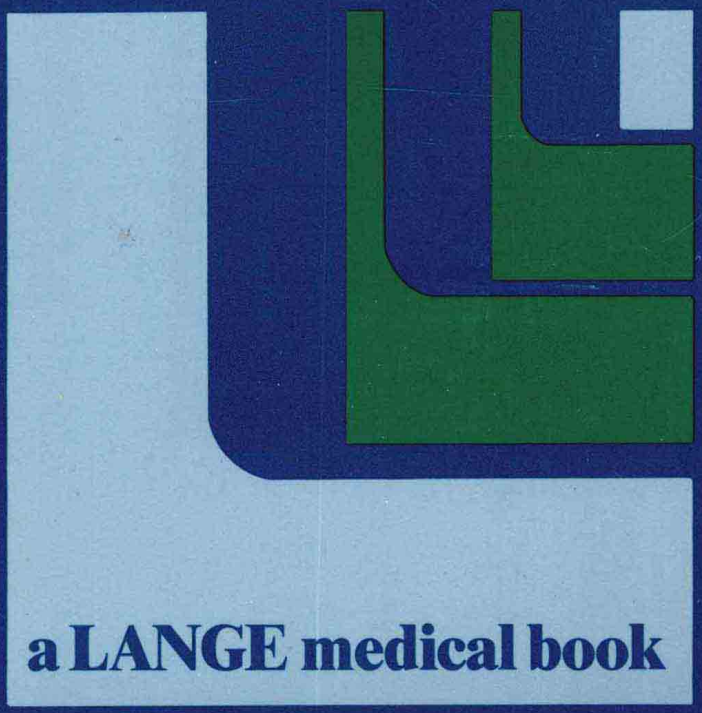
Examination and Board Review

Basic Histology



a LANGE medical book

Douglas F. Paulsen

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Basic Histology

Examination and Board Review

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This book is dedicated to my students at the Morehouse School of Medicine for their dedication to providing quality primary care to the economically disadvantaged and the medically underserved.

Preface

This book is intended to help you use your study time more effectively, both as you learn histology and as you review it for course or National Board (Part I or FLEX) exams. It is specifically designed as a companion to the sixth edition of *Basic Histology* by Junquiera, Carneiro, and Kelley. It contains many page references to this text that can help you find information quickly and easily. However, you can use this book successfully with any standard histology text.

Several distinctive features make this study guide useful and easy to understand. They include:

- A list of learning objectives for every chapter.
- A synopsis of each subject in outline format.
- A set of study-focusing questions for every chapter.
- A set of Boards-type multiple-choice questions and answers for every chapter.
- Four sets of Boards-type integrative multiple-choice questions and answers for related chapters.
- Page references to *Basic Histology,* sixth edition, for all of these questions.

Each chapter begins with a list of objectives that describe the most significant facts and concepts for that topic. The second section of each chapter is a synopsis of the topic presented in outline form.

The third section of each chapter consists of a set of study-focusing questions designed to direct your attention to the facts and concepts that most often appear on exams. Many of these questions can be answered with a word, phrase, list, or sketch. Others ask you to make comparisons and are best answered by making a chart or table and filling in the requested information. Every question asks for specific information and provides page references directing you to that information.

The last section of every chapter is a set of multiple-choice questions written in 4 of the formats commonly used by the National Board of Medical Examiners. In addition, this book contains 4 sets of integrative multiple-choice questions. The correct answers to all of the multiple-choice questions are included at the end of the book, along with page references to the sixth edition of *Basic Histology*. If you do not have this text, you can find the answers to these questions in any standard histology text by using the index. These page references will help you learn not only what the correct answers are, but why they are correct.

Douglas F. Paulsen, PhD
Atlanta, Georgia
August 1989

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Part I: Fundamental Concepts

Methods of Study 1

Objectives

This chapter should help the student to:

- Know the mathematic relationships among the units of measure commonly used in analyzing histologic specimens.
- Name the instruments and techniques commonly used in the preparation and study of histologic specimens.
- Know the basic steps in preparing specimens for the various types of light and electron microscopy.
- Know the advantages and limitations of the various histologic instruments and techniques and choose the appropriate ones to reveal specific microscopic features of cells and tissues.

Synopsis

I. GENERAL FEATURES OF HISTOLOGY & ITS METHODS

A. Goals of Histology: The term *histology* means "the study of tissues." Histology is a largely visual science that relies on microscopy to reveal cell, tissue, and organ substructures. It's goals include:

1. Understanding cell, tissue, and organ structure at levels not visible to the unaided eye, including 3-dimensional relationships among their biochemical constituents.

2. Understanding the relationship between the substructure and the normal functions of cells, tissues, and organs.

3. Establishing a basis for learning histopathology—the relationship between abnormal tissue and organ structure and functional defects.

4. Providing a basis for treating diseased and injured tissues and organs. In medicine, this is the ultimate goal.

B. Histologic Methods: Histology is classed as a subdiscipline of anatomy ("cutting apart"), because its methods involve dividing tissues and organs into pieces and preparing them for microscopic examination and chemical analyses. These methods fall into 4 main groups:

1. Microscopy The purpose of histologic methods is analysis of histologic specimens with the aid of a microscope. The several types of microscopy are classified in 2 main groups, **light microscopy** (IV) and **electron microscopy** (**EM**; see V).

2. Tissue preparation for microscopy The optical characteristics of each type of microscope are attended by certain requirements in preparing a tissue for analysis (III). Exam-

ples of common preparative procedures include **sectioning** to produce thin, translucent tissue slices and **staining** with dyes and chemicals to reveal the otherwise transparent substructure of the slices.

3. Cell, tissue, and organ culture These methods allow observation of the effects of certain treatments on the structure and function of isolated cells, tissues, and organs (VI).

4. Cell fractionation This technique involves mechanically breaking cells and then separating their components by centrifugation for electron microscopic or biochemical analysis (VII).

C. Advantages and Limitations of Histologic Methods: Only by understanding the advantages and limitations of histologic methods can one properly interpret the information they provide. The advantages result from making small and complex structures and processes accessible to observation. The limitations result from the fact that the methods themselves, especially dividing an organism into pieces, often halt the very life processes we wish to analyze.

D. Structure and Function: Tissue structure and tissue function are so closely related that neither can be fully understood without an understanding of the other. Their relationship should be the main focus of both your initial study and your review.

II. UNITS OF MEASURE

A. Ångström unit (Å)* = 10^{-10}m = 10^{-7}mm = $10^{-4}\mu$m = 10^{-1}nm
B. Nanometer (nm) = 10^{-9}m = 10^{-6}mm = 10^{-3} = μm = 10 Å
C. Micrometer (μm) = 10^{-6}m = 10^{-3}mm = 10^{3}nm = 10^{4}Å

III. PREPARATION OF TISSUES FOR MICROSCOPIC EXAMINATION.

Light and electron microscopy share the basic types of preparative methods described below but differ in important details of preparation. The similarities and differences are summarized in Fig 1–1.

A. Fixation:

1. Purpose The purpose of fixation is to preserve the structural organization of the tissue. There are 2 main types of fixation: chemical fixation and freezing (physical fixation).

2. Advantages and limitations

a. Chemical fixation A good chemical fixative will prevent bacterial and enzymatic digestion, insolubilize tissue components to prevent diffusion, and protect against damage from subsequent steps in tissue processing. However, chemical fixation may cause changes in chemical composition and fine structure. The chemical changes may induce staining artifacts during sensitive histochemical procedures (see Chapter 2). The structural changes include denaturing and cross-linking certain tissue proteins.

b. Freezing Freezing is faster, because it eliminates several preparative steps (Fig 1–1). It also avoids dissolving lipids and denaturing fixative-sensitive proteins such as enzymes and antigens. However, frozen specimens do not last as long, and obtaining serial sections from them is difficult.

3. Chemical procedures The best fixation is achieved with rapid penetration of living tissue with fixative, by immersion or perfusion.

*This unit may be encountered in older publications, but it is now being replaced by the nanometer for structures in this size range.

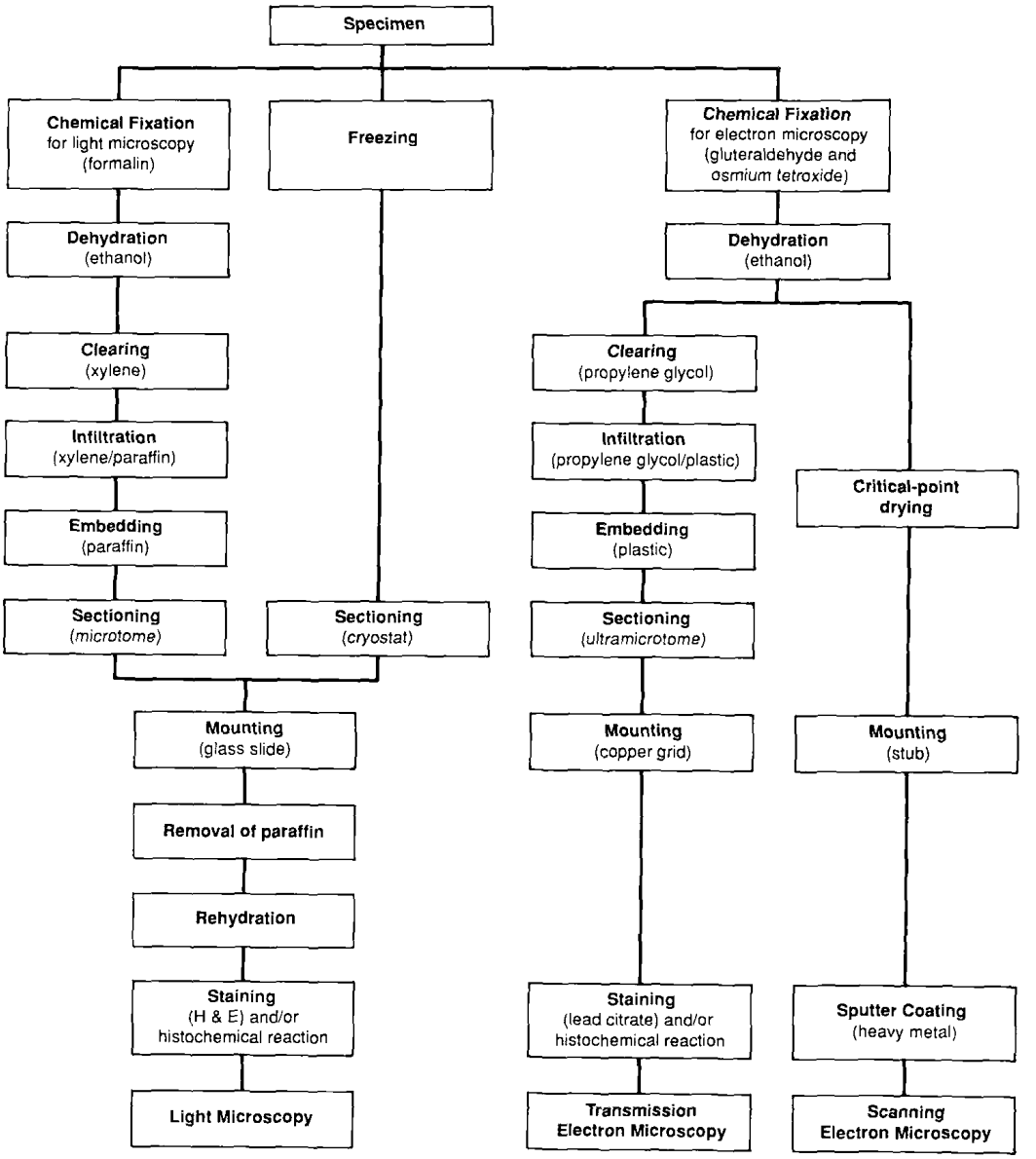

Figure 1–1. Steps in tissue preparation for microscopy. Common techniques and agents are shown in parentheses.

a. Immersion Small pieces of tissue may be fixed by simply immersing them in the fixative.

b. Perfusion Tissues and organs can be fixed before they are removed from the body by pumping fixative through the blood vessels that serve them. Perfusion is useful for fixing specimens too large to allow rapid fixative penetration by immersion (eg, in embalming).

4. Chemical fixatives

a. Aldehydes These react with amine groups, form cross-links among proteins, and cause coagulation (but not coarse precipitation) of tissue proteins. They may interfere with periodic acid-Schiff (PAS) and Feulgen staining specificity (see Chapter 2). Formalin (a solution of formaldehyde gas in water) is commonly used for light microscopy; glutaraldehyde is commonly used for electron microscopy.

b. Oxidizing agents These cross-link proteins and precipitate unsaturated lipids. Osmium tetroxide is an oxidizing agent often used with glutaraldehyde to fix tissues for

electron microscopy. It reacts with lipids to form a black precipitate and doubles as a stain for cell membranes. Potassium permanganate and potassium dichromate are oxidizing agents sometimes used in light microscopy.

c. Protein-denaturing agents Normal protein shape is maintained largely by ionic interactions with water molecules. Acetic acid, methyl alcohol, ethyl alcohol, and acetone denature protein by removing the associated water molecules, changing the protein's shape. In the absence of cross-linking agents, rehydrating the tissue can sometimes restore proteins to their original conformation.

d. Other chemical fixatives Mercuric chloride and picric acid are useful light microscopy fixatives whose precise mechanism of action is unknown.

5. Fixative mixtures No single fixative is ideal for all the structural details of all tissues. Mixtures are used to exploit the advantages of a variety of fixatives while minimizing their disadvantages. Bouin's fluid, used for light microscopy, contains picric acid, formalin, acetic acid, and water; Zenker's formalin (Helley's fluid), also used for light microscopy, contains formalin, potassium dichromate, mercuric chloride, and water. Karnovsky's fixative, used for electron microscopy, contains paraformaldehyde and glutaraldehyde in a buffered salt solution.

6. Double fixation In this procedure, used for electron microscopy, the specimen is fixed in buffered glutaraldehyde, washed with phosphate buffer, and postfixed in buffered osmium tetroxide.

7. Physical procedures Tissue may be frozen to preserve its structure for either light or electron microscopy. Prior embedding in a cryoprotectant (eg, glycerin) and rapid freezing at low temperatures in liquid nitrogen helps prevent formation of large ice crystals and related artifacts. Freezing allows the tissue to be sectioned (or fractured; see III.F) without chemical fixation, dehydration, or clearing.

B. Dehydration:

1. Purpose The purpose of dehydration, or **substitution,** is to prepare chemically fixed tissue for infiltration with an embedding medium by replacing the water in the tissue with an organic solvent.

2. Procedure Ethyl alcohol is the most common dehydrating agent. Fixed tissue is immersed in a series of alcohol-water mixtures with increasing alcohol concentration until 100% alcohol is reached. Alcohol may denature proteins. Removal of water usually causes shrinkage.

C. Clearing and Infiltration:

1. Purpose In clearing, the dehydrating agent is replaced with a clearing agent (a solvent of the embedding medium), which renders the tissue transparent. Infiltration replaces the clearing agent with the embedding medium. Successful clearing and infiltration prevent bubbles from forming in the tissue. Clearing agents may denature proteins.

2. Procedures

a. Clearing The dehydrated tissue is immersed in a series of clearing agent-alcohol mixtures with increasing clearing-agent concentration, or it is placed directly in the clearing agent.

b. Infiltration The cleared tissue is immersed in a series of clearing agent-embedding medium mixtures with increasing embedding medium concentrations, at medium-high temperature. The clearing agent gradually evaporates and is replaced by the embedding medium.

3. Common clearing agents Xylene (a paraffin solvent) is most commonly used for light microscopy. Propylene glycol (a plastic solvent) is most commonly used for EM.

D. Embedding:

1. Purpose The purpose of embedding is to make the tissue firm and prevent crushing or other tissue disruption during sectioning. This permits thin, uniform sectioning.

2. Procedure The infiltrated tissue is positioned in a mold filled with the embedding medium. The medium hardens into a block, which is removed from the mold and attached to a chuck. When placed in the microtome, the chuck holds the block in place for sectioning.

3 Embedding media

a. For light microscopy Paraffin is the most common embedding medium. Others are celloidin, plastics (eg, methacrylate), and polyethylene glycol (water-soluble) wax.

b. For electron microscopy Plastics and epoxy resins (eg, Epon and Araldite) are most commonly used. These embedding media often require a catalyst to harden (polymerize) them after infiltration. Harder embedding media allow thinner sectioning, a requirement for electron microscopy.

E. Sectioning:

1. Purpose Most tissues are too thick and opaque to permit microscopic analysis of their internal structure. Cutting thin slices allows light or electrons to penetrate the specimen and form an image.

2. Instruments and procedures

a. For light microscopy A standard rotary **microtome** with a steel blade is used to cut sections (usually 3–8 μm thick) of specimens embedded in paraffin, celloidin, or polyethylene glycol. A microtome equipped with a glass or diamond knife may be used to cut sections (usually 1–5 μm thick) from plastic-embedded tissues. Frozen sections (10–30 μm thick) are cut with a freezing microtome, or in a cryostat, a standard microtome housed in a refrigerated chamber.

b. For electron microscopy An **ultramicrotome** with a glass or diamond knife is used to cut very thin sections (0.02–0.1 μm thick, or up to 0.5 μm thick for high-voltage electron microscopy). The instrument is equipped with a stereomicroscope for viewing the cutting.

F. Cryofracture and Freeze Etching:

1. Purpose Together, cryofracture and freeze etching permit EM examination of tissues without prior fixation and embedding, and thus, without fixative-related artifacts. These methods allow verification of results obtained by conventional EM techniques.

2. Procedures The tissue is frozen at very low temperatures and fractured with a sharp metal blade (cryofracture). The specimen is kept in a vacuum while the ice crystals sublimate, lowering the ice level from the specimen surface to reveal additional structures (freeze-etching). The etched tissue is coated with a layer of fine carbon particles to form a replica, which is then sprayed at an angle with heavy-metal (gold or platinum) particles to give a shadowed effect. The tissue and replica are returned to atmospheric pressure, the tissue is dissolved in strong acid, and the replica is mounted on a grid. EM examination of the replica produces a shadowed-relief image and provides a limited pseudo-3-D view of cell and tissue components. Resolution is limited by the size of the carbon particles and the thickness of the coating.

G. Mounting:

1. Purpose The purpose of mounting is to ease handling of and decrease damage to the specimen during microscopic examination.

2. Procedure

a. For light microscopy Specimens (usually sections) are placed on glass slides, which may be precoated with a thin layer of albumin, gelatin, or polylysine to improve tissue attachment. After staining, the sections are covered with coverslips to preserve them for repeated examination.

b. For electron microscopy Specimens are mounted on copper grids, because the electron beam cannot penetrate glass. Only portions of the specimen lying between the crossbars of the grid will be visible.

H. Staining:

1. Purpose Most tissue substructure is indistinguishable even at high magnification. Stains, ligands with specific binding affinities and optical properties, and radiolabels are used to localize and distinguish among various cell and tissue components. Knowledge of the specificities of such substances (Table 1–1) provides additional information about the structure and composition of a specimen. General staining procedures for light microscopy and electron microscopy are described here; more specific stains and techniques are described in Chapter 2.

2. Procedure for light microscopy After paraffin sections are attached to slides, the paraffin is dissolved and the tissue may be rehydrated prior to staining. Plastic-embed-

Table 1–1. Examples of common stains and their affinities.

Stain	Type	Affinity
Light microscopy		
Hematoxylin	Basic dye	Basophilic tissue components; eg, DNA, RNA, and polyanions such as sulfated glycosaminoglycans.
Toluidine blue		
Methylene blue		
Alcian blue		
Eosin	Acidic dye	Acidophilic tissue components; eg, basic proteins in the cytoplasm.
Orange G		
Acid fuchsin		
Oil red O	Lipid-soluble	Long-chain hydrocarbons (fats, oils, and waxes).
Sudan black		
Periodic acid-Schiff (PAS) reaction	Multicomponent histochemical reaction	Complex carbohydrates (glycosaminoglycans, glycogen).
Feulgen's reaction		Nuclear chromatin (DNA and associated protein).
Transmission electron microscopy		
Uranyl acetate	Heavy metal (electron-dense)	Nonspecific; adsorb to surfaces and enhance contrast.
Lead citrate		
Osmium tetroxide		Actually a fixative, but binds to phosphate groups of membrane phospholipids, enhancing contrast.
Ruthenium red		Polyanions; eg, complex carbohydrates such as oligosaccharides of the glycocalyx and glycosaminoglycans of the extracellular matrix.

ded sections are stained without removing the plastic. Most stain affinities are based on reciprocal acid-base characteristics of the stain and tissue components. Acidic stains such as eosin bind to basic, or **acidophilic,** structures and compounds (eg, cytoplasmic proteins and hemoglobin). Basic stains such as hematoxylin bind to acidic, or **basophilic,** tissue components (eg, the nucleoproteins in ribosomes). Stain mixtures reveal multiple cellular components. Hematoxylin and eosin (H&E), the most common stain mixture for light microscopy, distinguishes the nucleus from the cytoplasm.

3. Procedures for electron microscopy There are 2 types of electron microscopy (V.D):

a. Transmission electron microscopy procedures Most staining (contrasting) methods for transmission electron microscopy (TEM) are based on the stains' electron-absorbing or -scattering qualities and their affinities for particular cellular components. Heavy-metal salts such as lead citrate and uranyl acetate are most commonly used. The fixative osmium tetroxide interacts with lipids to form an electron-dense precipitate and thus doubles as a stain for cellular membranes.

b. Scanning electron microscopy procedures Specimens being prepared for scanning electron microscopy (SEM) do not require staining per se. Before mounting and sputter coating, SEM specimens are subjected to **critical point drying,** which prevents the development of artifacts related to surface tension. After dehydration, the specimens are soaked in a liquid miscible with CO_2 or Freon and placed in the critical point chamber. The chamber is heated to a critical temperature (31°C), causing the pressure to rise to a critical level (73 atm) at which the gaseous and liquid phases exist without surface tension and the liquid escapes the specimen without altering its structure. The specimen is then mounted on a stub and sputter-coated (sprayed) with a fine mist of heavy metal particles (eg, gold) before viewing.

I. Radioautography:

1. Purpose Radioautography allows localization of radioactive elements in cells or tissues. It is especially useful in tracing radiolabeled precursors and the molecules into which they are incorporated from one part of a tissue or cell to another.

2. Procedure The tissue is usually incubated with radiolabeled precursors prior to fixation, eg, with ^{3}H-thymidine for DNA, ^{3}H-uridine for RNA, ^{3}H- or ^{14}C-leucine for protein. Sections mounted on a slide are cleared of embedding medium, covered with photographic emulsion, and left in the dark. The emulsion becomes exposed (silver bromide is reduced to elemental silver) in areas in contact with the radiolabel; when it is developed, black grains (light microscopy) or curling particle tracks (electron microscopy) appear over labeled structures. The number of grains or tracks in the emulsion is directly proportionate to the amount of radiolabel present. For light microscopy, developed sections are often stained with hematoxylin to reveal tissue architecture.

J. Problems in Interpreting Tissue Sections:

Histologic techniques yield images that differ from the living state in certain predictable ways.

1. Artifacts Structural changes induced by histologic techniques are called artifacts. Some common artifacts caused by tissue preparation are discussed here; for lens-related artifacts, see IV.C.

a. Of fixation Fixation may cause precipitation of proteins normally in solution. It may also alter the chemistry of some tissue components, changing their staining properties.

b. Of dehydration and clearing Dehydration and clearing can cause cell and tissue components to shrink unevenly, according to their relative water content. This creates spaces between the cells and tissue layers that are not present in life.

c. Of sectioning Sectioning with a dull knife can result in crushed or pinched tissue sections. A burr or nick in the blade can tear the tissue. Chatter, or wavelike variations in the thickness of a section, can result from the knife vibrating during sectioning.

d. Of mounting During mounting, tissue sections may develop folds, so that some regions appear to have higher cell densities and darker staining properties than others.

e. Of staining Because the colors in stained tissue are artifacts of staining, it is best to focus more on structure than on the color of tissue components.

2. Conceptual problems Tissue sections can seduce the observer into thinking of 3-dimensional structures in terms of 2 dimensions. To overcome this problem, tissues and organs are sectioned in several planes to allow conceptual reassembly into 3-dimensional structures. In addition, analyzing **serial sections** in the order in which they were cut can help provide a 3-dimensional picture. Since even mixtures of stains are unable to stain every tissue component, adjacent sections are sometimes treated with different stains.

IV. LIGHT MICROSCOPY

A. Microscope Lenses:

1. The **condenser lens** is located between the light source and the specimen. It collects light from the source and projects a cone of illumination through the specimen.

2. The **objective lens,** consisting of one or more lenses (IV.C), is located between the specimen and the ocular lens. It enlarges and resolves the image of the specimen and projects it toward the ocular lens. Several objective lenses, each providing a different level of magnification, are generally mounted on a rotating turret.

3. The **ocular lens** is located between the objective lens and the observer or recording device. It further enlarges the image of the specimen and projects it onto the observer's retina, a screen, or a photographic emulsion.

B. Optical Properties of Lenses:

1. Magnification increases the apparent size of the specimen and makes it appear closer. It is a property of both objective and ocular lenses. The value for total magnification is obtained by multiplying the power of the objective by that of the ocular lens.

2. Resolution is a measure of how close together 2 objects can be and still appear separate; the smaller the value, the greater the resolution. The resolution of the human eye is 200 μm; of a light microscope, 0.2 μm; of an electron microscope, 0.001 μm. Increased magnification is virtually useless without increased resolution. The **resolving power** of a microscope, a measure of the resolution it provides, is independent of magnification and determines the clarity and richness of detail of the image. Resolving power (R) is calculated from the numerical aperture (NA) of the objective and the wavelength of illumination:

$$R = \frac{0.61\ \lambda}{NA}$$

The greater the value of R, the greater the resolution.

3. The **numerical aperture** of the objective lens is related to the width of the lens aperture. The lower the NA, the greater the resolving power of the microscope.

4. Refractive index is a measure of the comparative velocity of light in a medium. When there is air between the lens and the coverslip, some of the light projected through the specimen is refracted (bent) because of the change in refractive index at the air-glass interfaces. At high magnifications, the accompanying loss of resolution has a significant effect on image quality. Using immersion oil (which has the same refractive index as glass) between the coverslip and a special oil-immersion objective lens avoids the change in refractive index and thereby improves resolution.

C. Lens-Related Artifacts: Modern objective lenses contain a series of glass lenses. The first (frontal lens) is spheric or hemispheric and magnifies the image; the others correct for the following artifacts of lens curvature:

1. Chromatic aberration Spheric lenses bring light of shorter wavelength into focus closer to the retina than light of longer wavelength, resulting in multiple blurred images. This chromatic aberration can be avoided by using **achromatic** or **apochromatic lenses.**

2. Spheric aberration Optical properties of the center of a spheric lens differ from those of the periphery. Apochromatic lenses completely correct for this spheric aberration.

3. Curvature of field Spheric lenses prevent simultaneous focusing on the entire field: either the center or the periphery is out of focus. **Planar lenses** correct this curvature of the field, providing flat-field focus.

D. Types of Light Microscope:

1. The **compound bright-field microscope** is the most common tool of histology and histopathology. It is described as compound (as opposed to simple) because it uses a series of lenses. It is called bright-field because the entire field is illuminated by means of an ordinary condenser lens. Specimens must be translucent and stained to provide contrast.

2. The **dark-field microscope** uses a special condenser lens to provide contrast in unstained material, allowing living specimens to be visualized. A disklike shield excludes the center of the light shaft projected by the condenser lens, so that the specimen is illuminated only from the sides. Only objects that deflect light into the objective lens are visible; these are bright on a dark background.

3. The **phase contrast microscope** uses a special lens system to transform invisible differences in phase retardation (light retardation) caused by the different refractive indexes of specimen components into visible differences in light intensity. This allows visualization of living specimens, because fixation and staining are not needed. The specimen must be thin and translucent. High resolution is difficult to obtain.

4. The **polarizing microscope** allows selective visualization of **birefringent** (anisotropic) **structures**—repetitive or crystalline structures such as collagen fibers or myofibrils. Staining is not necessary. Light from the light source passes through a polarizing filter, the condenser lens projects the polarized light onto the specimen, and birefringent structures in the specimen rotate the polarized light. The objective lens projects the image through the analyzer filter, which is oriented so that only light waves oscillating in a plane different from that of the original polarized light can pass to the ocular lens and be seen. Birefringent structures appear as bright, often colored objects on a dark background.

5. The **fluorescence microscope** allows localization of substances labeled with fluorescing compounds (fluorochromes). When stimulated by light of the proper wavelength, fluorochromes emit light of a longer wavelength. Fluorescence microscopes have a special light source and 2 special sets of filters. An ultraviolet light source is commonly used, and the emitted light is in the visible spectrum. An excitation filter between the light source and the specimen filters out all wavelengths except that needed to stimulate the desired fluorescence. A barrier filter between the objective and ocular lenses protects the eyes from ultraviolet rays.

6. The **interference microscope** provides contrast in unstained material, relying on differences in refractive index (IV.D.3). Unlike the phase contrast microscope, it quantitates the phase retardation induced by components of the specimen; it compares the refracted light with an unimpeded reference beam and provides an electronic readout of the data. Because refractive index and phase retardation are proportionate to mass, this instrument can be used to calculate the mass of cellular components.

V. ELECTRON MICROSCOPY

A. General Principles: The equation for resolution is the same as for light microscopy (IV.B.2). An electron beam (wavelength approximately 0.005 nm) is used instead of visible light (wavelength 397–723 nm), giving electron microscopes much greater resolution and allowing useful magnification up to 200 times that of light microscopes. Glass lenses are not transparent to wavelengths below 400 nm, but the negatively charged electron beam can be deflected and focused by electromagnets as it travels through a vacuum.

B. Major Components of Electron Microscopes:

1. Cathode and anode The cathode is a metallic filament that emits a spray of electrons when intensely heated in a vacuum by electric current. The anode is a positively charged metal plate with a small hole at its center. Because of the potential difference between the cathode and anode (60–100 kV), electrons accelerate toward the anode, and some of them pass through the hole in the anode to form the electron beam. Together, the cathode and anode are analogous to the light source of a light microscope.

2. Condenser electromagnet The condenser electromagnet induces an electromagnetic field that deflects the electron beam and focuses a cone of the beam at the object plane.

3. Object The specimen, or object, is typically an ultrathin section of tissue stained with electron-absorbing or -scattering substances to provide contrast. The image formed is actually the shadow of the contrast material.

4. Objective electromagnet The objective electromagnet induces an electromagnetic field that deflects the portion of the electron beam which has passed through the specimen, to form and magnify the image of the object.

5. Projector electromagnet The one or 2 projector electromagnets are analogous to the light microscope's ocular lenses. They further enlarge the image produced by the objective electromagnet and project it onto a fluorescent screen or photographic emulsion.

6. Screen The fluorescent screen is a plate coated with material that fluoresces as electrons strike it. Electrons deflected or absorbed by the specimen do not reach the screen, while those that pass through the specimen do. The result is a transmission image formed by shadows of the electron-dense components of the specimen.

C. Limitations:

1. Because the electron beam must travel to the specimen in a high vacuum, living tissue cannot be used.

2. Tissue sections must be very thin, or they will absorb or deflect the entire beam.

3. The electron beam may damage or alter specimen structure.

4. The image cannot be viewed directly but must be used to create a fluorescent or photographic image.

D. Types of Electron Microscope:

1. The **transmission electron microscope (TEM)** permits visualization of the internal ultrastructure of cells and tissues as well as extremely small structures within cells or in intercellular spaces. It operates as described above (V.B). Specimens are prepared as described in section III.

2. The **scanning electron microscope (SEM)** permits visualization of surface ultrastructure. After the specimen is coated with a thin layer of heavy metal (III.H.3.b), a narrow electron beam is directed across its surface in a point-by-point sequence, generating 2 major signals:

a. Secondary electrons are released from the specimen surface, collected on detectors, and converted electronically into an image that is displayed on a cathode ray tube. This image provides an apparently 3-dimensional representation of the specimen surface.

b. X-rays are generated when the electron beam strikes atoms heavier than sodium. Analysis of the x-ray signal can supply information regarding the concentration and distribution of certain elements in the specimen.

VI. CELL, TISSUE, & ORGAN CULTURE

A. Purpose: The purpose of cell, tissue, and organ culture is to study the function of living cells and tissues without the interference of the organism's normal homeostatic mechanisms. These methods permit easier control and manipulation of the cells' or tissue's environment. Cells and tissues isolated and grown in culture are referred to as **in vitro** ("in glass") and those in the intact organism as **in vivo** ("in the living"). Cells may react differently to a particular treatment in vitro and in vivo.

B. Culture Types:

1. Cell culture In suspension culture, cells are suspended in culture medium, either free or attached to the surface of floating beads. In plate culture, cells are attached to plastic or glass tissue-culture dishes. The dishes may be coated with substances that improve attachment: gelatin, collagen, polylysine, serum albumin, or extracellular matrix extracts. Plate-cultured cells behave differently at different densities. They may be cultured in confluent monolayers (the entire culture surface is covered with cells in contact with one another) or at clonal densities (the cultures are seeded at low density to avoid cell-to-cell contact). The latter method allows growth of individual cell colonies, or clones.

2. Tissue and organ culture Fragments of tissues or organs are removed from the body and grown as intact explants, usually at the air-medium interface. This method is often used to study embryonic differentiation and morphogenesis away from the complex environment of the embryo.

C. Culture Medium: The medium consists of a buffered isotonic saline solution to which is added an array of essential nutrients (eg, amino acids, vitamins, hormones) or rigidly controlled composition. Recent advances in knowledge of cell and tissue growth requirements have decreased the use of serum and tissue extracts of less well-defined composition to supplement the medium. Antibacterial and antifungal agents are often added to the medium.

D. Isolation and Study of Pure Cell Strains: Individual cell types may be isolated and studied in vitro in order to examine their separate contributions to tissue and organ function. Cell suspensions are commonly obtained from tissues by enzymatic digestion (eg, with trypsin, collagenase, or hyaluronidase) of the cellular and intercellular components that hold cells together. Cell types may then be separated on the basis of size and mass through specialized forms of **centrifugation** (elutriation, density gradient centrifugation). Newer methods use specific antibodies to isolate particular cell types from a heterogeneous population in suspension. Some such methods exploit differential binding of cells by antibodies attached to a culture surface; others use a fluorescence-activated cell sorter, which separates cells labeled with fluorescent antibodies from unlabeled cells.

VII. CELL FRACTIONATION.

Cell fractionation is used to isolate and collect cellular components in quantity to study their contributions to cell function. This procedure begins with the mechanical **homogenization** of cells and tissues to break plasma membranes and release the cell components into suspension. The components (individual organelle types) are then separated on the basis of size and density, using either of 2 centrifugation methods.