Overview of Histology and Approaches to its Study

Chapter Outline

INTRODUCTION TO HISTOLOGY

Histology (derived from two Greek words: *histos* meaning "tissue", and *logia*, a suffix generally used to denote the study of a subject or the branch of knowledge of a discipline "science") is the study of the microscopic anatomy of cells and tissues of plants and animals. Tissue was first used to describe the different textures of body parts being dissected by an anatomist. *Microanatomy* is the study of the structure (anatomy) of small (micro) things. Small things—cells and their arrangement—constitute tissues and the association among these form organs. Histology is a discipline that performs a scientific study dealing with the fine detail structure of biological cells and their formation into tissues and organs using microscopes to look at specimens of tissues that have been carefully prepared using special processes called **histological techniques**.

Histology is the study of the tissues of the body. But since the tissues are composed of cells and their products, histology also deals with the study of the structure and activities of the cell. Histology is a structural science and serves to complete the anatomical knowledge gained from dissection. Thus, it is intimately related to physiology and pathology. However, a thorough knowledge of normal histology is essential for the understanding of the altered structure seen in various conditions of disease. The term histology is used in a broad sense for the study of tissues as revealed by various techniques utilizing the methods of cytology, histochemistry, electron microscopy, and tissue culture. In a restricted sense, histology is a structural science and serves to complete the anatomical knowledge gained from dissection. Since tissues are composed of cells and their products, the structure of cells must necessarily form the basis of histology. All the technologies employed for the study of histology bring an insight into the details inside a cell with the use of microscopes. The

simplest and most common method in the study of histology by a student is to use his microscope for the examination of preparations termed *sections*. A section is a very thin slice of tissue, laid flat on a sheet of glass called a *slide*, stained and mounted in a medium of suitable refractive index and covered with another circular or square very thin piece of glass often called a *cover slip*. Because there are both living and non-living components in tissues of our body, it is logical to employ methods which would reveal the salient features of both these components. The methods utilized for the study of cell micro-anatomy fall basically in two groups: (i) Those employed for the living cells, and (ii) Those employed for the dead cells that have been fixed. In the modern histology lab immuno-logical and molecular (DNA) techniques are frequently utilized to provide accurate tumor identification which will aid the clinician in selecting a mode of therapy that offers that greatest probability of cure.

Histological Classification of Animal Tissues

Tissues are divided into four groups (epithelial tissue, connective tissue, muscle tissue, and nervous tissue). These groups are further subdivided into many subgroups of these four basic tissue types (for example, blood cells are classified as connective tissue, since they generally originate inside bone marrow). Also, for another example, the epithelial tissue is subdivided into **covering and/or lining epithelia** (outer layer of skin, inner surface of heart and blood vessels, inner surface of respiratory cavities, etc.) and **glandular epithelia** (most of the glands in the body).

- **Epithelium**: The lining of glands, bowel, skin, and some organs like the liver, lung, and kidney.
- **Endothelium**: The lining of blood and lymphatic vessels.
- **Mesothelium**: The lining of pleural and pericardial spaces.
- **Mesenchyme**: The cells filling the spaces between the organs, including fat, muscle, bone, cartilage, and tendon cells.
- **Blood cells**: The red and white blood cells, including those found in lymph nodes and spleen.
- **Neurons**: Any of the conducting cells of the nervous system.
- **Germ cells**: Reproductive cells (spermatozoa in men, oöcytes in women).
- **Placenta**: An organ characteristic of true mammals during pregnancy, joining mother and offspring, providing endocrine secretion and selective exchange of soluble, but not particulate, blood-borne substances through an apposition of uterine and trophoblastic vascularized parts.
- **Stem cells**: Cells with the ability to develop into different cell types.

Note

Tissues from plants, fungi, and microorganisms can also be examined histologically. Their structure is very different from animal tissues.

Short Definitions of Histology and Related Sciences

Some short definitions of the term histology are as follows:

- **Histology** is the scientific and microscopic study of cells and tissues of plants and animals using special staining techniques combined with light and electron micro-scopy. It is often carried out by examining a thin slice (called a "section") of tissue under a light microscope or an electron microscope. In order to distinguish different biological structures more easily and accurately histo-logical stains are often used to add colors to or enhance the colors of certain types of biological structures differently from other types of structures that may be located next to and/or in contact with each other.

Histology is an essential tool of biology, medicine and veterinary medicine.

- **Cell biology** is the study of living cells, their DNA and RNA and the proteins they express.
- **Anatomy** is the study of organs visible by the naked eye.
- **Morphology** studies entire organisms by whole mounts (Fig. 1.1).
- **Cytology** is the microscopic study of loose cells or clusters obtained from bodily secretions, aspirations, scrapes, swipes, or washings (Fig. 1.2). Cytology is a specialized department whose primary role is to detect cancerous and precancerous lesions on Pap smears and cancerous lesions in non-gynecological specimens including fine needle aspiration (FNA) biopsies, urine samples, sputum samples, CSF and other body fluids. All scientific and technical staff are highly trained with strict quality control procedures rigorously implemented to ensure high standards of reporting. This may involve a slide being examined under the microscope by several cytologists and pathologists before a final diagnosis is made.

Brief Historical Account

Marie François Xavier Bichat (1771–1802), born at Thoirette in Jura, France, was a French anatomist and physiologist who is best remembered as the **Father of modern histology and** descriptive anatomy. Despite working without a microscope (because he distrusted it), he was the first to introduce the notion of tissues as distinct entities, and maintained that diseases attacked tissues rather than whole organs. Bichat (Fig. 1.3) made significant contributions to medicine and physiology and opined that the diverse body

Fig. 1.1: Whole mounts: (A) of Fasciola hepatica, (B) of an insect, and (C) of mouse mammary gland as viewed with a microscope.

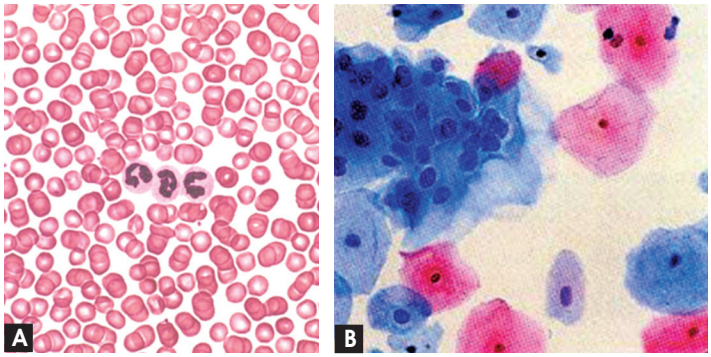

Fig. 1.2: Microscope views of smear preparations of (A) human blood from a normal individual, and (B) cervical smear from a case of uterine cervix malignancy

Fig. 1.3: Marie François Xavier Bichat (1771–1802) is regarded as Father of modern histology descriptive anatomy and pathology.

organs contain particular tissues or *membranes*. He described 21 such membranes, including connective tissue, muscle tissue, and nerve tissue. His analyses did not include any acknowledgement of cellular structure. Nonetheless, he formed an important bridge between the *organ pathology* of Giovanni Battista Morgagni and the *cell pathology* of Rudolf Ludwig Carl Virchow.

In the 19th century, histology was an academic discipline in its own right. The 1906 Nobel Prize in Physiology or Medicine was awarded to histologists Camillo Golgi and Santiago Ramon y Cajal. They had dueling interpretations of the neural structure of the brain based in differing interpretations of the same images. Cajal won the prize for his correct theory and Golgi for the staining technique he invented to make it possible.

Uses of Histology

The knowledge about histology may be utilized for applying it to the following causes:

- *Education:* Histology slides are often used in teaching laboratories to help students learn about the microstructures of human (and animal) *biological tissues*.
- *Diagnosis for treatment:* Biological tissue samples taken from a patient (that is, a specific person or animal's body) may be studied in detail to enable medical or veterinary experts to learn more about the patient's condition and hence perhaps understand its causes and make recommendations for treatment or management of the condition. Although the study of the microstructure of ***diseased*** cells and tissues (e.g. to help inform decisions about treatment in clinical medicine) is an aspect or use of histology because it uses histological techniques, study of diseased tissues is more accurately called *histopathology*.
- *Forensic investigations:* Forensic histology, immunohistochemistry and cytology involving microscopic study of biological tissues using various stains can help clarify the cause of sudden unexpected deaths and other issues in forensic science.
- *Autopsy:* Biological tissues from a deceased person or animal can be studied using histological techniques enabling experts (e.g. pathologists to diagnose unexplained death of a person) to learn about the circumstances and possibly cause of death. *Histologists* are people who have and use the special skills necessary to process samples of biological tissues in histology laboratories. Tissue obtained from the patient (or sometimes from a suspect in the case of forensic science labs) is processed using a series of techniques to prepare appropriate very tiny samples of tissue then mount them on slides and stain the tiny sample of tissue on each slide using chemicals called histology stains that have been carefully selected in order to help the people who will look at the slides to distinguish between the different types of biological material within the tiny sample on the slide.

HISTOCHEMISTRY, HISTOPATHOLOGY, AND CYTOCHEMISTRY

Histochemistry is the aspect of histology study of the identification and distribution of *chemical compounds* within and between biological cells

using histological techniques such as histology stains, indicators and *light* (optical) and electron microscopy. In order to fully understand these definitions of histochemistry, it is helpful to recall the definitions of *histology* and *chemistry*. Histology is the microscopic study of the structure of biological *cells* and *tissues* using special techniques including histology stains, combined with light and electron microscopy. Chemistry is the science of *matter* and the changes that occur between different kinds of matter, especially chemical reactions, during which types of matter are rearranged into other types of matter, e.g. liquid water splitting into the gases *hydrogen* and *oxygen*. So, whereas histology in general is the study of biological cells and tissues in fine microscopic detail using special histological techniques, histochemistry is concerned specifically with the chemicals within, between, and forming the biological cells and tissue themselves.

Histopathology (a sub-discipline within *pathology*) is the microscopic study of diseased biological tissue. Medical specialists who study and interpret diseased tissues in microscopic detail are called histopathologists. In addition, *histotechnicians* and *histotechnologists* are members of a laboratory team who employ technology to diagnose diseases, and to conduct research.

Histotechnologists play a fundamental role, in the allied health profession, by preparing suitable thin slices of human, animal or plant tissue for microscopic examination. This is an important part of the intricate process of scientific investigation used in establishing and confirming patient diagnosis (Fig. 1.4). Because of the histotechnologist's skillful application of sophisticated laboratory techniques, the seemingly invisible world of tissue structure becomes visible under a microscope. The tasks performed by the histotechnologist require patience, mechanical ability, knowledge of biology, immunology, molecular biology, anatomy and chemistry. It requires five basic steps, each an integral part of the histotechnologist's job. Histopathologists also provide frozen section analysis in operating theatres in hospitals allowing a prompt examination of small selected pieces of tissue while the patient is still in the operating room. The diagnosis of this tissue will predetermine the extent or continuance of the operation.

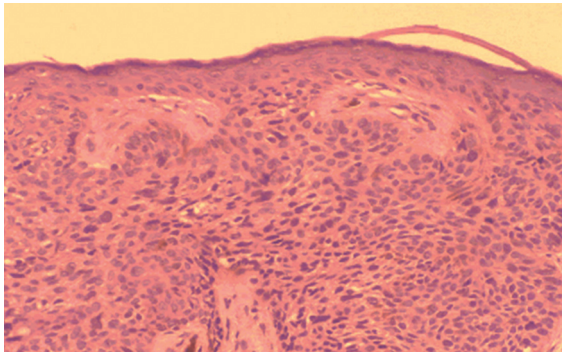

Fig. 1.4: A histopathology slide from tiny biopsies to entire organs that have been surgically removed. The thin sections are routinely stained with hematoxylin and eosin, and reviewed under a microscope by specialist pathologists.

Cytochemistry is the method for detecting different substances in tissue sections (Fig. 1.5). Most based on a specific chemical reaction or high-affinity interactions between molecules.

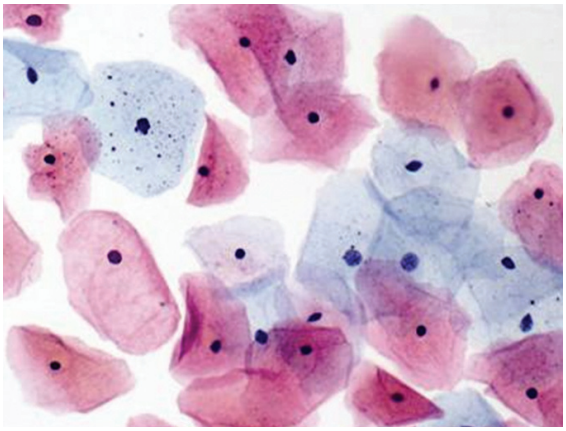

Fig. 1.5: Cells on a Pap smear are carefully examined by several specialists to detect cancerous and pre-cancerous lesions in non-gynecological specimens (of body fluids) before a final diagnosis is made.

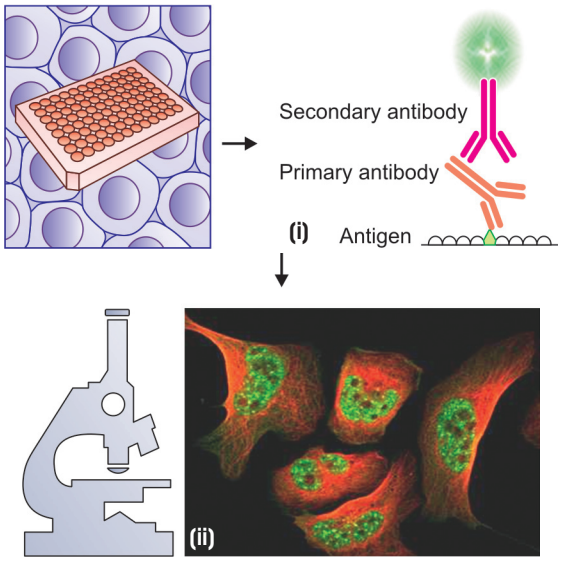

Fig. 1.6: Steps in immunocytochemistry: (i) Cell-seedling, fixation and immunostaining; (ii) imaging, annotation and image analysis. RNA binding motif protein 25 (RBM25) is localized in the nuclear speckles (green). Microtubules are stained in red.

Usually produce a colored or electron-dense compound.

Examples are:

- Phosphatases, peroxidases and dehydrogenases used to react with specific chemical bonds.
- Lipid soluble dyes like Oil red O and Sudan black used to detect lipids
- Periodic-Schiff (PAS) reaction transforms glycol groups into aldehydes for detection of polysaccharides.
- **Immunocytochemistry** depends on a specific interaction between an antigen and its antibody. Labeled antibodies can identify and localize specific proteins (Fig. 1.6).

METHODS OF STUDYING HISTOLOGY

The methods utilized for the study of cell microanatomy fall basically in *two* groups:

i. those employed for the living cells and tissues, and
ii. those employed for the dead 'fixed' specimens

Study of Living Cells and Tissues

Living cells are usually more difficult to prepare and the preparations can be retained temporarily, that too for a relatively short period only. The first human cells studied in the living condition were blood cells. Due to the fluid nature of the blood, a thin film of blood can be made between a glass slide and a cover slip, edges are sealed to prevent evaporation and the microscope stage is warmed to average body temperature. By this procedure, the blood cells are still surrounded by their natural environment—the plasma. Thus, the conditions inside the body are nearly achieved. However, the cells of organized tissues cannot be studied by this procedure as they are too thick for examination by transmitted light. Hence the cells from specimens, like spleen and lymph nodes are obtained from the cut surfaces of organs and tissues, mixed with an isotonic saline and examined in the same manner as the thin film of blood. Very small pieces from the subcutaneous tissues are generally examined after being teased to form thin 'spreads' on a glass slide. This depicts the normal arrangement of fibers, but only some cells because a few cells are destroyed. When the structure of a tissue is to be quickly studied, as for the histopathological diagnosis of a patient being operated, some quicker methods are followed. By these methods, the preparations cannot be preserved for recording purposes and the tissue deteriorates after sometime. Such preparations are termed temporary preparations, and these depend on the point whether the material is a liquid or a solid.

Study of Dead (or 'Fixed') Specimens

A *temporary* or a *permanent* preparation may be needed to demonstrate the structural details.

1. Temporary Preparations

Temporary preparation from a solid material may be made for histological examination by selection of any of the following procedures.

a. ***Scraping***: The material is scraped with a glass slide or cover slip, and the scraped out tissue is spread on a slide as smear, fixed, and examined under microscope, either as such or after staining.
b. ***Teasing***: The tissue is placed over a glass slide with a little normal saline solution. By means of mounted needles, the tissue is spread, fixed, and examined.
c. ***Maceration***: In this method, the tissue is dissociated by chemical agents such as 33 per cent alcohol, and nitric acid 1 in 4 dilutions with glycerine–water mixture. After keeping the tissue in any of the above solutions, for about 24 hours, 35 per cent potassium hydroxide solution is used to macerate a tissue.
d. ***Sectioning*** (or ***slicing***): Thin slices of the tissue are cut either by hand or with a knife, or by a freezing microtome (or cryostat if available) after the material has been treated in gum solution.

Besides the above four conventional methods for making the temporary preparations of a solid material, there are a few other methods as indicated below:

- *Tissue culture is* a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ. Cells in culture may multiply; change size, form, or function; exhibit specialized activity (muscle cells, for example, may contract); or interact with other cells (Fig. 1.7). In practice, the term "cell

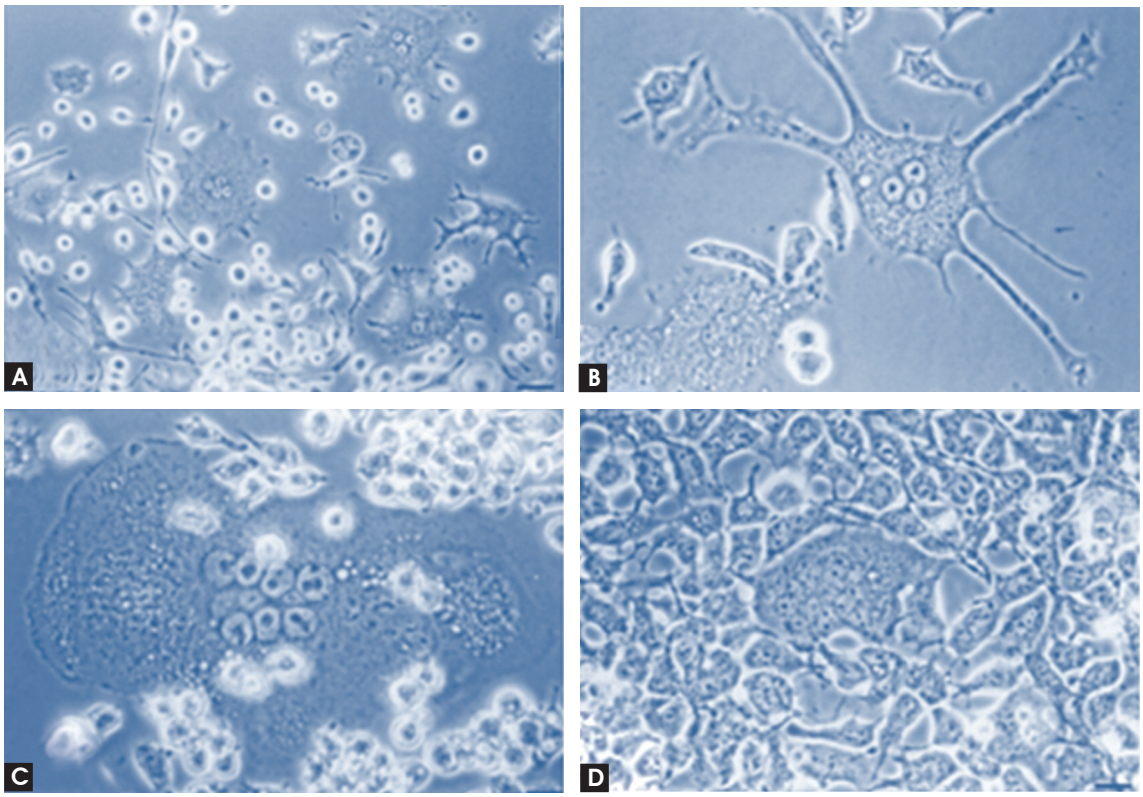

Fig. 1.7: Photomicrographs depict formation of multinucleated giant cells in a tissue culture. Photographs were taken with a 20X objective in A and a 40X objective in B–D. Bars: (A) 50 μm; (B–D) 25 μm.

culture" now refers to the culturing of cells derived from multicellular eukaryotes, especially animal cells, in contrast with other types of **culture** that also grow cells, such as plant **tissue culture**, fungal **culture**, and microbiological **culture** (of microbes).

- *Micro incineration* (in which microscopic sections are incinerated in a crucible and the 'ash' left behind is examined for qualitative chemical analysis of inorganic constituents)
- *Freezing–drying* method of tissue fixation in which a specimen may be frozen rapidly using carbon dioxide snow and then dehydrated in a vacuum. Sections may then be cut for microscopic examination.

2. Permanent Preparations

When a permanent histological preparation is required, the tissue needs to be what is called *fixed* or *dead*. A fixed tissue after subsequent processing can be sectioned into thin slices and many morphological and histochemical staining methods can also be employed.

It may be noted that only a fixed material can be examined with an electron microscope. Also, tissues fixed, unlike the fresh ones, many years earlier can be examined. However, a small delicate tissue for better handling and to avoid its distortion during section cutting, is placed in a supporting medium which varies in its consistency at varying temperature, liquid at a higher temperature, and solid at a lower temperature, for smooth cutting. When the liquid embedding medium is allowed to solidify in a special container, a small *block* of the given specimen is formed. A block is sectioned with an instrument called the *microtome* (Chapter 8), the tissue together with its supporting medium cuts as one mass. This procedure prevents distortion making thin sections that can be examined with a *light microscope* (Chapter 16, Part 2). In routine work, *paraffin-wax method of embedding* is followed and only this method is described in detail in the present book. Specimens are usually received in fixative (preservative) but sometimes arrive fresh (Fig. 1.8) and must be immediately fixed.

Before specimens are accepted by a laboratory the identification (labeling) and accompanying documentation will be carefully checked, all details recorded and "specimen tracking "commenced. It is vital that patient or research specimens are properly identified and the risk of inaccuracies minimized. The preparation of sections is the most technically complicated of these methods as it requires specialized equipment and considerable expertise. Although the methodology for preparing sections from both animal and plant material is similar, the following description relates to animal (human) tissues.

a. The tissue can be *rapidly frozen* and kept frozen while sections are cut using a cryostat microtome (a microtome in a freezing chamber). These are called "**frozen sections**". Frozen sections can be prepared very quickly and are therefore used when an intra-operative diagnosis is required to guide a surgical procedure or where any type of interference with the chemical makeup of the cells is to be avoided (as in some histochemical investigations).
b. Alternatively specimens can be infiltrated with a liquid agent that can subsequently be converted into a solid that has

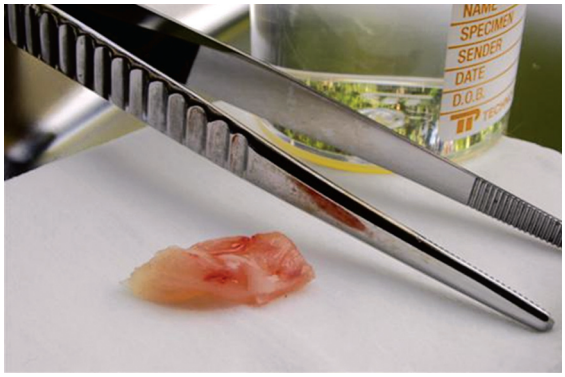

Fig. 1.8: A fresh, unfixed specimen after surgical removal. To prevent degeneration or drying-out the specimen should be fixed as soon as possible.

appropriate physical properties that will allow thin sections to be cut from it. Various agents can be used for infiltrating and supporting specimens including epoxy and methacrylate resins but paraffin wax based histological waxes are the most popular for routine light microscopy. This produces so-called "paraffin sections". These sections are usually prepared with a "rotary" microtome. "Rotary" describes then cutting action of the instrument. In all histopathology laboratories paraffin sections are routinely prepared from almost every specimen and used in diagnosis.

3. Cinematography

Cinematography is a technique to maintain permanent records of cell activity by taking the cinematograph taken through the objectives of a microscope. The technique of television microscopy (Fig. 1.9) is the only available technique for the direct quantitative measurement of skin capillary blood flow. Television microscopy has applications in both research and clinical practice, and has attracted considerable interest from clinical groups attempting to study capillary blood flow. The critical requirements for a functioning system are difficult to ascertain from the published literature.

The exposures are taken at intervals of several seconds and thrown on the screen at the usual speed. This method is extremely useful for noticing certain cellular stages like the process of cell division by mitosis, the shifting of the nucleus during the prophase, and separation of chromosomes. These steps cannot be appreciated by any other means of cell examination.

TISSUE PREPARATION STEPS FOR LIGHT MICROSCOPY

Routine Standard Techniques

To process any given specimen for microscopy, following *four* types of preparations can be made:

1. *Whole-mounts:* The whole-mounts are prepared in those cases where an entire organism or structure is small enough or

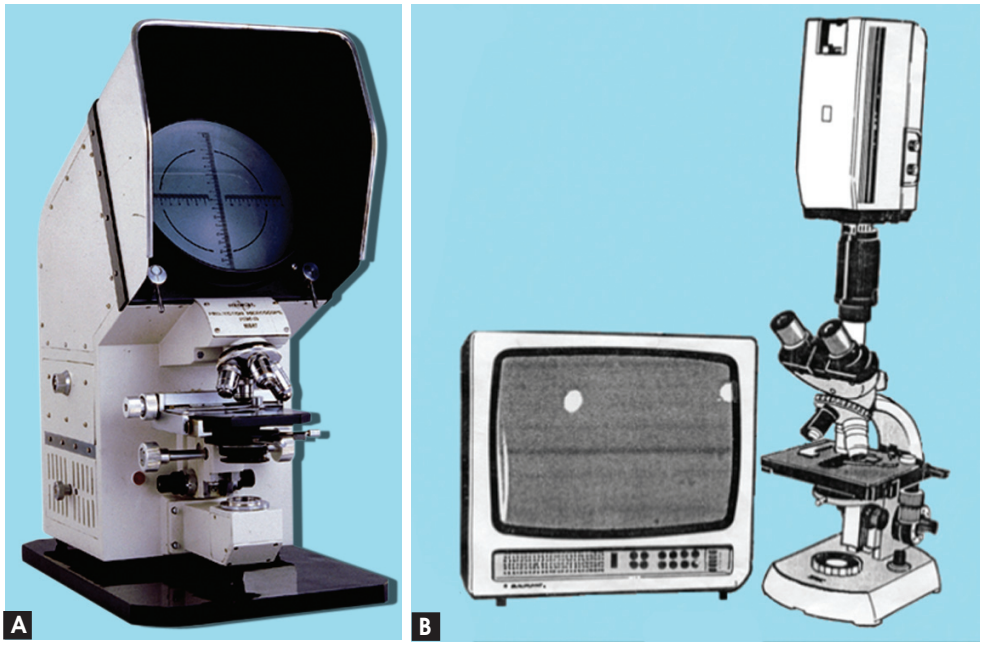

Fig. 1.9: (A) Projection microscope and (B) equipment for television microscopy as tools for histology teaching

thin enough to be placed directly onto a microscope slide. For example, a small unicellular or multicellular organism or a membrane that can be stretched thinly on to a slide (*see* Fig. 1.1). The whole-mounts preserve the structural relationships between individual cells and extracellular components.

2. *Squash preparations:* In a 'squash' preparation whole-mounts cells are intentionally squashed or crushed onto a slide to reveal their contents, e.g. botanical specimens such as root tips where cells are disrupted to reveal chromosomes (Fig. 1.10).
3. *Smears:* Smears are made in those specimens that consist of:
   a. Cells suspended in a fluid (e.g. blood, semen, cerebrospinal fluid, or a culture of micro-organisms)
   b. A situation where individual cells have been scraped brushed or aspirated from a surface
   c. From within an organ (exfoliative cytology) Smears are the basis of the well-known "Pap test" that is used to screen for cervical cancer in women. However, smears and squash preparations provide detail about individual cells and relative cell numbers, but structural relationships are lost.
4. *Section preparation:* Most fresh tissue is very delicate, easily distorted and damaged and it is thus impossible to prepare thin sections (slices) from it unless it is supported in some way whilst it is being cut. Usually the specimen also needs to be preserved or "fixed" before sections are prepared.

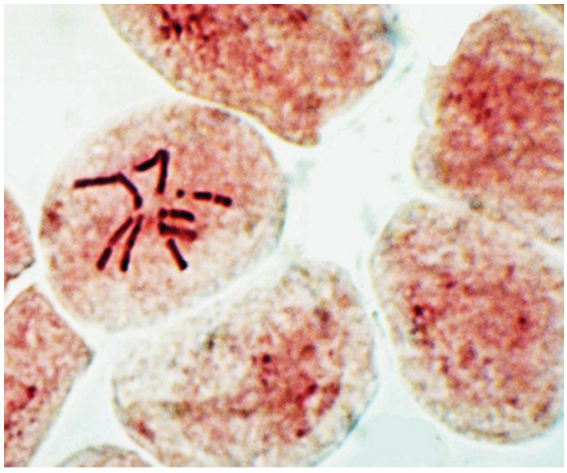

Fig. 1.10: Different phases in a squashed root tip of a plant.

Broadly there are two strategies that can be employed to provide this support. The section cutting is done in such situations where the specimen cannot be examined by the above three methods. Sections very thin slices can be cut from them, mounted on slides, and stained are prepared using an instrument called a "microtome" (Chapter 8).

EQUIPMENT IN A HISTOLOGY LABORATORY

Sterilization and Cleaning of Glassware

Sterilization

The sterilization can be done by the following methods:

1. **Dry Heat** sterilization has a limited value. Prolonged exposure may cause damage.
2. **Hot air oven** where heat is transferred by convection, conduction or radiation.
   a. The temperature of 100° C for one hour can destroy the non-sporing organism.
   b. Fungal spores need 115° C for one hour.
   c. While for other all bacteria 160°C temperature is needed for one hour.
3. **Incineration** where the flame is an effective way of sterilization. Flame heat is needed for the loop for culture.
4. **Moist heat** is the most reliable method of sterilization. This is the most lethal agent to kill microorganism. Microbial death is due to coagulation and denaturation of the protein and enzyme.
5. **Boiling** is not effective to kill spore bearing bacteria and for surgical instruments.

6. **Steam sterilization** or tyndallization is exposure to steam at 100° C for 90 minutes. This good means to sterile the media which contain sugar.
7. **The autoclave** is heating water under pressure which boils at progressively higher temperature. This method is good for rubber material and surgical instruments.
8. **Membrane filters** are Millipore filters. Filters with the pore of 0.22 micrometer are sufficient for the bacteria.
9. **Seitz filter** are disposable asbestos pad filter.
10. **Flaming** when the material is wetted by alcohol and then flamed. This method is rapid.
11. **Ultraviolet light** causes damage to bacteria.
12. **Radiation** in form of beta and gamma X-rays used for the surgical pads.
13. **Supersonic and ultrasonic** waves, 9000 cycles per second or above are used to rupture and disintegration of the cells.

Cleaning

Different glassware in a histology laboratory, brushes commonly used, and bottle brush, bent for cleaning laboratory glassware are shown in Fig. 1.11.

Cleaning of Micro slides and Cover slips

Micro Slides

The slides available in gross (1 gross= 12 dozens) are usually rectangular in shape. Their thickness varies (Fig. 1.9). Thin slides are less than 1 mm in thickness; the medium ones

Fig. 1.11: (A and B) Different glassware in a histology laboratory. (C) Laboratory brushes commonly used, (D) Bottle brush, bent for cleaning laboratory glassware.

are between 1 and 1.25 mm; and the thick ones are more than 1.25 mm thick. These slides are available in different sizes.

For very large sections 82 × 82 mm slides or even photographic emulsion plates are used. The most common size of slides in laboratory is 76 mm × 25 mm (3" × 1"). Other sizes are 76 × 50 mm; 76 × 32 mm and 76 × 40 mm, i.e. 3" × 2", 3" × 1.25" and 3" × 1.5" respectively.

Cover Slips

Unlike glass slides, the cover slips are purchased in ounces or grams. These are circular, square, or rectangular in shape; and their sizes are also variable. According to their thickness, the cover slips are numbered from 0 to 3. Number '0' and '1', suitable for oil-immersion and photomicrographic work are 0.07 to 0.13 mm and 0.13 to 0.17 mm thick respectively. For ordinary microscopy, cover slips number '2' (0.17 to 0.25 mm thick), and number '3' (0.25 mm and above) are used. The average size of cover slips is 32 mm at its diameter, range being 0.155 to 0.185 mm. The more viscous the mounting medium used, the thinner should be the cover slip.

SUMMARY

What is Histology, Histochemistry, and Histopathology?

Histology is a structural scientific study of the microscopic anatomy of cells and tissues of animals and plants gained from dissection. *Marie Francois Xavier Bichat* is remembered as **Father of Modern Histology** and Descriptive Anatomy. Histologically the tissues are in *four* groups: Epithelial tissue (covering and lining the body tubes/tubular structures), connective tissue (mainly filling the spaces between the organs), muscular tissue (with inherent contractility), and nervous tissue (with characteristic property of irritability for conduction of impulses).

Histochemistry is the aspect of histology study of the identification and distribution of *chemical compounds* within and between biological cells using histological techniques such as histology stains, indicators and *light* (optical) and electron microscopy.

Histopathology (a sub-discipline within *pathology*) is the microscopic study of diseased biological tissue. Histopathologists also provide frozen section analysis in operating theatres in hospitals allowing a prompt examination of small selected pieces of tissue.

Histological Techniques

The term is generally used for performing a scientific study to enable a specimen prepared in such a way so as to study the detailed structure of its constituent cells and/or tissues by cutting it into sufficient thin slices—called *sections* with the help of an instrument called a *microtome*.

1. ***Study of Living Cells and Tissues***: Blood cells still surrounded by their natural environment—the plasma. However, cells of organized tissues cannot be studied by this procedure because of their excessive thickness to allow light transmitted through them.
2. ***Study of Dead or 'Fixed' Cells and Tissues***
   a. Specimen may be *rapidly frozen* and kept frozen while sections are cut
   b. Specimen can be *infiltrated with a liquid agent* that can subsequently be converted into a solid *block*. Thin *sections* can be cut later at will.

Preparations from a solid material may be made for histological examination by any of the following procedures:

a. *Scraping*: The material is scraped with a glass slide or cover slip, and the scraped-out tissue is spread on a slide as a smear, fixed, and examined under a microscope, either as such or after staining.
b. *Teasing*: The tissue is placed over a glass slide with a little normal saline solution. By means of mounted needles, the tissue is spread, fixed, and examined.
c. *Maceration*: In this method, the tissue is dissociated by chemical agents such as 33 per cent alcohol, and nitric acid 1 in 4 dilutions with glycerine-water mixture. After keeping the tissue in any of the above solutions, for about 24 hours, 35 per cent potassium hydroxide solution is used to macerate a tissue.
d. *Sectioning* (or *slicing*): Thin slices of the tissue are cut either by hand or with a knife, or by a freezing microtome (or cryostat if available) after the material has been treated in gum solution.

Steps in Section Preparation for Light Microscopy

- **Fixation**—carried out preferably by perfusion method in a living anaesthetized animal to make preparation almost as in life
- **Dehydration**—through increasing graded strengths of ethanol
- **Clearing** or **dealcoholization**—for removal of alcohol and transfer the specimen in a medium acting as a paraffin wax solvent
- **Infiltration**—with molten paraffin wax
- **Embedding**
- **Paraffin block making**
- **Section cutting** (or microtomy) *see* Chapter 9 for procedure and precautions
- **Ribbon of sections**/or single section flattened on water bath
- **Affix** on clean (pg. 13) egg-albumin (adhesive) coated glass sides
- **Slides**
  - For normal routine work 76 × 25 mm slides are universally used.
  - 1.0–1.2 mm thick slides are preferred because these do not break easily.
- Slides are air-dried and stored till routine/or other selective staining is done
- For details of staining steps—*refer* to Chapter 10; *see* Chapter 11 for special staining procedures
- Mount, cover, and label stained sections (*refer* to Chapter 12).
- Examine the stained section with proper use of light microscope (Part-II, Chapter 17).

Sterilization and Cleaning of Glassware

Sterilization

The sterilization can be done by following methods:

1. **Dry heat** sterilization has limited value. Prolonged exposure may cause damage.
2. **Hot air oven** where heat is transferred by convection, conduction or radiation.
   a. The temperature of 100° C for one hour can destroy the non-sporing organism.
   b. Fungal spores need 115° C for one hour.
   c. While for all other bacteria 160° C temperature is needed for one hour.
3. **Incineration** where the flame is an effective way of sterilization. Flame heat is needed for the loop for culture.
4. **Moist heat** is the most reliable method of sterilization. This is the most lethal agent to kill microorganism.
   - Microbial death is due to coagulation and denaturation of the protein and enzyme.
5. **Boiling** is not effective to kill spore bearing bacteria and for surgical instruments.
6. **Steam sterilization** or tyndallization is exposure to steam at 100° C for 90 minutes. This good means to sterile the media which contain sugar.
7. **The autoclave** is heating water under pressure which boils at progressively higher temperature. This method is good for rubber material and surgical instruments.
8. **Membrane filters** are Millipore filters. Filters with the pore of 0.22 micrometer are sufficient for the bacteria.
9. **Seitz filter** are disposable asbestos pad filter.
10. **Flaming** when the material is wetted by alcohol and then flamed. This method is rapid.
11. **Ultraviolet light** causes damage to bacteria.
12. **Radiation** in the form of beta and gamma X-rays used for the surgical pads.
13. **Supersonic and ultrasonic** waves, 9000 cycles per second or above are used to rupture and disintegration of the cells.

Cleaning

1. Cleaning of large glassware

For cleaning use sterilized disposable plasticware or submerge the glassware in a cleaning solution:

- Potassium dichromate ..... 2 g
- Distilled water ..... 200 ml

Dissolve dichromate in distilled water; when cool, ***add very slowly***

- Sulfuric acid (concentrated) ..... 9 parts
- Potassium dichromate solution (2%) ..... 1 part

For removal of grease from the glassware used in a histology laboratory, following procedure is advised:

- Soak for several hours in a mixture of sulfuric acid and excess amount of potassium dichromate
- Wash in running tap-water, preferably overnight
- Clean with suitable brush (*see* Fig. 1.10)
- Rinse in distilled water.

2. Cleaning of glass slides and cover slips

Slides are to be properly cleaned; otherwise the attached sections on them tend to fall during subsequent steps of routine histological procedures.

a. **Soak in any one of the following solutions**

i.	Old xylene	1 part
	Methylated spirit	1 part
ii.	Acid alcohol	0.5% or 1.0%
	Alcohol (70%)	1000 cc
	HCl (conc.)	5 cc (for 0.5%) or 10 cc (for 1.0%)
iii.	Concentrated aqueous solution of sodium hydroxide	
iv.	Concentrated nitric acid (HNO_3)	
v.	Sulfuric acid or nitric acid (conc.)	1 part
	Potassium dichromate	9 parts

b. Wash in running tap-water

c. Rinse in distilled water

d. Rinse in 0.5 per cent acid alcohol

e. Dip in 90 per cent alcohol

f. Wipe dry each slide with a soft, lintless cloth.