Chapter

1

Introduction

- Introduction
- Types of specimen received in histopathology
- Methods of tissue preparation
- Methods to study living cells
- Equipment required in histopathology laboratory

Histology *'Study of Tissue'*

We study a detailed microscopic structure or architecture of various tissues.

Histopathology *'Study of Diseased Tissue'*

To study the disease process, we require various investigations including study of tissue under microscope which is very vital to conclude the diagnosis in certain pathological conditions. In different disease processes, there are variations and alterations in cell structure, relative arrangement and their functions. To study these specific changes in the tissue under the microscope, tissue has to be prepared in such a way that it retains almost normal or near to normal structure. For this purpose, thin slices of the tissue, called as **'sections'** are prepared and stained with suitable dye (stain) to demonstrate various tissue components.

Practically 'Histopathology' Includes

- Gross examination (naked eye examination) of specimen removed after surgery.
- Microscopic study of the tissue.
- Various other techniques to establish the diagnosis like special staining methods, enzyme histochemistry, immunohistochemistry, electron microscopy, etc.

Types of Specimen Received in Histopathology

Common ones are biopsies, surgical specimens and frozen sections.

a. **Biopsy:** 'Tissue sample or specimen taken from pathological lesions'
   - *Incisional biopsy*: When only a small part of the lesion is surgically taken out for diagnostic purpose.
   - *Excisional biopsy*: When entire lesion is removed and submitted for histopathological studies. Total excision of the lesion serves the therapeutic purpose also.

Biopsy material should be carefully handled in the laboratory. Any crushing, scrapping or squeezing should strictly be avoided.

b. **Surgical specimens:** The entire organ or specimen removed after surgery.

Note: In routine clinical practice, the term biopsy is used for small surgical specimens.

Surgical specimens can further be divided into soft tissue specimens and bony specimens.

c. **Frozen sections (intraoperative surgical specimens):** Mean when the patient is still in operation theatre. Frozen sections are required to decide quickly:
   - The exact nature of the lesion.
   - Whether the surgical margins are clear of disease process or not.
   - Sometimes to decide whether the submitted tissue contains adequate material to establish the diagnosis later on.

   'Cryostat' is used to section the tissue quickly and with good quality.

Types of tissue
- Biopsy: Incisional and excisional biopsy
- Surgical specimens: Bony and soft tissue
- Frozen section

Methods of Tissue Preparation

Direct examination: When the material is in liquid or fluid form, e.g. cells suspended in blood or lymph can be studied directly by examining a drop of the fluid.

Teasing: When the tissue is thick, place it over a glass slide with some fluid like normal saline, tease the tissue with needle so that the cells are separated from each other in fluid medium.

Smear: Commonly used method to study the cells as in cases of exfoliative cytology, aspiration cytology, aspiration of bone marrow and blood cells. Place a drop of fluid containing cells, e.g. blood, aspirated marrow or any body fluid, etc. on the glass slide. Prepare a smear or film. Fix the smear, stain it with appropriate stain and study under microscope.

Imprint preparations or touch preparations: Rapid method to study the cells and to some extent architectural arrangement of cells in cases of some organs like lymph nodes, spleen, bone marrow, etc. Method can be used to evaluate surgical margins instead of taking intraoperative frozen sections. Press the cut surface of the fresh organ or tissue onto a slide. Then fix it and stain it.

Squash smear: It is a recent modality of studying tissues where a 1–2 mm piece of tissue is gently crushed between two glass slides and the smear made, stained and examined. This methodology is being used frequently for CNS biopsy.

Tissue culture: Living cells are preserved for a relatively longer period outside the body by keeping the fragments of tissue (removed from the body) in a physiological medium at a suitable temperature. Now with 'Hanging Drop' preparation method growth, multiplication, and differentiation can be studied directly under the microscope.

Sectioning method: Standard, convenient and most commonly used method to study the fixed or dead tissue. These preparations are permanent and can be kept for years. A small fixed piece of tissue is cut into very thin, translucent slice called 'section'. These are two dimensional sections and are approximately one cell layer thick. A good quality thin section can be obtained by preparing the tissue in such a way that it can be cut with the help of a machine known as 'Microtome'. Preparation of the tissue for microtomy is known as 'Tissue Processing'. Tissue processing is done either by preparing the paraffin wax blocks or done by freezing the tissue as used in frozen section technique.

Methods to Study Living Cells

Special staining techniques are required to demonstrate living organisms or living cells.

Supravital staining: Staining dye is added to a medium containing cell which is already removed from the body and preserved in a tissue culture medium, e.g. demonstration of RNA in reticulocytes with either new methylene blue or brilliant cresyl blue (Fig. 1.1).

Vital staining: Staining dye is injected directly into the living organism to study parts of living cells especially cytoplasmic elements. Nucleus is resistant for vital stains or dyes. Basic principle of this method is that some parts of the cells either absorb or phagocytise the staining material used, e.g. trypan blue stains macrophages because of the ability of the macrophages to phagocytise foreign material. Hence, macrophages can be demonstrated in various reticuloendothelial system. This method is used in experimental studies.

Equipment Required in Histopathology Laboratory

In routine histopathology laboratory, following areas are important: Grossing area, tissue processing, section cutting, staining area including special staining, and other specialized areas, e.g. immunohistochemistry, frozen section.

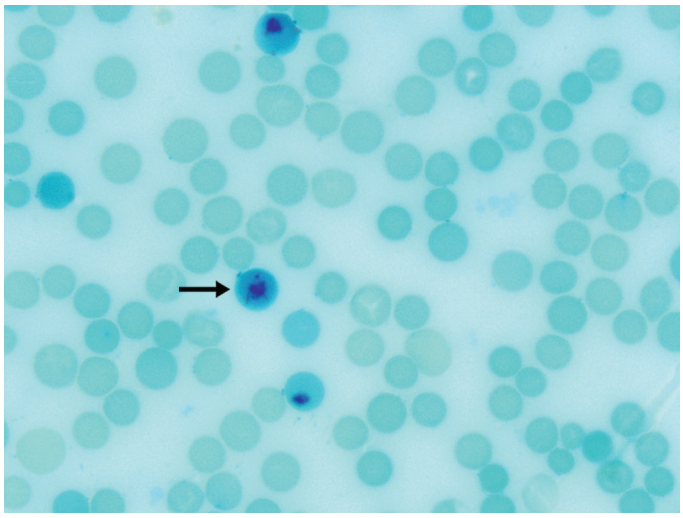

Fig. 1.1: Smear showing **reticulocytes** using **supravital stain (methylene blue)**

For gross examination and sampling of tissue, an adequate size, properly ventilated room is required. Gross room should contain the following:

- Cutting board with ready access to sink so that all the fluid flows directly into the sink.
- Hot and cold water facility with sink.
- Instruments including various sized smooth and toothed forceps, large and small scissors, a scalpel handle with disposable blades, various sized knives, malleable probe, ruler, weighing machine, band saw, cassettes, labels, filter paper, lead pencil, etc. (Figs 1.2 to 1.4).

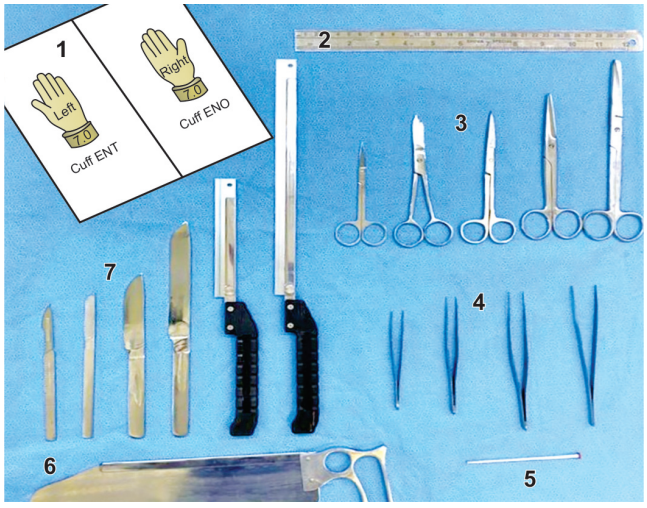

Fig. 1.2: Equipment for grossing: (1) Gloves, (2) ruler, (3) scissors, (4) forceps, (5) probe, (6) bone saw, (7) assorted size knives

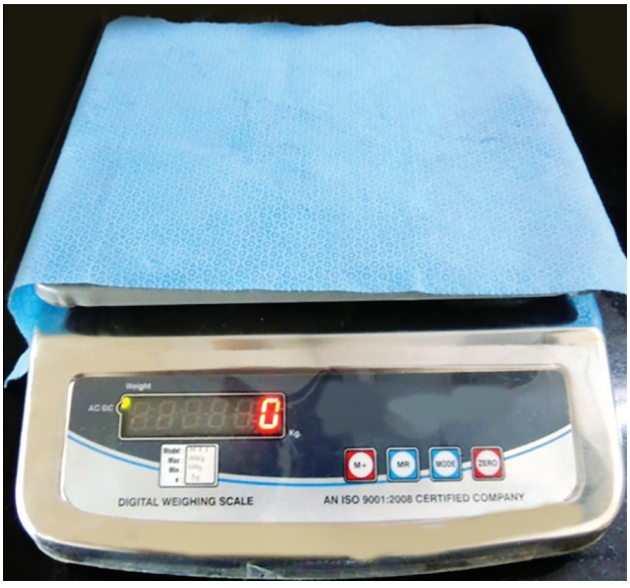

Fig. 1.3: Weighing machine to weigh organs/tissue

Fig. 1.4: Grossing technique: (1) Specimen container, (2) specimen cassette, (3) technique for grossing, (4) filter paper, (5) eosin, (6) paint and brush for inking

- Shelves for keeping specimen containers
- Containers for various fixatives
- 4°C refrigerator
- Balances
- X-ray view box
- Photographic facility

Note: Grossing station with attached photography units are commercially available.

Various equipments required for tissue processing, section cutting and staining are discussed in appropriate sections.