RICHARD S. SNELL, M.D., Ph.D.

CLINICAL AND FUNCTIONAL HISTOLOGY FOR MEDICAL STUDENTS

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PREFACE

Histology is the study of the normal microscopic structure of the body. A medical student must master this subject, because only by doing so will he be able to understand how the different tissues function. Moreover, histology is basic to the understanding of cell and tissue disease; it is impossible to study the abnormal before the normal is known.

Most existing textbooks are either too long and too detailed for present-day curricula or too short and only superficially concerned with those tissues that are commonly diseased.

The primary objective of this book is to provide the student with a basic understanding of the structure and function of those tissues of the body that are often diseased. Throughout the book, the practical application of histological and physiological facts to clinical medicine is stressed. At the end of each chapter is a series of clinical problems that require histological knowledge for their solution; the answers are placed at the back of the book.

The illustrations consist of photomicrographs of different tissues, taken at various magnifications, and simple explanatory line diagrams. It is recommended that the student have access to histological slides and to an atlas of histology to provide further examples of tissue structure.

The writing of this book would not have been possible without the work of anatomists, physiologists, and physicians too numerous to mention, and I gratefully acknowledge their assistance.

I thank the many medical students, clinical colleagues, and friends who stimulated me to write this book. I am most grateful to my colleagues in anatomy and cell biology in this country and other parts of the world who provided me with photographic examples of histological material.

I also thank Mrs. Mike Barnard and Lois Gottlieb for their invaluable technical assistance in preparing the hundreds of histology slides examined during the preparation of this book. I thank the staff of the audiovisual service of the George Washington University School of Medicine and Health Sciences, and in particular Jill Weinstein, B.S., for her skillful preparation of the many photomicrographs. I extend my sincere appreciation to my artist, Myra Feldman, for the very fine drawings. Special acknowledgment is due to Michele Boyd, Sandra Kosha, and Patricia Keogh for their skill and patience in typing the manuscript. Finally, to the staff of Little, Brown and Company go my gratitude and appreciation for their friendly assistance throughout the preparation of this book.

R. S. S.

CONTENTS

INTRODUCTION

Before beginning the study of histology, a freshman medical student should first become familiar with the general structure and use of a microscope. The student should also understand the basic principles involved in the preparation and staining of tissues so that he or she will be able to interpret histological sections more accurately. It has been my experience that many students are so awed by the fact that they have at last gained entrance into medical school after years of hard work that they are tempted to plunge immediately into the subject with little knowledge of the "tools of the trade."

LIGHT MICROSCOPE

A *simple microscope* has a single lens and produces only a moderately magnified image of the object you are studying. A *compound microscope* consists of a series of lenses and produces a far greater magnification. You will be using a compound microscope in your histology course.

The compound microscope (Fig. I-1) consists of mechanical and optical parts. The mechanical part has a *base*, which provides a stable foundation for the microscope, a *pillar*, which extends upward from the base, and a *stage*, on which the object to be studied is placed. The optical parts are attached to the pillar above and below the stage. They consist of the eyepieces or oculars, the objectives, the condenser, and the mirror. In many microscopes, the mirror and the illuminator are securely housed in the base of the instrument.

The *eyepieces* consist of a combination of lenses, which are inserted into the upper end of the tube of the microscope. The engraved value, such as "12.5×," indicates the magnification of the eyepiece. The *objectives* (there may be three, four, or five) are a combination of lenses attached to the lower end of the tube of the microscope. The engraved value, such as "10×," indicates the magnification of the objective. A 10× objective used with a 12.5× eyepiece gives a total magnification of 125×. The different objectives are attached to the *nosepiece*, which in turn is attached to the lower end of the microscope tube. One changes from one objective to another by rotating the nosepiece so that one objective is moved away and another is moved into position.

The *condenser* is a combination of lenses situated below the stage. It projects a cone of light onto the object being observed. The condenser can be raised

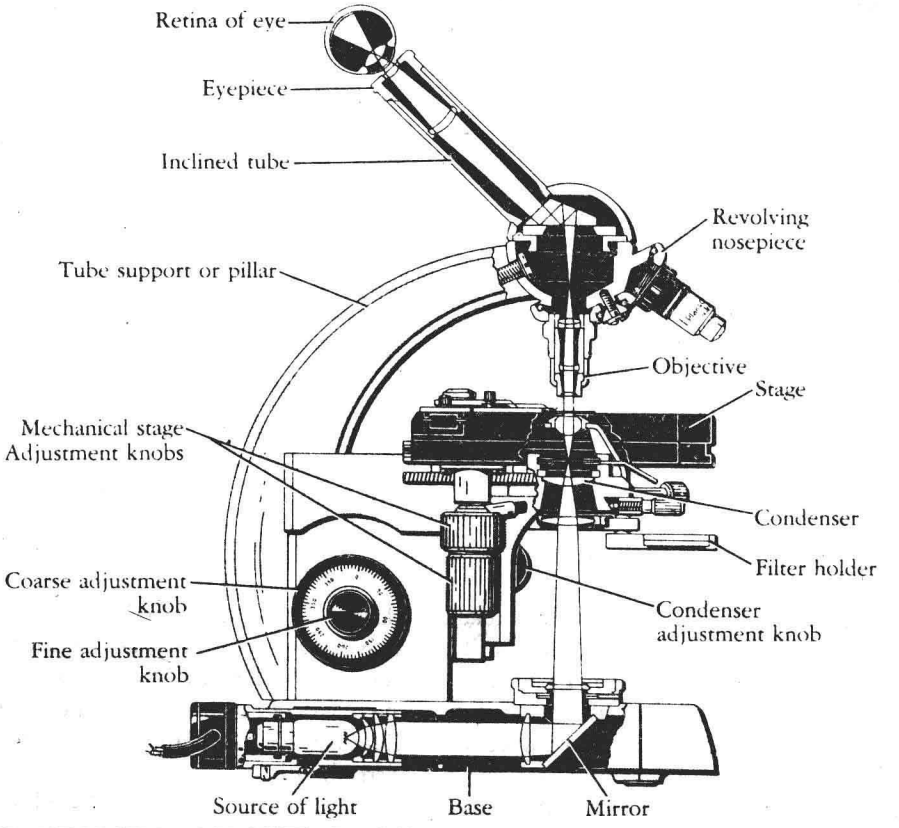

Fig. I-1. A student's light microscope and its main parts. The pathway of the light from the lamp in the base to the eye of the observer is shown. (Courtesy of Carl Zeiss Co.)

or lowered by a rack-and-pinion mechanism so that the light can be focused on the object. The passage of marginal rays into the condenser is prevented by the *iris diaphragm.*

The *mirror* that is situated below the condenser reflects light rays coming from the light source. Situated between the mirror and the condenser there is usually a swing-out holder for *light filters.*

How Does the Light Microscope Work?

The object to be studied is mounted on a glass slide, which is placed on the stage of the microscope. The object is moved into position beneath the objective either by hand or by using a *mechanical stage.* The object is brought into correct focus either by raising or lowering the stage or by raising or lowering the microscope tube, to which are attached the eyepieces and the objective. The light rays originating from the light bulb in the illuminator are reflected from the mirror and pass through the condenser. Here they are bent so that they converge on the object. The light rays then enter the objective and pass through its lenses, which cause them to converge and cross. From this point the light rays pass through the lenses of the eyepiece, where they are again bent. Having emerged from the eyepiece, the light rays are directed toward the pupil of the eye; they then impinge on the retina. If the eye is relaxed, as in long-distance vision, a clear image of the object should be obtained when the objective is in the position of exact focus. The position of the

lenses of the microscope in relation to the object can be changed by adjusting the coarse and fine adjustments. The *coarse adjustment* brings about long-range movements, whereas the *fine adjustment* is a delicate mechanism bringing about small movements.

The compound light microscope is thus a two-stage magnifying system. The object is magnified first by the lenses in the objective and then again by a second set of lenses in the eyepiece. The total magnification is the product of the magnifications of the objective and the eyepiece. A compound microscope produces an image that is upside down and laterally reversed. The reversal is easily demonstrated: if you move the specimen to one side, the image moves in the opposite direction.

Magnification, Definition, Resolution, and Depth of Focus

Magnification is the increased size of the image as compared with the object. The total magnification of a compound microscope, as explained previously, is the degree of image magnification produced by the objective lenses multiplied by the magnification produced by the lenses of the eyepiece. Always use a low-power objective when you begin your examination of an object; it permits you to observe a wider field, and it is useful for scanning.

Definition is the sharpness of the image when the lens system has been adjusted correctly. A blurred image usually means that the lenses have been adjusted incorrectly or that they are dirty. Another common cause is inadvertently placing the glass slide on the stage with the wrong side up.

Resolution is the ability of a lens to visualize separately two points that appear as a single point when seen with a lower magnification. A good objective has the ability to separate adjacent points and thereby to permit minute details to be seen distinctly. An objective with a high resolving power has lenses that have been carefully made so that the rays of light passing through a given point do not get mixed up with rays passing through adjacent points.

The resolving power of an objective depends on an important factor called the *numerical aperture*. This may be defined as the angle between the light rays entering the center of the objective and those entering its outer, peripheral margin. The magnitude of this angle is not indicated in degrees but in the form of a sine value, that is, a numerical value. The numerical aperture (NA) of the lens is engraved on each objective, beside the magnification. The numerical aperture determines the ability of the objective to collect diffracted light from fine details in the object.

Depth of focus is the ability of the lens to show structures that are related to one another but lie at different levels in the specimen. The depth of focus diminishes as the magnifying power and the numerical aperture of the objective increase.

Objective Lenses and the Oil-Immersion Objective

The ordinary compound microscope is equipped with three or four objective lenses: 2.5× (low power), 10× (medium power), 40× (high dry objective), and 100× (oil immersion). These lenses will, for example, give magnifications of 25×, 100×, 400×, and 1000×, respectively, when combined with a 10× eyepiece. A wider range of magnification can be obtained by replacing the 10× eyepiece with others ranging from 5× to 20×.

To change objectives, rotate the nosepiece until the new objective clicks into position. Even though the objectives are parafocal, slight focusing may be necessary with the fine adjustment. With the high-power objectives, extreme care should be exercised to prevent damage to the thin glass coverslip covering the specimen or to the objective. Using the coarse adjustment, move the specimen (or objective) until the objective almost touches the coverslip. Then look through the eyepiece and use the fine adjustment to bring the object into focus.

The oil-immersion objective is used as follows: First, the area of the specimen to be examined is brought to the center of the field of vision with the use of a low-magnification objective. The stage of the microscope is lowered, and the nosepiece is rotated to swing the oil-immersion objective into place. A small drop of immersion oil is then placed on the coverslip above the object. The stage of the

microscope is then gradually raised until the objective just touches the drop of oil. Looking through the eyepiece, you then gradually raise the stage, using the fine adjustment, until the object comes into view and is in focus. When you are examining tissues with oil-immersion objectives, repeated focusing is necessary, because of the extremely shallow depth of focus.

The immersion oil tends to cling to the lower end of the objective, so the slide may be moved about on the stage as different fields of vision are explored. After you have completed your examination, the oil should be removed from the objective and the coverslip. Wipe the surfaces with lens paper or a soft cloth moistened with 90% alcohol.

PHASE CONTRAST MICROSCOPE

When a piece of unstained tissue is examined with an ordinary light microscope, the detailed structure cannot be visualized. The reason for this is that the refractive indices of the cellular components are very similar, resulting in a lack of contrast. The phase contrast microscope is an instrument that converts small differences in refractive index that cannot be seen into differences in intensity that are visible.

Light waves traveling through cellular components of different optical densities will do so at different speeds. Thus, light waves traversing nuclei, mitochondria, and cell inclusions will emerge at different times, out of phase with one another. There are special apertures with absorbing and phase-shifting plates situated within the condenser and the objective lenses of the phase contrast microscope that convert phase differences into intensity differences. The phase contrast microscope is particularly useful in studying unstained tissues and living cells.

INTERFERENCE MICROSCOPE

The interference microscope uses two separate beams of light that pass through the specimen. One beam passes through the object being studied, and the second passes through another, neutral area. The two separate beams are then combined in the image plane. Because the object being studied has a greater optical density than the neutral area, the beam of light passing through it will have been retarded or interfered with to a greater extent than the beam passing through the neutral area. The degree of interference can be used to measure the refractive index, the thickness, and the dry mass per unit area of the object.

POLARIZING MICROSCOPE

Polarization is a phenomenon that occurs when light passes through certain substances, such as crystals, and is divided so that two light rays emerge that are derived from one. These substances have two refractive indices and are said to be *birefringent.* In the polarizing microscope, the light is polarized below the stage of the microscope by a Nicol quartz prism called the *polarizer.* The polarized light then passes through the specimen. A second prism, called the *analyzer,* is located next to the eyepiece within the microscope tube. When the position of the analyzer and polarizer prisms is adjusted so that the two polarized light beams are traveling in parallel directions, a normal image can be seen through the eyepiece. If the analyzer is then rotated so that its axis lies at right angles to the polarizer, no light reaches the eyepiece and nothing can be seen. The placing of an amorphous (monorefringent) object on the microscope stage with the prisms in the same right-angle position will result in nothing being seen, because the light rays have not been split by the object. If now a crystalline or birefringent object is placed on the stage, a light image will appear on a dark background. Thus, for biological materials to alter the direction of polarized light, and thus be visualized with a polarized microscope, their submicroscopic structure must consist of oriented, asymmetrical molecules. Muscle fibers, connective tissue fibers, and lipid droplets exhibit birefringence and have been studied extensively using polarizing microscopes.

FLUORESCENCE MICROSCOPE

In this form of microscopy, ultraviolet light is used to illuminate the specimen. Certain biological substances emit visible light when they absorb ultra-

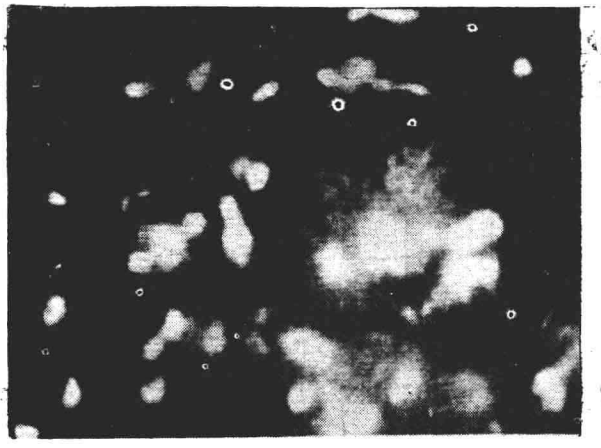

Fig. I-2. Photomicrograph of Kupffer cells of liver that have phagocytosed fluorescent latex particles. (Courtesy of Dr. R. S. McCuskey.)

violet light and are said to exhibit fluorescence. The observed image gives the appearance of being self-luminous. Fluorescence may take place with naturally occurring compounds such as vitamin A. Also, fluorescent dyes can be introduced into the specimen (Fig. I-2), where they may bind to specific compounds or be coupled with specific antibodies.

TRANSMISSION ELECTRON MICROSCOPE

The transmission electron microscope (TEM) differs from the light microscope in that it uses a beam of electrons rather than a beam of visible light (Fig. I-3). One of the great disadvantages of the light microscope is the long wavelength of visible light, which limits the maximum resolving power to about 0.2 μm. A stream of electrons has a very short wavelength, and resolutions of about 0.2 nm can be obtained with modern electron microscopes.

In the electron microscope, the electrons are emitted by a heated tungsten filament called the *cathode.* Because electrons are charged particles and would collide with air molecules and thus be absorbed and deflected, the entire optical system of an electron microscope must operate in a vacuum. The *anode* is a metallic plate with a small hole in its center. A potential difference of 40 to 100 kV between

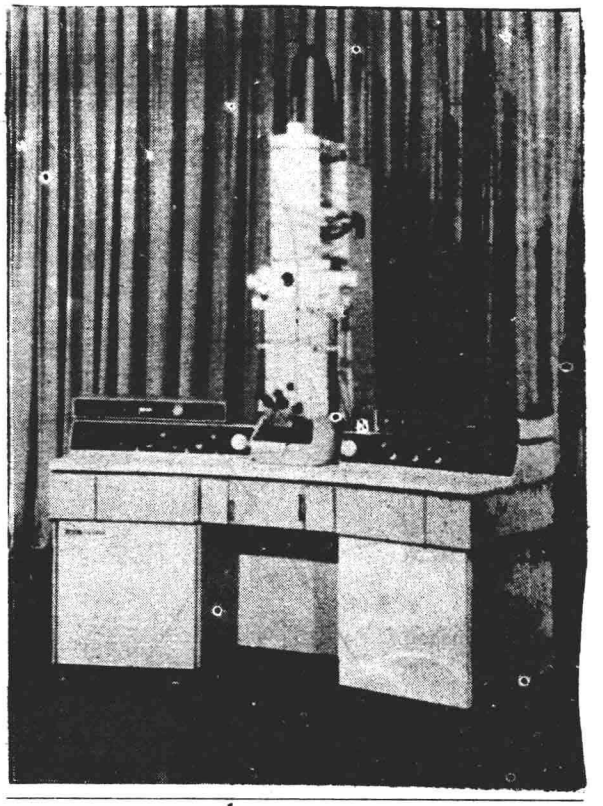

Fig. I-3. The JEOL model JEM-100 CX transmission electron microscope. (Courtesy of JEOL, Ltd.)

the cathode and the anode accelerates the electrons as they pass from the cathode to the anode. On reaching the anode, many of the electrons pass through the hole in its center to form a beam. The electron beam then passes through a series of electromagnetic lenses similar to the glass lenses found in the light microscope (Fig. I-4). The electromagnetic lenses serve to focus the beam of electrons, and the strength of the magnetic field produced by the lenses can be changed by altering the amount of current passing through the coils of wire in the lenses. In this way the condenser focuses the beam on the object. As the electrons leave the object, they are focused by the objective lens, and a magnified image is obtained. The image is further enlarged by one or two projection lenses. Because

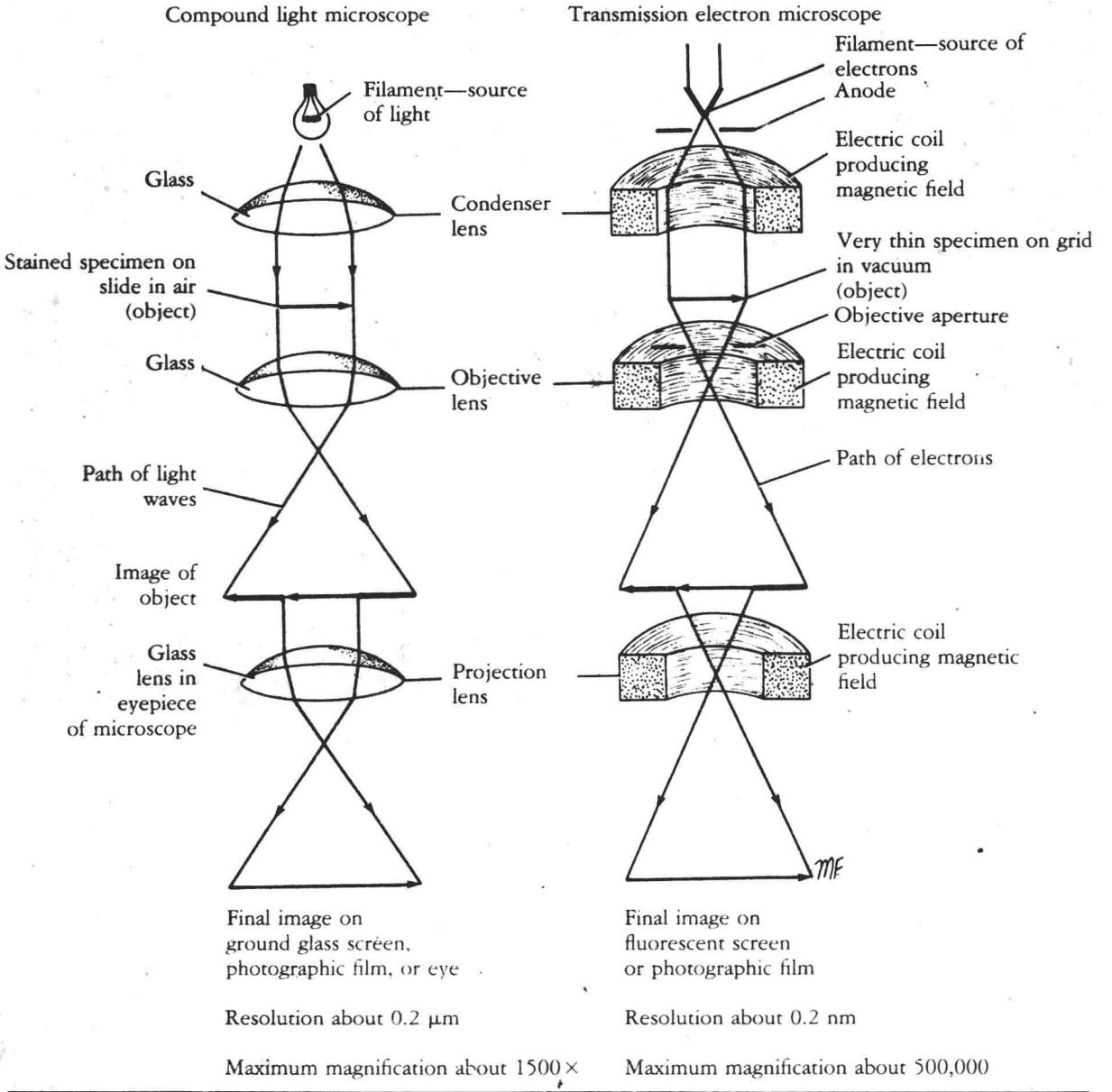

Fig. I-4. Comparison of the paths taken by a beam of light in a compound light microscope and a beam of electrons in a transmission electron microscope.

electron beams are invisible to the eye, the image is revealed by causing the electrons to project onto a fluorescent screen or photographic film.

Unfortunately, electron beams have a very poor penetrating power, so specimens must be cut in extremely thin slices (0.02–0.1 μm). Because the thin sections have very little contrast, they have to be stained with heavy electron-absorbing metals (such as uranium and lead) to increase the contrast.

The penetrating power of electrons is augmented by increasing the accelerating voltage. It is now possible with accelerating voltages of a million volts to use thicker sections (1–5 μm) and at the same time have a higher resolution.

SCANNING ELECTRON MICROSCOPE

The scanning electron microscope (SEM) examines the surface of the tissue; the electron beam does not pass through the specimen (Fig. I-5). A narrow electron beam is directed onto the surface of the specimen and scans back and forth in a regular manner. As the electron beam strikes the surface of the specimen, secondary electrons are emitted from the surface. The secondary electrons are caught by detectors, creating an electrical signal, which is displayed on a television screen. The scanning beam of electrons impinging on the specimen travels in synchrony with the image-producing beam on the television screen. In this way, a three-dimensional image of the surface of the specimen can be built up on the television screen. Micrographs can be obtained by photographing the image.

The tissue is prepared for SEM first by fixation and then by careful dehydration. The surface of the specimen is then coated with a thin layer of metal, such as gold, gold-palladium, or carbon, to assist in the scatter of the electrons.

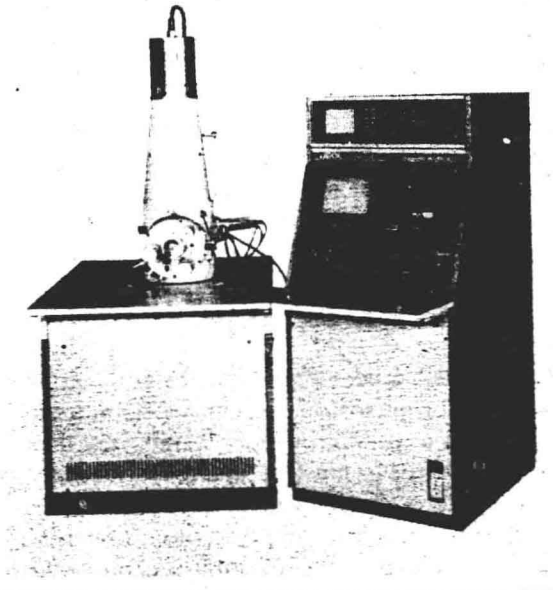

Fig. I-5. The JEOL model JSM-35 scanning electron microscope. (Courtesy of JEOL, Ltd.)

PREPARATION OF TISSUES FOR LIGHT MICROSCOPIC EXAMINATION

Obtaining the Specimen

Human tissues to be examined are usually from surgical specimens that include normal as well as diseased tissue. Small samples of normal tissue, only a few millimeters thick, are carefully cut from the specimen by using a pair of forceps and a sharp razor blade. It is important at this stage that the tissue not be damaged or distorted by excessive handling or by using dull cutting instruments.

Fixation

The tissue block is then rapidly immersed in a solution of chemicals to preserve the protoplasm. The chemical substances used to preserve tissues are called *fixatives*. Fixatives cause the precipitation of proteins, and most inactivate cell enzymes and inhibit autolysis. Tissues become harder as a result of fixation. Tissues removed from the body should be rapidly fixed, and this is best accomplished by using a small block of tissue and a large volume of fixative. This ensures a quick penetration of the tissue by the fixative. Failure to fix the tissue adequately permits intracellular enzymes to continue to function and

to break down the cellular structure, a condition known as *postmortem degeneration.*

There are many different fixatives, and the choice among them will depend on the particular structures you wish to study. Not all fixatives are compatible with all staining methods. For example, one fixative may act as a mordant for staining, whereas others inhibit staining. The fixative that preserves glycogen and allows it to be stained is absolute alcohol; the fixative of choice for fat is formalin. The most commonly used fixative is a 10% solution of formalin in saline.

Processing

After the tissue has been preserved, the next step is to prepare it for microscopic examination. In order to permit light to pass through the tissue, very thin sections of the tissue must be cut. Unfortunately, although the process of fixation does harden the tissue, the material does not become sufficiently firm or cohesive to permit perfect thin sections to be cut. For this degree of firmness to be achieved, the tissue must be completely impregnated with some supporting medium that will hold the cells and intercellular structures together. The supporting materials used are referred to as *embedding materials.*

Some embedding materials, such as Carbowax and gelatin, are water-soluble, and tissues do not have to be dehydrated before their use. The most commonly used embedding materials are paraffinlike substances that are not miscible with water; when they are to be used, tissues must be dehydrated prior to impregnation. Examples of such substances are Paraplast and Tissue Prep.

Dehydration

Before an embedding material such as paraffin can penetrate fixed tissue, the water content must be removed. Dehydration is carried out by immersing the tissue block in increasing concentrations of ethyl alcohol. The use of graded strengths of alcohol gradually removes the water from the tissue without causing cellular damage and replaces the water with alcohol. Alcohol has the advantage of further hardening the tissue.

Clearing

Impregnating the tissue with an embedding medium is impossible at this stage, because the paraffinlike substances used for embedding are immiscible in alcohol. The tissue must therefore be immersed in a chemical in which both alcohol and paraffin are soluble. The chemical xylene is commonly used. Such a chemical is often referred to as a *clearing agent,* because it makes the tissue transparent as a result of its high refractive index. The block of tissue is removed from the absolute alcohol and passed through successive changes of xylene until all the alcohol is replaced with xylene.

Embedding

Once the tissue is impregnated with the clearing agent, the block of tissue is placed in melted paraffin. The tissue is transferred through two changes of paraffin to ensure the replacement of all the clearing agent with paraffin. The tissue block and the paraffin are then poured into a mold and allowed to harden. Excess paraffin is then trimmed away.

Sectioning

The small block of paraffin containing the tissue is then mounted on an instrument that is designed to cut thin tissue slices. The instrument, called a *microtome,* has a sharp steel knife, capable of regularly cutting paraffin sections 4 to 6 μm thick (Fig. I-6). The paraffin sections are caused to flatten by floating them on warm water; they are then transferred to glass slides coated with adhesive.

Staining

Most unstained tissues are almost transparent, and the recognition of structures under the light microscope is difficult, if not impossible, in such tissues. Staining methods were introduced in the middle of the nineteenth century, and their use immediately led to enormous advances in our knowledge of histology.

The different staining techniques result in the differential dyeing or metallic coating of the various

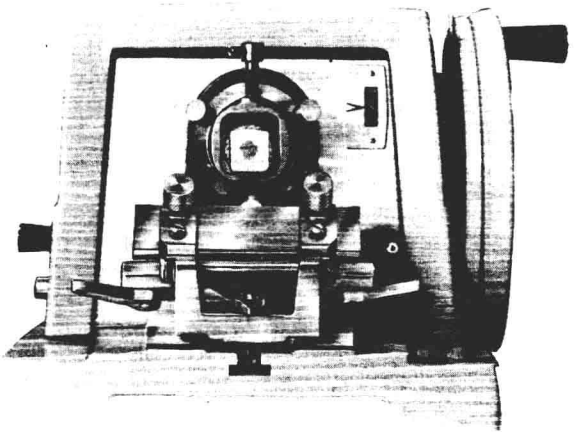

Fig. I-6. Microtome for cutting paraffin-embedded tissues. Each turn of the wheel seen at the right advances the specimen block, which then strikes the knife edge, cutting the sections. (Courtesy of American Optical Corporation.)

tissue components. Unfortunately, the chemistry involved in the majority of techniques is not understood, and many staining techniques were discovered by accident. There are three broad groups of biological stains. The first consists of the general tissue stains, which use one or two dyes to differentiate the nucleus from the cytoplasm of cells. The second group involves special staining procedures, for example, those used to demonstrate collagen and elastin in connective tissue. The third group includes heavy metal impregnation methods, in which metal salts are deposited on tissues and the salts are then converted to metal.

It is unnecessary for a medical student to have an encyclopedic knowledge of the different staining techniques now available to make different tissue components visible under the light microscope. Table I-1 summarizes some of the common techniques used in the preparation of microscope slides. Examples are shown in Figure I-7.

The most common combination of dyes used for histology and histopathology is *hematoxylin* and *eosin* (H&E). Hematoxylin is a natural dye obtained from the bark of logwood trees. It is not a dye as such, and must be oxidized to hematein to become a stain. Furthermore, the resulting dye (hematoxylin-hematein) has no affinity for tissues. A mordant, such as aluminum or iron, must be used with the hematoxylin mixture before it can stain tissues. The dye mixture stains a purple-blue color. Eosin is a synthetic dye and gives a pink to red color.

In cells stained with H&E, the nucleic acids present in the nucleus are stained with hematoxylin, giving the nucleus a purple-blue color. Eosin is attracted to the basic elements of protein in the cell cytoplasm, staining the cytoplasm pink to red. Tissue components that stain readily with basic dyes are called *basophilic*; those with an affinity for acid dyes are termed *acidophilic*. Hematoxylin behaves as a basic stain and therefore stains nuclei basophilically. Eosin is an acid dye and stains the basic protein elements in the cytoplasm acidophilically.

Metachromasia

Certain dyes react with tissue components and stain them a different color from that of the dye solution. The color change in the dye is called *metachromasia*. Examples of single dyes that exhibit metachromasia are methylene blue, toluidine blue, and thionin. With blue dyes, the color shift is toward red. A good example is the staining of mast cells with methylene blue. The cytoplasmic granules will stain purple-red, whereas the remainder of the tissue will be blue. The cause of metachromasia is not fully understood, but it has been suggested that polymerization of the dye molecules is responsible. The presence of macromolecules with electronegative radicals in the tissue is thought to facilitate the polymerization and bring about a color change.

Process of Staining and Mounting Sections

Before a tissue section can be stained, the paraffin in which the tissue is embedded must be removed. The section, which is adherent to the glass slide, is passed through xylene, and this dissolves the paraffin. Because many stains are dissolved in water, it is now necessary to remove the xylene from the

Table I-1. Stains commonly used in histology

Method	Composition	Result
Hematoxylin and eosin (H&E)	Hematoxylin, hematein, metal mordant	Nucleus—blue; cytoplasm—varying shades of red; collagen—very pale pink; cartilage and calcium deposits—dark blue; red blood cells—bright red
Heidenhain's iron hematoxylin	Iron alum, hematoxylin	Muscle striations—blue-black; nuclei—blue-black; mitochondria—blue-black
Masson's trichrome stain	Iron hematoxylin, Biebrich scarlet, acid fuchsin, phosphomolybdic-phosphotungstic acid, aniline blue	Nuclei—black; cytoplasm, keratin, muscle fibers—red; collagen, mucin—blue
Mallory's aniline blue collagen stain	Acid fuchsin, aniline blue, orange G	Nuclei—red; collagen—blue; ground substance, cartilage, mucin—blue; red cells, myelin—yellow; elastic fibers—pink, yellow, or unstained
Verhoeff's elastic stain	Hematoxylin, ferric chloride, iodine, sodium thiosulfate	Elastic fibers—blue-black to black
Orcein elastic stain	Orcein, alcohol, hydrochloric acid	Elastic fibers—dark brown
Weigert's elastic stain	Hematoxylin, resorcin-fuchsin	Elastic fibers—blue-black to black
Metallic impregnation of reticular fibers	Silver nitrate or silver hydroxide	Reticular fibers—black
Wright and Giemsa stains for blood	Methylene blue, methylene violet, azure A, azure B, eosin	Nuclei of white cells—purple; basophilic cytoplasm of lymphocytes, monocytes—blue; eosinophilic granules—pink to orange; neutrophilic granules—pink to purple; basophils—dark blue cytoplasmic granules; red blood cells—pink

tissue and replace it with water. The section is immersed in a series of diminishing concentrations of ethyl alcohol until it is filled with water. After the section has been stained with the appropriate dye solution, it is passed through alcohols of increasing concentration to remove all the water once again. Finally, the section is immersed in xylene before being mounted in a mounting medium soluble in xylene. A thin glass coverslip is then placed over the section to protect it and to make a permanent preparation.

PREPARATION OF TISSUES FOR ELECTRON MICROSCOPY

The same general principles are involved in the preparation of tissues for electron microscopy as for light microscopy, with the following important differences.

Fixation

Most fixatives used for light microscopy produce a coarse precipitation of protein and loss of ultrastructural detail; they are therefore unsatisfactory for electron microscopy. A double fixation process is

Fig. I-7. Photomicrographs of sections, showing the use of common stains. (A) Transverse section of esophagus stained with hematoxylin alone; the nuclei are blue. (B) Transverse section of esophagus stained with eosin alone; the cell cytoplasm shows varying shades of red. (C) Transverse section of esophagus stained with hematoxylin and eosin (H&E); the nuclei are blue, and the cytoplasm is red. (D) Transverse section of esophagus stained with Mallory's stain; cytoplasm is pink, the collagen is blue. (E) Transverse section of small artery stained with Weigert's elastic stain; elastic fibers are black. (F) Section of lymph node, showing reticular fibers impregnated with silver; reticular fibers are black.

now commonly employed. First, a very small piece of tissue (just a few cubic millimeters) is fixed in buffered glutaraldehyde solution. In the case of animal tissues, it is sometimes preferable to perfuse the organ before death to ensure that the minimum of postmortem degeneration occurs. The tissue is then exposed to a second fixative of buffered osmium tetroxide. Osmium tetroxide not only fixes the tissue but also "stains" many of its components. Staining here means that the reduced osmium becomes adherent to some of the cell components and, because of its density, increases the contrast of the image.

Embedding

Because electrons have very little penetrating power and are scattered by passing through a specimen, electron microscopy requires that very thin sections be used. For this to be possible, the tissue must be embedded in material that is harder than paraffin. The epoxy resins Epon and Araldite are commonly used.

Sectioning

Ultrathin sections, 50 to 100 nm thick, are necessary for electron microscopy. Compare this thickness with the 5 to 10 μm required for the light microscope. The very thin sections are cut on an ultramicrotome (Fig. I-8) with a knife made of plate glass or diamond. The sections are floated on water and mounted on copper grids. The grids support the delicate sections and permit the passage of the electrons through those portions of the section that lie suspended over the grid perforations.

Fig. I-10. Photomicrographs of sections, showing the use of some common histochemical stains. (A) Vertical section of white skin treated with dopa reagent and counterstained with eosin; the melanin granules are brown. (B) Section of cortex of kidney, Jones's periodic acid–methenamine silver stain; the basement membrane is black. (C) Section of submandibular salivary gland, mucicarmine stain; the mucin is red. (D) Section of liver, acid phosphatase stain; black lead phosphate precipitate at site of enzyme activity. (E) Adipose tissue cells, osmic acid stain; fat is stained black. (F) Liver hepatocytes, Best's carmine stain; glycogen is red.

Staining

Staining is a misnomer in electron microscopy, because colored dyes are not used. In order to obtain a greater contrast in the black-and-white images of tissue components on the fluorescent screen or photographic film, however, substances are used that adhere to the different cellular components and make them more electron-dense. Solutions of salts of heavy metals such as uranyl acetate or lead citrate are commonly used for this purpose.

Freeze-Fracture Etching

Freeze-fracture etching is a method of preparing tissues for electron microscopy without using chemical fixatives or dehydrating and embedding agents. The tissue is rapidly frozen at very low temperatures (−160°C) and then fractured with a metal blade. The cut surface of the tissue is not produced by the metal blade cutting through the tissue; rather, the striking of the frozen tissue by the blade initiates a fracture line that spreads across the tissue, much like a fracture line running across broken glass. The exposed surface of the tissue is then kept in a vacuum at a low temperature, which permits the frozen water on the surface of the tissue to sublimate into the vacuum. This is the etching phase of the process, and it leaves the various cell components standing out in relief on the frozen surface (Fig. I-9). Carbon and platinum are then deposited on the frozen-fractured-etched surface of the tissue at an angle to produce a shadowed effect, similar to the way driven snow is blown over a group of houses: the snow piles up on the near sides of the houses but leaves clear spaces or shadows on the far sides of the houses. The tissue is now brought back to normal atmospheric pressure and temperature. The carbon-platinum replica is placed on a copper grid after the tissue has been digested by a strong acid and can then be examined with a transmission electron microscope. Freeze-fracture etching has been most useful in the study of cell membranes and their junctional complexes.

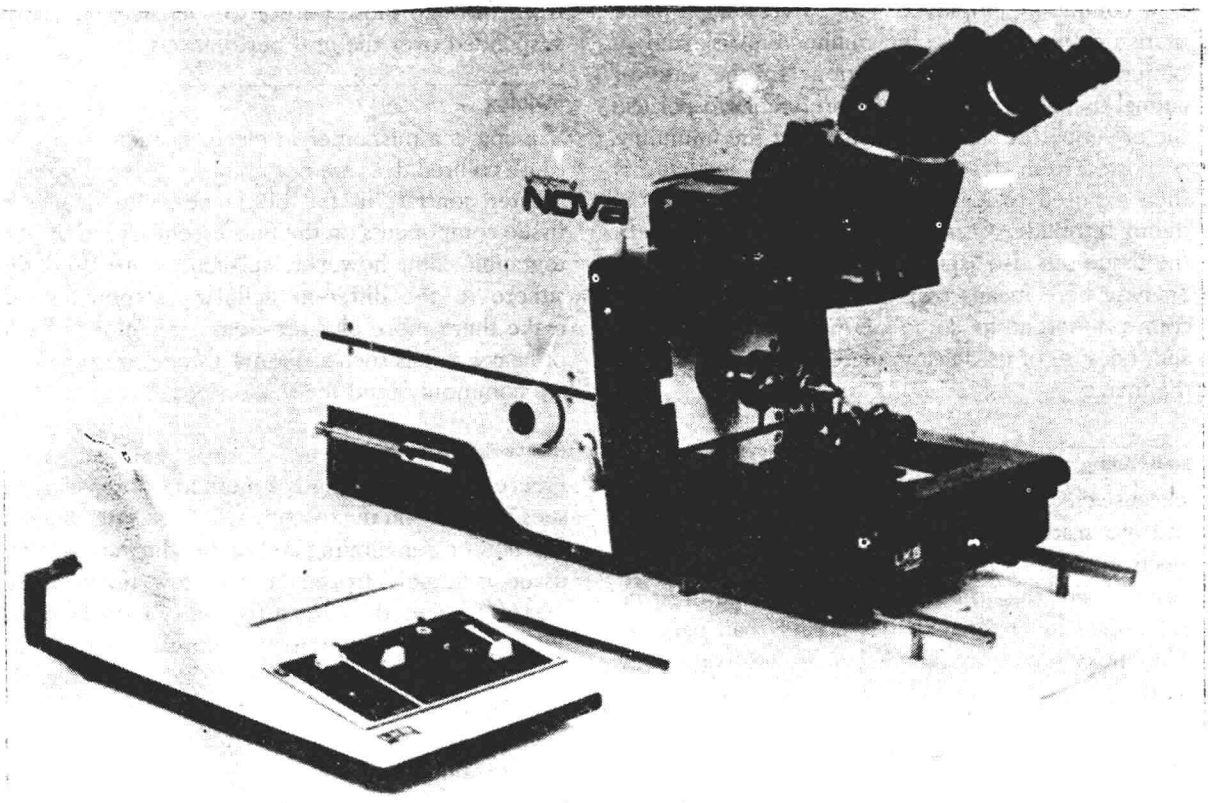

Fig. I-8. Ultramicrotome for cutting epoxy-embedded tissues. The knife is plate glass or diamond, and the sections are floated on water. The tissue block is advanced by heat expansion of a metal rod or by a delicate mechanical mechanism. The cutting procedure is closely watched through a low-power binocular microscope. (Courtesy of LKB.)

HISTOCHEMISTRY AND CYTOCHEMISTRY

Histochemistry and cytochemistry are the study of the chemical processes that take place within cells and tissues and the identification of the structural sites where these chemical reactions take place. Using a tissue section, one attempts to localize a particular chemical component by means of a chemical reaction whose end product is visible with the microscope. At first, the use of histochemistry was limited to light microscopy, but the methods have been so refined that, for example, it is now possible to localize glucose-6-phosphate in the rough and smooth endoplasmic reticulum with the electron microscope.

At this point you may ask yourself what the difference is between a histochemical staining method and a morphological staining method that has been used traditionally by histologists for demonstrating such structures as nuclei, Golgi complexes, and nerve fibers. A histochemical technique is one based on fully understood inorganic or organic chemical reactions, whereas the use of ordinary dyes for staining involves reactions that are not always understood and may involve a multitude of physicochemical phenomena.