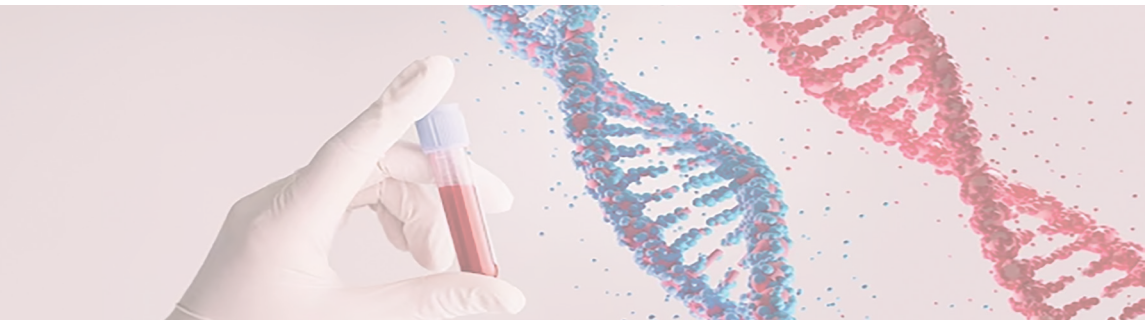

Chapter 1

Introduction to Pathology

LEARNING OBJECTIVES

After the completion of the chapter, the readers will be able to:

- Develop a clear idea about the field of pathology.
- Understand the importance of studying pathology.
- Know the important branches within pathology.
- Awareness of important terms used by pathologists,used throughout this book.
- Understand various staining techniques and their utility.

CHAPTER OUTLINE

- Introduction
- Aspects of Disease
- Important Terminology in the Study of Pathology
- Historical Background
- Importance of Studying Pathology
- Branches of Pathology
- Histopathological Examination

KEY TERMS

Cytopathology: Cytopathology refers to specialized diagnostic procedures for studying individual cells removed from tissues.

Disease: A derangement in the structure/function of an organ or system adversely affecting a part of or the entire body.

Histopathology: Histopathology is the diagnosis and study of diseases of the tissues, and involves examining tissues and/or cells under a microscope.

Microtome: A microtome is a specialized precision cutting tool that cuts sections from a block of implanted tissue repeatedly and accurately.

Pathology: Pathology (from the Greek word *pathología*, meaning the study of suffering) refers to the speciality of medical science concerned with the cause, development, structural/functional changes, and natural history associated with diseases.

Sectioning: Sectioning refers to cleanly and consistently cutting paraffin embedded or frozen tissue into a thin slice.

INTRODUCTION

Pathology is the branch of medicine which deals with the study of disease. This includes identification (diagnosis) of the patient's disease, recognizing the most likely causes, understanding the underlying cellular and molecular mechanisms leading to disease development and predicting treatment (therapeutic) response.

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ASPECTS OF DISEASE

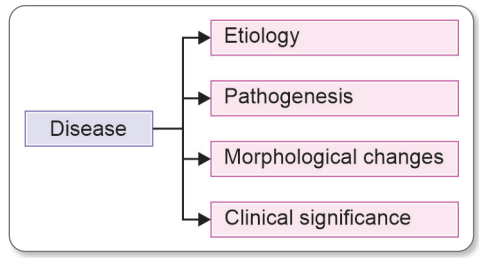

Figure 1.1: Four major aspects of pathology

The four aspects of a disease which comprise pathology are as follows (Fig. 1.1):

1. **Etiology:** It is referred to as 'cause' of a disease.
2. **Pathogenesis:** It is a process/mechanism leading to disease.
3. **Morphological changes:** The structural alterations in cells or tissues that are often characteristic of the disease process.
4. **Clinical significance:** It is referred to as functional consequence of morphological changes.

Advancements in pathology, especially over the last decade have been tremendous with the advent of molecular diagnostics and the incorporation of genetic testing. The pathology laboratory serves as the ultimate link between clinical medicine and experimental science, thus making it one of the most exciting and innovative areas to work in for a healthcare professional. Some of the most critical questions essential for successful management of patient disease are answered in the pathology laboratories.

Before proceeding, it is important to know about the key terminology that has been used throughout the text, commonly used in all healthcare settings, especially in the context of pathology and diagnostics.

IMPORTANT TERMINOLOGY IN THE STUDY OF PATHOLOGY

- **Disease:** A derangement in the structure/function of an organ or system adversely affecting a part of or the entire body.
- **Symptoms:** The physical or mental disturbance experienced by the patient himself/herself, indicating the presence of disease, e.g., fatigue, vomiting and shortness of breath.
- **Signs:** Evidence of disease detected by the physician, e.g., an enlarged liver.
- **Diagnosis:** To know/identify the disease.
- **Lesion:** Changes in cells/tissues/organs occurring due to disease that is the actual pathology, e.g., a skin boil.
- **Etiology:** The why of disease or the cause of disease.
- **Pathogenesis:** The how of the disease or the mechanism of how a disease develops.
- **Etiopathogenesis:** To understand the cause and mechanisms behind disease.
- **Morphology:** The visual study of the structural features of a disease. This can be macroscopic or microscopic.
- **Gross examination/macroscopic features:** The structural changes seen in an organ/lesion in a diseased state, visualized *by the naked eye*. For example, the presence of an enlarged kidney.
- **Microscopic examination:** The structural changes seen in cells/tissues/organs in a diseased state, visualized *under the microscope, e.g., fatty change of the liver*.
- **Light microscope:** The most commonly used type of microscope which uses visible light to magnify structures otherwise invisible to the naked eye.
- **Electron microscope:** A type of microscope using a beam of accelerated electrons rather than visible light to visualize structures after enhanced magnification. This is used to examine structures not seen with the light microscope such as mitochondria, endoplasmic reticulum.
- **Ultrastructural features:** Features of subcellular organelles seen using an electron microscope.
- **Prognosis:** To predict the outcome of disease on the basis of the clinical/diagnostic features.
- **Therapy/Treatment:** Steps taken to cure/bring relief to a diseased condition.

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HISTORICAL BACKGROUND

- **Hippocrates** (460–370 BC) is considered the Father of Modern Medicine. He proposed the modern theory of disease.
- **Giovanni Morgagni** (1682–1771) described the finding of over 600 autopsies. He correlated his pathological findings with the clinical features exhibited by the patients prior to their demise.
- **Antonie van Leeuwenhoek,** 'the Father of Microbiology', used handcrafted microscopes to observe and report on what he described as animalcules.
- **Robert Hooke** (1636–1703) later used the microscope to document the existence of 'little boxes' or cells.
- **Thomas Willis** (1621–1675) pioneered the best qualitative analysis of urine at this time. He was the first to notice the characteristic sweet taste of diabetic urine.
- **Richard Lower** (1631–1691) was the first to perform direct transfusion of blood from one animal to another.
- Moving on to the nineteenth century, many scientists were making great strides in bacteriology, microbiology and histology. Improvements in the microscope allowed further exploration of the cellular and microbial worlds.

IMPORTANCE OF STUDYING PATHOLOGY

- **Diagnosis of disease:** The word "diagnosis" has been derived from the Greek root word (dia+ gignosko), which means *to know apart or to thoroughly know.* The most important role of the pathologist is to diagnose or identify a disease. This is done by testing samples taken from patients (blood/sputum/tissue/pus/fluids, etc). Sometimes, the clinician suspects a particular disease on the basis of the patient's symptoms and signs, which requires confirmation, whereas in other cases, pathologists give a diagnosis which was not suspected at all.
- **Understanding the cause of disease:** Disease can be caused due to any of or a combination of multiple factors, like infectious agents, genetic mutations, deficiency or excess of hormones/nutrients, reduced blood supply, etc. In some instances, removal of an injurious agent or addition of a specific nutrient can completely cure disease. For example, supplementation of vitamin C can cure scurvy. Identifying the cause is thus important, as appropriate therapy can be given ensuring optimum management.
- **Understanding the mechanisms of disease:** This is essential for developing pharmacotherapeutic and other dietary/lifestyle interventions which can aid in better treatment.
- **Predicting the course of disease:** With various advances in the field of molecular genetics, we have entered the era where we can also predict the course of disease or *prognosis* in different patients.
- **Identifying the molecular profile of a disease:** This is of increasing relevance, especially in the field of cancer therapy. Scientists observed that patients with a similar tumor diagnosis, e.g., breast carcinoma were performing differently with treatment. Different molecular types of various tumors have now been described enabling targeted therapy aiding in better outcomes.
  Understanding the fundamentals of both pathology as well as genetics is critical for managing patients well as well as predicting treatment outcomes and is an essential foundation to become a successful nursing practitioners.

BRANCHES OF PATHOLOGY

- **General pathology:** General pathology deals with understanding the general mechanism of injury in cells and tissues, as well as the body's response to these injurious stimuli.
- **Systemic pathology:** The study of pathological changes in disease specifically involving a particular organ/body system, e.g., myocardial infarction (heart attack).

- **Histopathology:** The study of pathological changes occurring in tissues, seen by examining both gross and microscopic features.
- **Surgical pathology:** The study of pathological changes occurring in tissues—either small pieces (biopsies) or entire organs/parts (resection specimens) during the course of surgery to remove a disease.
- **Cytopathology/cytology:** The study of pathological changes at a cellular level. It involves examination of fine needle aspirates (FNAC)/fluids into which cells are shed off (effusion cytology).
- **Hematology:** The study of pathological changes occurring in the blood and/or bone marrow as part of a disease process.
- **Autopsy:** The study of pathological changes in a tissue/organ which is removed after the death of a person for evaluation with the aim of understanding the cause of death.
- **Forensic pathology:** This involves the application of pathological techniques to aid in the investigation of crime. This includes autopsy work, DNA studies for confirming parentage, identity and toxicological study of samples where poisoning is suspected.
- **Immunopathology:** The study of pathological changes, specifically in diseases caused by abnormalities in the functioning of the immune system.
- **Clinical pathology:** The branch of pathology dealing with the study of body fluids including blood, semen, urine, cerebrospinal fluid with a specific clinical context.
- **Chemical pathology:** Chemical pathology deals with the biochemical basis of disease and the use of biochemical assays for screening, diagnosis, prognosis, and management.
- **Genetic pathology:** It includes cytogenetics, molecular genetics and biochemical genetics. Cytogenetics involves the microscopic analysis of numerical and structural chromosomal anomalies such as increase or decrease in the number of chromosomes. Molecular genetics deals with analysis of structure and function of genes at the molecular level. Biochemical genetics deals with evaluation of molecular aspect of genetic evolution, mutation, gene action and regulation.
- **Molecular pathology:** The study of detecting and diagnosing pathological changes at the level of DNA/RNA/proteins.

HISTOPATHOLOGICAL EXAMINATION

Steps of Histopathological Examination

Specimen Receiving

A histology requisition form with complete patient information, clinical findings, a provisional clinical diagnosis, and previous investigations must accompany every tissue specimen brought into the surgical pathology lab. The individual accessing the specimen must match the information on the requisition form and specimen container. A unique number should be given to each specimen in order to identify it.

Gross Examination

The specimen should be subjected to a thorough examination in a clean area with good ventilation, adequate lighting, and convenient tissue disposal facilities. Gross examination entails carefully inspecting the item, describing it on the requisition form, and packing it entirely or partially into a small plastic cassette that is then placed in an appropriate fixative.

Fixation

Fixation of tissue can be done by physical or chemical means. Physical methods include heating, microwaving and cryopreservation. Chemical fixation is achieved by immersing the specimen in fixative. Fixation is done to provide stability to the tissues for easy handling.

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MUST KNOW

Oxidizing agents: It includes potassium permanganate, potassium dichromate, osmium tetra-oxide. They have a definite action such as osmium tetra-oxide is used for electron microscopy.

Tissue Embedding

It is a process of infiltrating or surrounding the tissue with a medium, which is similar in density to that of the tissue. Paraffin wax is the most commonly used followed by celloidin, nitrocellulose and various type of resins. It can be done manually or by automation. Paraffin-embedded tissue can be sectioned at a thickness varying from 4 µm to 10 µm.

Sectioning

Once the tissue has been embedded, it must be sliced into sections with appropriate thickness. Microtomes are the mechanical devices used for cutting uniform section of tissue of desired thickness (Fig. 1.2).

Figure 1.2: Microtome for tissue cutting

Staining

The staining process uses a variety of dyes to check their ability to stain cellular components. Hematoxylin and eosin (H&E) stain is used routinely. The sections should be "deparaffinized" by treating them with xylene to alcohol and then with water. Routine staining is referred to as Hematoxylin and eosin (H&E) stain, special stains are employed in specific situations according to the diagnostic needs.

Visualizing tissue and cell architecture requires the use of histological staining. There aren't many natural colors in living or fixed cells, and there aren't many variations in the refractive indices of their individual sections. Pathologists and scientists can view tissue morphology (structure) or search for the existence or prevalence of specific cell types, structures, or even microorganisms under a microscope by staining tissue sections.

Both standard and unique staining are used in histology. The H&E stain is the primary example of routine staining. Special staining is the term used to describe additional staining techniques to the standard H&E stain that provide the precise information needed by researchers or pathologists.

Routine Staining

Tissue-based diagnosis is built on routine H&E staining. The nucleus and a few other components are stained blue, and the cytoplasm, and eosinophilic structures are then counterstained with an aqueous or alcoholic solution of eosin for a preliminary microscopic examination of the tissues. If used correctly, this method can offer extraordinary insights about tissue composition and cell structure. For tissue-based diagnosis, particularly in the identification and categorization of cancer, this information is crucial. The primary technique in histology is H&E staining, which is also known as the "gold standard" for brightfield microscopy.

Special Stains

In addition to routine histopathological evaluation which is performed with hematoxylin and eosin staining (commonly referred to as H&E staining), a number of ancillary or "special" stains are used by pathologists to arrive at a correct diagnosis.

Special stains may further be:

Histochemical: In which special combinations of specific reagents are applied on tissue slides to delineate morphology.

Immunohistochemical: In which specific antibodies are used to detect antigenic proteins on cells within a tissue. This is done by using the principle of specificity of antigen-antibody binding, if an antigen is present the binding will occur, which is visualized by a chromogenic reagent commonly 3,3′-Diaminobenzidine (DAB).

Common histochemical stains include:

- **Periodic acid-Schiff (PAS):** This is used to detect the presence of glycogen, fungal structures, neutral mucin (seen in the stomach and duodenum), basement membranes. PAS positivity is highlighted by a magenta color.
- **Alcian blue:** This stain is used to highlight acidic mucins (both sulfated and sialated). Alcian blue stain is typically done at different pH. Staining at pH 0.5 picks up the sulfated acid mucins of the large intestine (goblet cells). This capability gives it the ability to diagnose Barrett's metaplastic change. Staining at pH 2.5 picks up the sialomucins of the small and large intestine. Alcian blue positivity is reflected by a beautiful deep turquoise blue color. Alcian blue also picks up stromal origin mucins comprising of hyaluronic acid.
- **PAS-AB:** This is a combination stain with a dual color of magenta and turquoise blue serving as a pan-mucin stain covering both neutral and acidic mucins.
- **Mucicarmine:** Used to highlight epithelial mucin and gives a rose color to mucin. It is sometimes used for the diagnosis of mucoepidermoid carcinoma of the salivary gland.
- **Masson's trichrome**: As the name suggests, the word trichrome means three colors. This stain is a connective tissue stain used to highlight fibrosis. It is also used to highlight muscle from surrounding connective tissue, muscle taking up a red stain and the collagen either blue or green depending on the counterstain used. It is commonly used on liver biopsies to document the presence or absence of cirrhosis.
- **Reticulin stain:** This is a silver impregnation technique commonly used to highlight fibrosis. It helps highlight reticulin, which are actually type III collagen fibers and form the framework of organs such as the liver and spleen. Reticulin stain is commonly used to confirm cirrhosis and demonstrate collapse as seen in liver failure. Another frequent application is to highlight fibrosis in the bone marrow (myelofibrosis).
- **Van-Gieson (VG stain):** It is a stain used to highlight collagen using picric acid and acid fuschin. Collagen is highlighted as red in this technique.
- **Perl's stain (Prussian blue):** Stains iron in the ferric form bound to protein (hemosiderin). This is based on the reaction of ferric iron with potassium ferrocyanide generating an insoluble potassium ferri-ferrocyanide complex which stains deep blue.
- **Sudan black, Oil red O:** Both stains are used to highlight fat, however, tissue used must be fresh. Frozen sections are usually used for this purpose. This is because lipids dissolve in the organic solvents used in tissue processors.
- **Masson's fontana:** It used to highlight melanin, which is seen as black in color.
- **Congo red:** It used to stain amyloid.
- **Toluidine blue:** It used as a rapid staining alternative as this is quick to perform. Used to highlight mast cells.
- **Grocott-Methenamine stain:** It is used to demonstrates fungi, nocardia and actinomyces.
- **Gram's stain:** Gram-positive bacteria typically stain blue to purple, while Gram-negative bacteria stain pink or red.
- **Ziehl-Neelsen staining:** Acid-fast bacteria, such as *Mycobacterium tuberculosis*, that do not stain with Gram's staining are stained using the Ziehl-Neelsen method. Methylene blue serves as a counterstain while carbol fuchsin serves as a stain (Fig. 1.3).
- **Lepra stain (Wade Fite stain):** It used to highlight *Mycobacterium lepra*.

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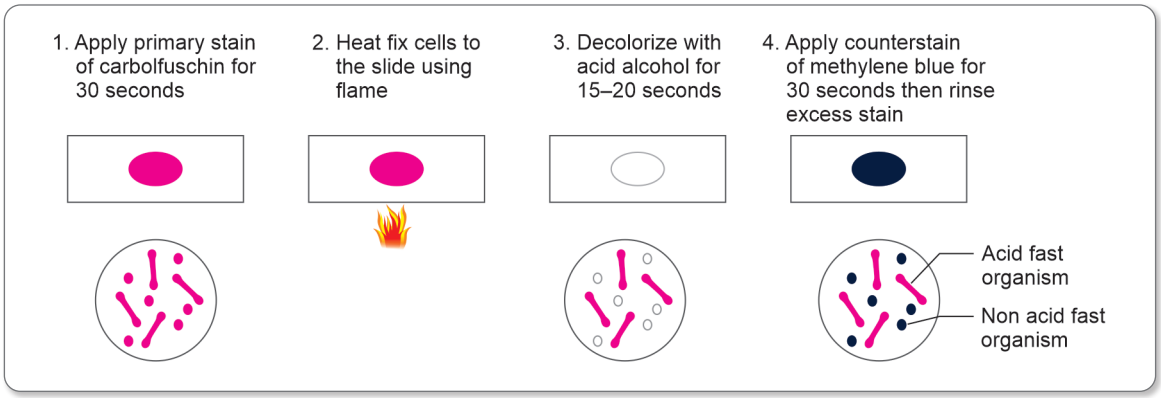

Figure 1.3: Procedure of Ziehl-Neelsen staining

Mounting

This is the final step which involves placing the prepared specimen onto a microscope slide, often with the addition of mounting medium and a coverslip for further microscopical study.

MUST KNOW

Xylene (DPX) or Canada Balsam, Permount, Glycerol, Gelatin, Fluoromount, Vectashield, Mounting Medium with DABCO, Clear Mounting Media, Entellan, Hoyer's Solution are some commonly used mounting media.

SUMMARY

- Pathology is the *branch of medicine which deals with the study of disease*.
- Histology is the study of pathological changes occurring in tissues, seen by examining both gross and microscopic features.
- Systemic pathology is study of pathological changes in disease specifically involving a particular organ/body system, e.g., myocardial infarction ("heart attack").
- Histopathology is study of pathological changes occurring in tissues, seen by examining both gross and microscopic features.
- Surgical pathology is study of pathological changes occurring in tissues—either small pieces or entire organs/parts during the course of surgery to remove a disease.
- Cytopathology/cytology is the study of pathological changes at a cellular level.
- Hematology is the study of pathological changes occurring in the blood and/or bone marrow as part of a disease process.
- Autopsy is the study of pathological changes in a tissue/organ which is removed after the death of a person for evaluation with the aim of understanding the cause of death.
- Forensic pathology involves the application of pathological techniques to aid in the investigation of crime.
- Immunopathology is the study of pathological changes, specifically in diseases caused by abnormalities in the functioning of the immune system.
- Clinical pathology is branch of pathology dealing with the study of body fluids including blood, semen, urine, cerebrospinal fluid with a specific clinical context.
- Genetics is the branch of medicine dealing with the study of hereditary disease.
- Molecular pathology is study of detecting and diagnosing pathological changes at the level of DNA/RNA/proteins
- Bacteria can be classified as Gram-positive or Gram-negative based on the two different forms of Gram's staining.
- Fixation is done to provide stability to the tissues for easy handling.
- Carbohydrate molecules are stained using the Periodic Acid-Schiff (PAS) method.

ASSESS YOURSELF

Long Answer Questions

1. What is staining? Explain the types of staining techniques.
2. Discuss the importance of studying pathology.

Short Answer Questions

1. Write the types of staining.
2. Write short notes on:
   i. Branches of pathology
   ii. Importance of studying pathology

Multiple Choice Questions

1. **Which of the following fixative is used in tissue fixation for electron microscopy?**
   a. Formaldehyde
   b. Glutaraldehyde
   c. Zenker's fixative
   d. Bouin's fixative
2. **A microtome is a device used for:**
   a. Processing of tissue
   b. Sectioning of tissue
   c. Staining of tissue
   d. Disposal of tissue
3. **All of the following are clearing agents; except:**
   a. Methanol
   b. Xylene
   c. Chloroform
   d. Carbon tetrachloride
4. **The following is most commonly used as an embedding agent:**
   a. Paraffiin wax
   b. Colloidin
   c. Nitrocellulose
   d. Resins
5. **All of the following are the components of surgical pathology; except:**
   a. Gross pathology
   b. Immunohistochemical analysis
   c. Molecular pathology analysis
   d. Cytopathology

Answer Key

1. b **2.** b **3.** a **4.** a **5.** d

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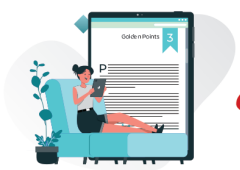

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