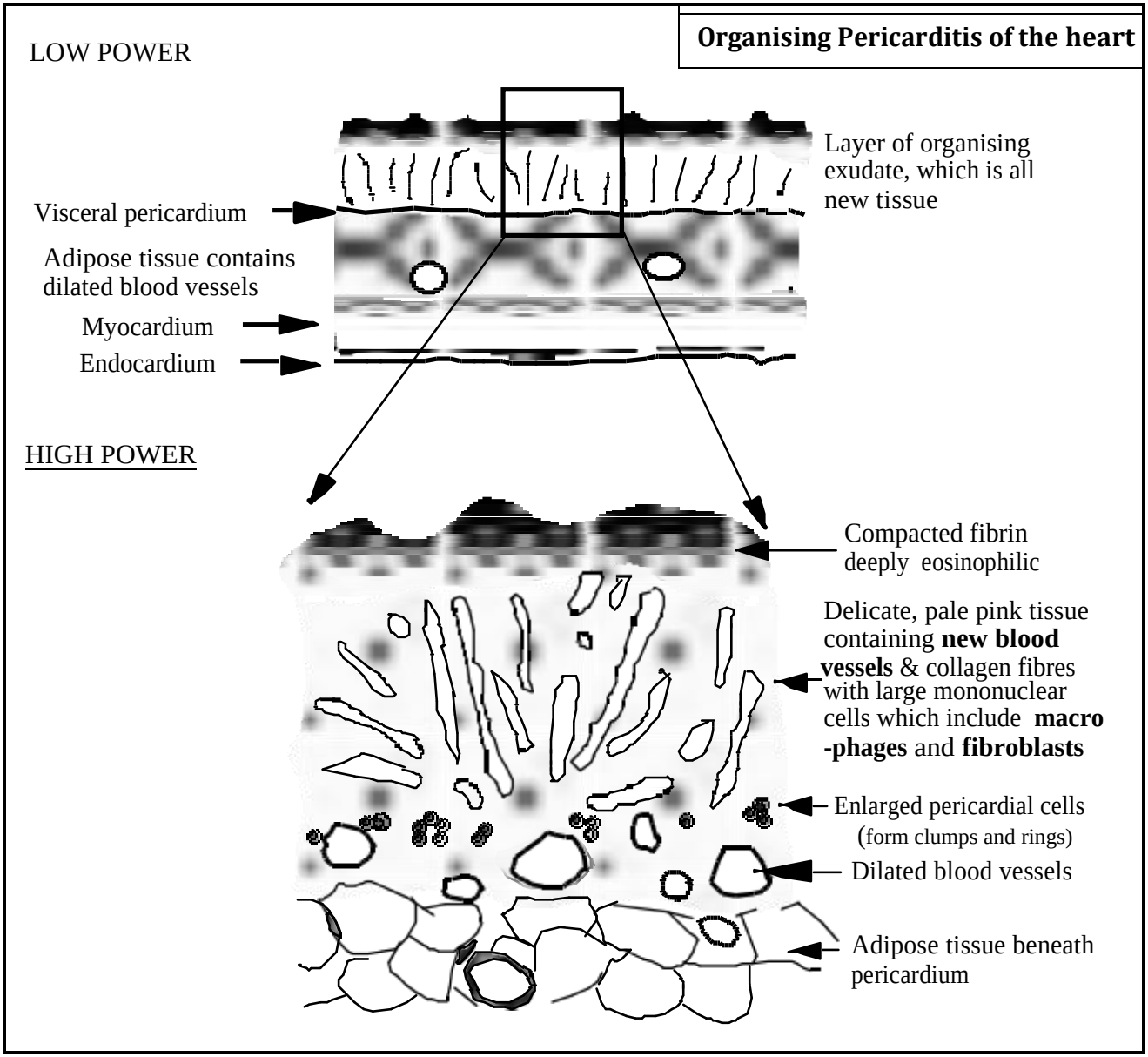
No history is available.

Acute fibrinous pericarditis - P77.693

This followed a recent myocardial infarction. The presence of necrotic cells causes release of inflammatory mediators, which affect surviving blood vessels causing dilatation, increased permeability and the other processes of acute inflammation. Fibrinogen escaped with other plasma proteins and was converted to fibrin, forming this yellowish film over the surface of the heart.

Fibrino - purulent pericarditis – 28.200

No history is available



3.2. Organisation of exudate within the lung (H&E and Elastic Ponceau-S)

NDP Images: 3.3: 65.93; 3.4: 65.93 (Elastic Ponceau-S)

Glass Slides: 3.3: 65.93; 3.4: 65.93 (Elastic Ponceau-S)

Image Maps: A_CI_ON_LU_57; A_CI_ON_LU_13

Lung tissue several weeks after a case of pneumonia which failed to resolve.

Lung has many small air spaces, which may fill with inflammatory exudate during pneumonia. Macrophages often clear the exudate completely, leading to

resolution, but if the injury is prolonged, the exudate may not be cleared rapidly by absorption, coughing and macrophages. Then the inflammatory exudate undergoes repair / organisation with conversion into fibrous tissue. The **basic process** is the same as before, **only the pattern is different**.

New capillaries grow inwards, followed by fibroblasts. As the exudate shrinks, fibroblasts arrange themselves over it and secrete a 'basketwork' of collagen fibres. A special stain, Elastin Ponceau-S, can make this clearer. The pre-existing framework of lung tissue contains **elastic fibres**, which stain **black** with the stain, whereas the newly synthesised **collagen fibres** are **dull red.** Look for the outline of the pre-existing alveolar walls lining the air spaces in **black** (as they contain elastin fibres), whereas within some of the alveolar spaces there are loose **'baskets' of reddish collagen fibres** resulting from organisation of exudate (see diagram). Other alveolar spaces have been cleared of exudate and contain only macrophages.

Q21. What would be the effect on lung function of permanent thickening of the alveolar walls by fibrous tissue?

Museum Specimens
PLEURA: fibrous adhesions - R60.393
Delicate fibrous adhesions bind the layers of the pleura together. This is the end result of a previous pleurisy (inflammation of the pleura).

PLEURA: fibrous adhesions - P80.809
Fibrous adhesions bind the surface of the lung to the pleura overlying an adjacent rib. Many such adhesions were found incidentally in an elderly man, probably due to past episodes of pneumonia with pleurisy.

Numerous fibrous adhesions are present involving the parietal (outer layer of the two layers of) the peritoneum. Some areas of the visceral peritoneum (the inner layer which overlies and adheres tightly to the small intestine,) also appear dull, due to the presence of a delicate, off-white layer of fibrinous exudate. Here we see both fibrinous and fibrous material. Which is likely to have come first chronologically?

3.3. Organising purulent exudate forming an abcess in adipose tissue.

NDP Images: 3.5: 89.757 & 72.667
Glass Slides: 3.5: 89.757 & 72.667
Image Maps: A_CI_AB_SK_08; A_CI_AB_SK_37

The most conspicuous feature is an irregular central area (often slit-shaped) of **purulent exudate** (central pus), mainly **neutrophils**, with a few **macrophages**

phagocytosing them. There are some new capillaries running through the surrounding paler tissue of the abscess wall. The capillaries are made up of large, plump endothelial cells surrounding vascular spaces and you may have difficulty

in recognizing them at first. Around the edges, the process of **organisation** is occurring and there is fibrous tissue (collagen laid down by fibroblasts), forming the abcess wall that separates the central pus from the surrounding adipose tissue. A smaller number of **chronic inflammatory cells (lymphocytes and plasma cells)** can be seen in the abscess wall, including a few lymphoid aggregates (in some but not all sections). **This is another example of chronic inflammation following on from acute inflammation**. The chronic inflammation then leads on to **repair** in a sequential process.

Q22. What is the central cell co-ordinating the process of organisation of angiogenesis with fibrosis?

4.1. Repair of a skin wound – a common example of healing

4.2. A recent wound of the skin

NDP Images: 3.6: 87.1080 & 81.158
Glass Slides: 3.6: 87.1080 & 81.158
Image Map: A_CI_HW_SK_11

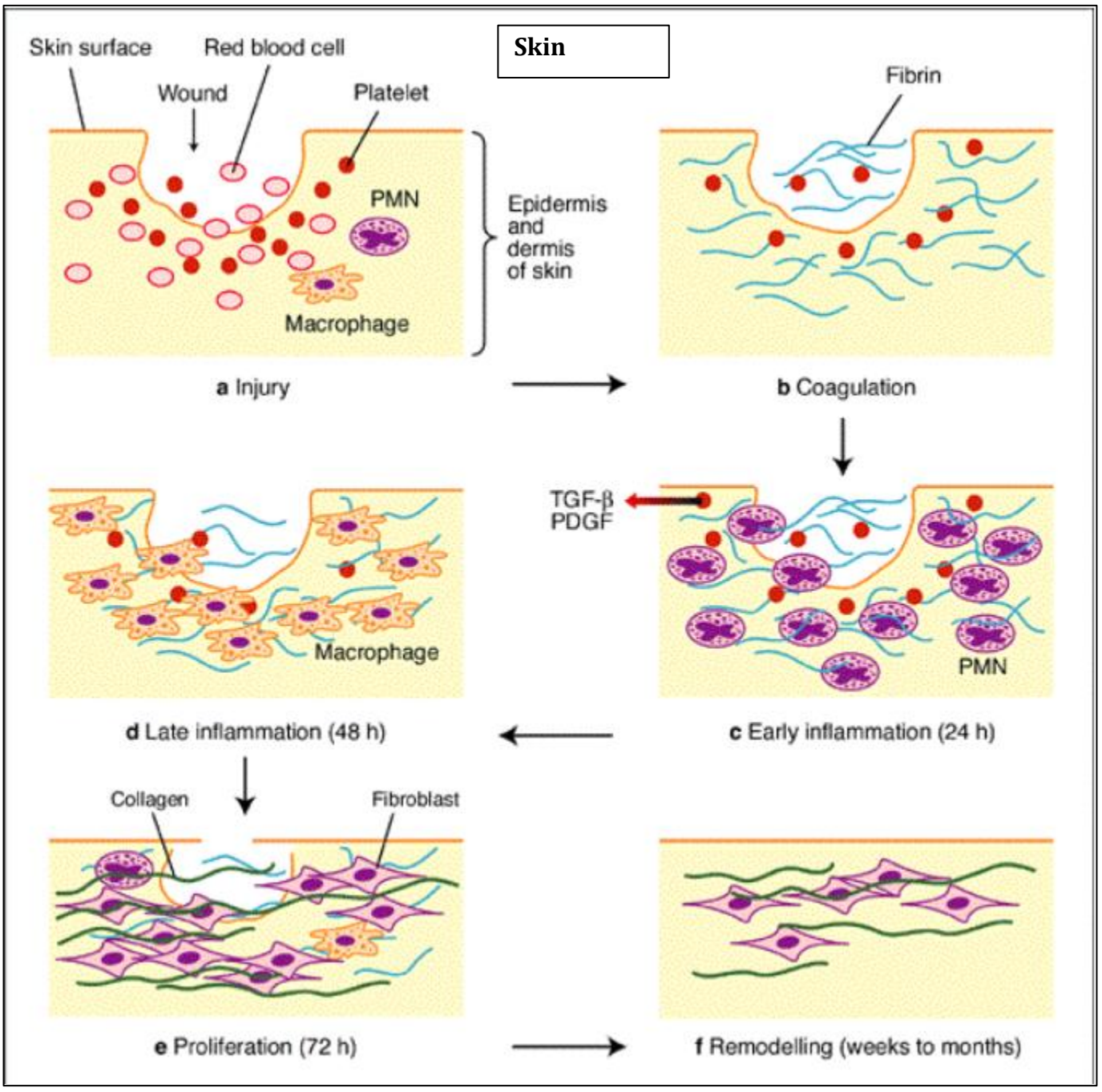
Seventeen days earlier, what was thought to be a pigmented mole had been excised from the shoulder of a 38 year old woman by her G.P. It proved to be a type of skin cancer known as a malignant melanoma, which develops from the melanocytes of the epidermis. Therefore a wider excision was done and the original sutured wound is included in this section (an example of **skin healing**, [see diagrams below] in which the two sides of the wound are stitched together with only mild tissue damage and mild inflammation).

The exact appearance of the wound varies in different sections, but the regenerated epidermis has a flat inferior border, lacking normal rete ridges. Where the skin edges were brought together, there is irregularity and islands of epidermis have been pushed downwards into the dermis. This contains some apparently empty areas, which contain **oedema fluid**.

In the superficial part of the wound, **fibroblasts are beginning the repair process**. Deeper in, are pieces of suture material, (brown or refractile), surrounded by neutrophils and also, some large, multinucleated macrophages. The latter are formed by the **fusion of macrophages** and are called **foreign body GIANT cells**, responding to the suture material (acting as a foreign body that is not easily phagocytosed or broken down by ordinary macrophages).

Q23. Where is the REPAIR taking place? What cells types are involved in REPAIR?

Q24. Where is the REGENERATION taking place? What cells types are involved in REGENERATION?



Do not confuse the terms **fibrinous** and **fibrous:**

Fibrinous exudate = fibrinogen leaking from blood vessels forms fibrin threads

Fibrous = fibroblasts synthesising and secreting collagen fibres into the extracellular matrix

Please examine photograph P3.2: Small intestine, examined at post mortem.

Numerous fibrous adhesions are present involving the outer layer (peritoneum) of the intestines. There are several off-white bands of fibrous material, which adhere tightly to the small intestine. Sometimes the intestines may twist around such adhesions and become obstructed. This is a good example of how previous injury has

healed by repair forming adhesions.