BASIC HISTOLOGY

A Review with Questions and Explanations

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First Edition

Library of Congress Catalog Card No. 82-82839
ISBN 0-316-92358-3
Printed in the United States of America
HAL

Preface

Knowledge of the microscopic anatomy of the human body is a fundamental building block in understanding the function of organs in health and disease. It is knowledge that is presumed in physiology, pathology, pharmacology, and clinical therapeutics textbooks, and thus, a solid foundation in the basics of histology must be laid early in a medical student's training.

This medical review text explains the structure and organization of basic tissues. The histology of the specialized organ systems is not addressed, since it is outside the province of this book. The book's format integrates didactic discussions with question-and-answer sections designed both to test the reader's comprehension of the information just read and to be an integral part of the process of imparting information. The format requires active participation by the reader, thus aiding both in initial insight and in information recall. We hope the active role of the students in reading the book will enhance their enjoyment of the learning process. The format of the book should prove especially useful to those preparing for board examinations, since the questions are similar to those they may confront on the "boards."

The material for the book was developed from a syllabus of the Anatomy Department of the University of Western Ontario, and we are grateful to all our friends in the department, especially R. C. Buck, M.D., Professor of Anatomy and former chairman of the department. We also gratefully acknowledge all those concerned with manuscript and illustration preparation. We would especially like to thank Mr. Cliff George and the people in the Audio Visual Services Division of the Faculty of Medicine at Memorial University of Newfoundland for their assistance with the drawings.

B. A. W.
B. J. J.

1 Light Microscopy Methods Used in the Examination of Cells and Tissues

A SHORT HISTORY

The invention of eyeglasses for the improvement of failing vision occurred in about the fourteenth century. However, the obstacle to further development of scientific optics was the association of optics with magic and conjurors' tricks; thus, doubt was cast on the validity of objects seen through lenses. The astronomer Johannes Kepler, who published his major work *(Astronomia nova)* in 1609, also investigated the structure of the eye and showed that it acted as an optical instrument. This corrected earlier misperceptions of its nature and opened the way to acceptance of the fidelity of images formed by lenses.

Who the actual inventor of the microscope was is uncertain, but many primitive instruments were in existence in the first half of the seventeenth century. Galileo possessed a compound light microscope in 1609, and another early microscope was demonstrated by Cornelis Drebbel in London in 1621. The **field lens** next appeared and was described by Huygens to be present on a microscope made by Johann Wiesel at Augsburg. The grinding of lenses that are very small yet perfectly shaped was a difficult task at which few investigators were successful. Anton van Leeuwenhoek was able to produce lenses that had a resolving power of 1.4 μ at a magnification of 270×. Between 1673 and 1716, he published observations on protozoa, bacteria, and many other subjects.

Some advances were made in the next half century, one of the main ones being the introduction by John Cuff, in 1744, of a central pillar to support the various parts of the instrument. Advances present in Cuff's microscope were an accessible stage, a substage mirror, and a focusing screw, as well as the central pillar still present in today's instruments.

The poor illumination of the specimen gave rise to obscure image formation, which resulted in certain aberrant optical phenomena inherent in the instrument. These phenomena were described as being part of the object observed. Thus, Leeuwenhoek assumed that the image distortion caused by diffraction was a primary component of all matter, rather than what it in fact was—an artifact due to the instrument. Although Sellique was not the first to advance the idea of combining two, three, or four low-power achromatic doublet lenses, his ideas were taken up by instrument manufacturers and incorporated into the newer microscopes.

The earliest compound light microscopes developed together with the telescopes and consisted of two lenses. The objective lens, the one closest to the object observed, gives the initial magnification; the lens of the eyepiece, or the projector lens or ocular lens, magnifies the image a second time.

Carl Zeiss made his first compound microscope in 1857. Earlier, after settling in Jena in 1846, he had been supported and advised by botanists. The manufacture of a compound microscope's objective lens was at this time a te-

dious process of trial and error. Zeiss therefore decided to seek a scientific method for its manufacture, and he persuaded Ernst Abbe to join him in this venture. Abbe established the **theory of image formation** and designed new types of objective lenses and methods of testing them. Otto Schott, a glass chemist, joined Zeiss and Abbe. With new types of glass Abbe was able to construct **apochromatic** (corrected for chromatic and spherical aberration) objective lenses. The original microscope lenses were not corrected for chromatic aberration at all. Apochromatic lenses are corrected for the entire visible spectrum. **Achromatic** lenses eliminate chromatic aberration within the spectral range of green, yellow, and orange.

The compound light microscope as improved by Zeiss, Abbe, and Schott contributed to the discovery of the tubercle bacillus by Robert Koch, an event that may be said to mark the beginning of modern bacteriology.

There are two ways of viewing objects under the compound light microscope: (1) by transmitted light and (2) by reflected light. When an object is viewed under the microscope by reflected light, the surface of the object reflects light, and those rays that enter the objective lens make up the image observed. When an object is observed by transmitted light, light from the microscope lamp is either reflected from a substage mirror or passes directly from a light source through a condenser to a focus on the object viewed.

LIGHT MICROSCOPY

The microscope is one of the most important instruments that the student of medicine will encounter. One should thoroughly understand the structure of the instrument and the theory behind it and should be skilled in its manipulation.

The Microscope

A light source is essential for ordinary light microscopy. The light from the microscope lamp passes through or is reflected by (in order) the mirror, the substage condenser, the prepared specimen sample, the objective lenses in the nosepiece of the microscope, and the ocular lenses in the eyepiece.

The **mirror** collects light and reflects it into the optical axis of the microscope. Some modern microscopes do not have a mirror. Instead, the light source is aimed directly at the substage condenser lens.

The **substage condenser lens** collects and focuses light rays onto the thin section of the object being studied. An iris diaphragm similar to that which regulates a camera lens aperture (f-stop setting) is attached on the condenser lens mounting and serves to control the amount of the light passing into the substage condenser lens from the mirror.

The **objective lenses** are small, planoconvex lenses mounted in the nosepiece of the instrument. Usually, three are mounted on a turret: a low-power, a high dry-power, and an oil-immersion lens. Each "lens" mounting contains a series of lenses that together produce a magnification equal to that of a simple lens with a focal plane of 16 mm in the case of the low power. Effective magnification is up to 1500×.

The **oculars,** or eyepiece lenses, further magnify the image and produce the final image at the eye point of the ocular. The final magnification is the product of the initial magnification of the objective lens and the magnification of the ocular lens. Hence the term **compound.** A **simple microscope** is a single magnifying lens.

QUESTION

Draw a compound light microscope, showing the mirror, substage condenser, microscope slide and object, objective lens, and ocular lens. Indicate the light path through the microscope. (Compare your drawing with that given in the Appendix.)

ANSWER

The simple hand lens or magnifying glass has been called a **simple microscope.** The compound light microscope consists of a magnifying device that uses the light rays to illuminate the subject of observation; the **electron microscope** is a magnifying device that uses an electron beam to "illuminate" the subject. The electron microscope has a range up to 300,000× magnification. Further, even the position of atoms in macromolecules can be worked out from x-ray diffraction patterns on photographs.

It is important to get a bright image, and, for this reason, there are devices beneath the stage of the microscope designed to concentrate a beam of light on the object on the microscope slide. Light, either directly from a lamp or reflected from a mirror, is aimed into the condenser lens. This condenser lens, in turn, gathers the light into a beam focused on the particular area under the objective lens. The condenser lens is fitted with an iris diaphragm similar to those in cameras.

QUESTION

What are the causes of poor definition of an image seen in the microscope?

A. Improperly focused condenser
B. Dirt
C. Both A and B
D. Wrong position of microscope slide
E. All of these

ANSWER

Answer: C. Two of the common causes of poor image definition are an improperly focused condenser and dirt. Other reasons for poor definition are improper opening of the condenser diaphragm and a poor specimen. The specimen on the microscope slide may have no contrast or it may be too thick to observe with transmitted light.

Resolution

The best **resolution** of the average eye is about 200 μ, and dimensions of cells are of the order of 10 to 100 μ. Microscopy with an ordinary optical lens system is limited by the nature of light and the lenses.

The amount of information obtained by means of a microscope from a section of tissue depends on (1) the resolving power of the microscope and (2) the contrast between various components in the stained section.

The **resolution distance** is the least distance by which two points may be separated and still be recognized as two after magnification. Since it is impossible to resolve two transparent objects in a clear homogeneous matrix, which affects light similarly (of similar refractive index), contrast by means of staining is essential in normal histologic work.

Light is visible radiant energy that behaves as follows:

1. Light travels in straight lines in homogeneous media and behaves both as particle and wave.
2. The light wave moves in three dimensions from its source.
3. Waves from a point source that are put out of phase with each other and then recombined interact and reinforce (increase amplitude) or cancel each other (decrease amplitude). Diffraction fringes produced when light passes through a slit or a hole are interference phenomena.

The wavelength of visible light is 0.5 μ (one micron [μ] is 1/1000 millimeter). The resolution of a light microscope is 0.2 μ.

QUESTION

Can the resolution of a light microscope be further improved?

A. Yes, through improvements in the condenser lens system.
B. Yes, through improvements in the objective lens system.
C. No, the resolution is limited by the wavelength of light.
D. No, the condenser lens system and objective lenses have been greatly improved and no further improvement is possible.

ANSWER

Answer: C. The resolution of a light microscope cannot be improved further because the resolution is limited by the wavelength of light.

One cannot expect to accurately measure an object when the smallest measuring unit of the measuring device is larger than the object. In the same way, if the wavelength of the emission is larger than the object to be illuminated, the image will be blurred.

One of the main conditions for good resolution is that the objective lens

admit much diffracted light from the object observed. Diffraction of light will always occur when a light wave encounters an object that is not self-illuminating.

Numerical Aperture

Closely associated with resolution is numerical aperture. Let us consider a group of particles on a slide under the microscope. If one completely opens the iris diaphragm of the substage condenser lens, only a portion of the clump of particles will be in focus, no matter how one attempts to adjust the other microscope controls. Now, if one slowly closes the diaphragm, one will reach a position at which the whole depth of many clumps will appear to be in focus simultaneously. This leads to the concept of numerical aperture.

The **numerical aperture** of a lens is determined by the size of half the angle of the light cone entering the lens. The resolving power of an objective lens is directly proportional to the numerical aperture.

The formula for determining the numerical aperture (NA) of an objective lens equals the refractive index (n) of the medium between the object and the lens times sine of half the angle of the light cone entering the lens (a):

$$NA = n \times \sin a$$

The numerical apertures of the low-power, high dry, and oil-immersion objectives are usually 0.25, 0.65, and 1.25 or 1.3, respectively.

QUESTION

Why does the substage condenser lens have an iris diaphragm?

A. To vary the total illumination of the object on the microscope slide
B. To vary the amount of light entering the substage condenser
C. To vary the angle of light entering the objective lens

ANSWER

Answer: C. The substage condenser lens has an iris diaphragm so that the light entering the substage condenser and, **hence,** the objective lens, can be varied.

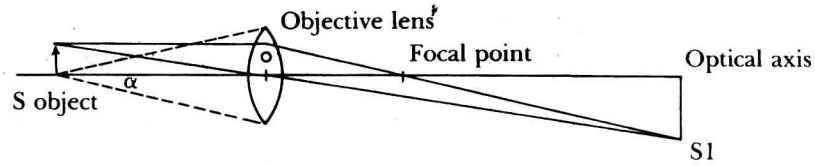
The **total illumination** of the object on the microscope slide should not be varied by moving the substage condenser or altering the iris diaphragm. The **rheostat** attached to the electrical supply to the lamp should be altered to adjust the total illumination. Choice B is correct as far as it goes, but the essential purpose of all the substage equipment of the microscope is to illuminate the object so that optimal conditions for viewing it are obtained.

The condenser lens below the stage permits light to illuminate the object through an angle that is consistent with the equality of the objective lens; i.e., it maintains the numerical aperture of the optical pathway.

The condenser lens is itself adjustable, because it is used with objective lenses of different numerical aperture and it must suit each. If too high a numerical aperture is provided by the condenser, excessive scatter or glare is produced, with loss of resolution.

Let us consider the image formation of a point source by the objective lens of the microscope by examining Figure 1-1.

Figure 1-1



The tip of an object S is imperfectly reproduced at S1 because only a portion of the light wave front is admitted to the lens and transmitted. This portion is determined by the solid angle α. The greater α, the more perfect the image at S1. α cannot be greater than 90 degrees, otherwise no light would enter the objective lens. The refractive index (n) between the subject S and the objective lens and the focal length of the lens are also of great importance to the equality of the reproduction of the subject S at S1.

All these conditions are described by the concept of **numerical aperture:**

$$NA = n \sin \alpha$$

The greater the numerical aperture, the better the image.

QUESTION

What is the maximum attainable numerical aperture for immersion lenses (1) in air, where refractive index = 1.0, and (2) in oil, where refractive index = 1.5?

A. 1.0
B. 1.3
C. 1.4
D. 1.5

ANSWER

Answer: D. The maximum attainable numerical aperture for oil-immersion lenses is 1.5.

$$NA = n \sin \alpha$$

where NA is the numerical aperture and n is the refractive index. The greatest angle α can be is 90 degrees.

$$\text{Sine } 90 \text{ degrees} = 1.0$$

$$n = 1.5$$

Solving the equation,

$$\text{Numerical aperture} = 1.0 \times 1.5 = 1.5$$

Refraction

Refraction is the phenomenon of a ray of light or heat being diverted or deflected from its previous course in passing obliquely out of one medium into another of different density, or in traversing a medium not of uniform density.

Refractive index is the ratio between the sines of the angles of incidence and refraction of a ray of light passing from some medium (usually air) into the given medium. There is a linear relationship between the refractive index of an object or solution and the concentration of solids contained in it.

The refractive index of a solid equals the refractive index of the solvent plus α times the concentration of the solution.

The concentration (K) is the concentration of the dissolved substance in grams per deciliter of solution and is a constant. α is defined as an increase in refractive index resulting in the concentration of the solid by 1 gram percent. Most substances found in living cells have a value of α very close to 0.0018.

The nucleus contains more solids per unit volume than the cytoplasm.

QUESTION

From the above equation, what is the refractive index of the nucleus?

A. Lower than that of the cytoplasm
B. Equal to that of the cytoplasm
C. Higher than that of the cytoplasm

ANSWER

Answer: C. The nucleus has a greater dry mass per unit volume than the cytoplasm. Thus, substituting in the equation:

Refractive index of object equals the refractive index of solution plus 0.0018 K. When K, the concentration of the solids in the solution, increases, the refractive index of the object or solution increases.

It is often very difficult to determine whether a specific object within a living cell is a vacuole or a more solid structure, such as an organelle.

If one were able to determine whether the object has a higher or lower dry mass than the surrounding material one would be able to distinguish between these two types of structure within the living cell.

QUESTION

This might be done by determining the refractive index of the given structure. Thus, when compared with the surrounding cytoplasm, the refractive index of an organelle such as a mitochondrion would be _____.

A. Equal to that of a vacuole
B. Lower than that of a vacuole
C. Lower than that of the cytoplasm
D. Higher than that of the cytoplasm

ANSWER

Answer: D. The refractive index of an organelle such as a mitochondrion would be higher than that of the cytoplasm.

The other choices (equal to or lower than that of a vacuole) are wrong. If anything, it would be much **higher** than that of a vacuole. The refractive index of a vacuole, however, would be **lower** than that of the cytoplasm.

The differences in the refractive indexes as explained in the preceding answer are the basis of the **Becke test.** A high dry objective lens and a stopped-down condenser are used. Under these conditions there is a thin band of light visible that outlines the periphery of the cell. If the focus is **raised,** the bright

halo moves **toward** the medium of higher refractive index; if the focus is **lowered,** the halo moves toward the medium of **lower** refractive index.

QUESTION

Using a high dry objective lens (×40) and a stopped-down condenser, observe a living cell. There is a structure in the cell that one thinks might be a vacuole or a mitochondrion. When one lowers the focus, the bright halo moves from the structure to the cytoplasm. The structure is _____.

A. A vacuole
B. A mitochondrion
C. A region of cytoplasm

ANSWER

Answer: B. When one lowers the focus **under the above conditions,** the bright halo moves from a region of high refractive index to one of low refractive index, that is, from a region of high dry mass to one of low dry mass. A vacuole has a lower dry mass than an equal volume of cytoplasm. A mitochondrion has a higher dry mass than an equal volume of cytoplasm. The structure therefore is a mitochondrion.

Immersion Refractometry

The above rules have been used in a technique known as **immersion refractometry.**

The technique is based on the fact that a living cell, if surrounded by a nonpenetrating and nontoxic medium of identical refractive index, will become almost invisible. The concentration of solids in the bathing fluids is then equal to that inside the cell. This is an indirect method for determining the concentration of solids within the cell. The method is valid only if the following conditions are also fulfilled: The solid has the same α as that within the cell, and the solvent has the same refractive index as the solvent within the cell (Refractive index of solid = refractive index of solvent + $\alpha \times K$).

In reference to the light microscope, the objective lens and the ocular, or eyepiece, lens both magnify the object on the microscope slide. The calculation of the magnification of the virtual image that is seen when one looks down a light microscope is made according to the following formula:

Objective lens magnification × ocular lens magnification = virtual magnification

The magnifications of the three most commonly used objective lenses are 10×, 40×, and 100×. The most commonly used ocular, or eyepiece, lenses are 6× and 10×.

QUESTION

What is the maximum magnification of a microscope with lenses as listed in the above paragraph?

A. 2400
B. 600
C. 1500
D. None of these

ANSWER

Answer: D. The maximum magnification of a microscope with the lenses (1) objectives 10×, 40×, 100×; and (2) oculars 6× and 10× is neither 2400 nor 1500. It is 1000; i.e., the 100× objective coupled with the 10× ocular.

Oil-Immersion Microscopy

In the first half of the nineteenth century, Amici and Ross both noticed the impaired image that occurred at higher magnifications owing to the thickness of the coverglass (coverslip) then available. Ross solved the problem by making one of the pairs of lenses in his objectives movable in respect to the other. Amici decided to try immersion of the lens. He first tried water-immersion lenses, but then used sassafras oil, the refractive index of which (1.53) is similar to that of cedar oil (1.51), which was later recommended by Abbe for homogeneous immersion.

With present-day instruments, the purpose of using oil between the lens and the coverslip with the highest power objective is to maintain the numerical aperture of the system and thereby permit resolution consistent with the optical quality of the lenses: It allows more light to enter the lens.

The working distance of the average oil-immersion objective is about 0.13 mm, and that of the low-power objective 4.0 to 4.5 mm. (The **working distance** is the distance between the bottom of the objective lens and the object when it is in focus.)

QUESTION

If the thickness of the preparation and coverslip above the object is greater than the working distance of the oil immersion lens one should _____.

A. Turn the slide over and view it from the opposite side.
B. This information is irrelevant.
C. Use oil between the oil-immersion lens and the slide.
D. Use the high dry objective lens for maximum magnification.

ANSWER

Answer: D. If one uses the oil-immersion lens, the object will not come into focus and one risks scratching or fracturing the lens and breaking the glass microscope slide. An oil-immersion lens, if it is used at all, must be used with cedar oil.

Magnifying Power

The **magnifying power** of a lens is always expressed in linear dimensions. The term implies that the relative length or "diameter" of an object, and not its area, will be magnified a given number of times.

QUESTION

What is the magnification of the objective lens in a microscope if a square whose side is 8 μ has a virtual image of 22 sq mm?

A. 10×
B. 40×
C. 100×
D. 110×
E. 45×

ANSWER

Answer: C. The magnification of the objective lens in a microscope the ocular lens of which is 6× and which produces a virtual image of 22 sq mm from a square of length 8 μ is 100×. The image of the square is 22 sq mm in area. Its side is therefore the square root of 22 sq mm (i.e., 4.8 mm or 4800 μ).

The magnification is therefore 4800 divided by the size of the side of the square, i.e., 8 μ.

$4800 \div 8 = 600\times$ magnification.

The total magnification of a microscope is equal to the magnification of the objective lens multiplied by that of the ocular lens.

Therefore, the objective lens in a system that has an ocular lens 6× and a total magnification of 600× is 100×.

Distortion

Distortion results from a lens surface's having differing magnifications at the marginal and central portions of the image. It is most frequently seen in low-power lenses and is best observed with a wire mesh as object.

QUESTION

If an objective lens system acts as a cylinder of glass and some of the elements of the objective lens system are not optically centered, the combined defects result in _____.

A. Spherical aberration
B. Diffraction disk
C. Astigmatism
D. Chromatic aberration
E. Coma
F. Coma and astigmatism

ANSWER

Answer: F.

CONTRAST AND STAINING

The early microscopists looked at thick sections with transmitted light. Although the objects were present, they were difficult to see. This was partly due to the thickness of the sections and the poor construction of their microscopes, but it also was because there was not enough contrast.

For a tissue to be stained, a colored dye must react with it. Most tissues are colorless, and their various components can be seen in detail only if they are first stained.

With each procedure one adds in the processing of tissue for staining, the more there is that is artificial in the final product. By **artificial** is meant alteration from what would be the appearance of the cell or tissue **in life.** A careful watch has to be kept for divergence from what is believed to be the appearance in life. The preparation on the slide therefore may show certain features that do not reflect the structure of the tissue or cell during life but only indicate the nature of the damage that has been inflicted on it by the very process of preparation. These defects are called **artifacts.**

Observations of material in the living animal, and correlation of these with observations of the sections for microscopy discourages the tendency to interpret artifacts as being structural defects of the cell.

QUESTION

When a tissue is stained what is seen?

A. The unaltered stain in contact with the tissue
B. The stain, which has reacted with the tissues and is fixed to certain components
C. Both A and B
D. None of the above

ANSWER

Answer: B. When a tissue is stained and then observed under the microscope, the contrast is due to the unstained portion and the stain, which has reacted with the tissues and is fixed to certain components.

The stains used in histology are complex organic chemicals that, for effective use, require that the tissues be carefully prepared, often in very specific ways. If the coloring property is in the basic radical of the neutral salt of the dye, the dye is referred to as a **basic dye;** if it is in the acidic radical, an **acidic dye.**

Acidophilic Versus Basophilic Staining

Substances that stain with basic dyes are called **basophilic;** those that stain with acidic dyes, **acidophilic.** The most commonly used stain in histology is **hematoxylin and eosin** (H & E). In this staining procedure, the basophilic elements of the cell are stained by the basic dye hematoxylin. These elements are the nucleic acids of the nucleus and the ribonucleic acid of the cytoplasm; they combine with the basic dye, so they are basophilic. The cytoplasm stains with the acidic dye eosin. The final appearance consists of nucleus stained blue (by the hematoxylin), and the cytoplasm stained pink by the eosin.

A substance stained by a dye may take a color that differs from that of the dye. It is then said to stain **metachromatically;** the property of the substance is called **metachromasis.** This property may be due to concentration of the dye at its sites of affinity or by a slight reactive alteration of its molecular structure at its affinity sites.

QUESTION

Why do histologic staining procedures use both a basic and an acidic dye?

A. To ensure that all basophilic and acidophilic structures are stained
B. To show up small basophilic structures in the cytoplasm of the cells
C. To contrast the basophilic structures of the nucleus and cytoplasm with the general acidophilic background of the cytoplasm

ANSWER

Answer: C. Most histologic staining procedures use both a basic and an acidic dye to **contrast** the basophilic elements of the nucleus and cytoplasm with the general acidophilic background of the cytoplasm.

The primary purpose of staining procedures is to obtain a **contrast** between components within the cell and between one cell and another. This permits the resolution properties of the microscope lenses to be used to the best advantage.

If appropriate dyes are used, both acidophilic and basophilic elements can be stained the same color.

Although obtaining contrast is an important reason for the existence of histologic staining, further information concerning the nature of tissues and cells is made available by the use of stains.

QUESTION

What sort of information might this be?

A. Contrast of tissues
B. Structure of tissues
C. Chemistry of tissues

ANSWER

Answer: C. Other information that has become available through the study of histologic staining procedures is a knowledge of tissue chemistry.

A large number of stains now exist that stain specific groups of chemical substances. A structure in a thin section of tissue with known positive and negative control sections can be put through various staining procedures, and its reactions to a battery of tests noted. From these reactions, it is often possible to work out the chemical constitution of the structure. This type of histochemical procedure is a further aid in the identification of structures that previously have been identified by morphologic criteria only.

SUGGESTED READING

Bradbury, S., and Turner, G. L'E. Historical aspects of microscopy. *Proc. R. Microscop. Soc.* 2, 1967.

Brown, E. B. *Modern Optics.* New York: Reinhold, 1965.

Joseph, A., and Leahy, D. J. *Programmed Physics. Part III. Optics and Waves.* New York: Wiley, 1965.

Rochow, T. G., and Rochow, E. G. *An Introduction to Microscopy by Means of Light, Electrons, X-rays, or Ultrasound.* New York: Plenum, 1978.

Rooseboom, M. The history of the microscope. *Proc. R. Microscop. Soc.* 2:266, 1967.

2 Preparation of Tissues for Light Microscopy

CELL DEATH AND AUTOLYSIS

We have already discussed the concepts of resolution and contrast in the observation of specimens under the light microscope.

The major use of the light microscope consists of viewing thin stained sections by transmitted light. We shall now discuss the usual steps involved in processing a piece of tissue to prepare thin sections for examination by transmitted light microscopy.

Cells and tissues taken out of the body die, and part of the dying process is the cessation of production of energy by the cells. This lack of energy and the loss of direction from the nucleus results in the cell's failure to maintain the structure of its internal biologic membranes. These membranes rupture and release their compartmental contents into the cytoplasm. In the cell are **lysosomes**, organelles that contain potent digestive enzymes. The release of these enzymes into the general cytoplasm allows them to digest other cell organelles. Buildup of metabolic acids also occurs because, with the cessation of the cell's respiration and its free interaction with the bloodstream, these acids accumulate in the cell and are not further processed. For example, as a result of this accumulation, mitochondria swell and may appear as cloudy droplets in the cytoplasm.

The above process is called **autolysis,** or **self-digestion.** It results in alterations in the cell structure that are very great compared to those that take place during life; it occurs if cells and tissues die slowly.

There are a number of fast-acting cell poisons that do not allow the cells and tissues to undergo autolysis. Freezing will slow autolysis, whereas warming tissues or cells will accelerate it.

QUESTION

When we examine tissues under the light microscope, we want their structure to appear as close to that in life as possible. What procedure would be most desirable after the removal of a specimen for observation under the light microscope at a surgical operation?

A. Keeping specimen at room temperature before sectioning it
B. Keeping the specimen warm in an incubator
C. Treating the specimen with a slow-acting cytoplasmic poison
D. Treating the specimen with a fast-acting cell poison

ANSWER

Answer: D. If the specimen is allowed to remain at room temperature the processes of autolysis and, eventually, putrefaction will occur. If the specimen is treated with a slow-acting cytoplasmic poison, autolysis may still proceed, and, in any case, the preservation of structural detail will be poor. The best procedure is to treat the cells or tissue with a fast-acting cell poison. Structural detail of the cell is then preserved.

PREPARATION OF THE TISSUE

Fixation

The method of rapidly killing the cell with a fast-acting cell poison is called **fixation.** The usual chemical fixative used for light microscopy is **formalin.** Many chemical fixatives are used, including formalin, alcohol, glutaraldehyde, and osmium tetroxide. There is no ideal fixative. Secondary benefits that result from chemical fixation are inhibition of bacterial growth and, sometimes, an increase in the affinity of the cell for certain stains.

Ideally, the fixative solution should have certain properties: It should contain sufficient chemical fixative to stop cell metabolism rapidly, but not so much as to injure the fine structure of the cell to the extent that it produces excessive protein precipitation and cell shrinkage.

QUESTION

Which of the following solutions should the fixative be in?

A. A solution of lower osmolarity than that of normal bathing fluid of the cell
B. A solution of higher osmolarity than that of normal bathing fluid of the cell
C. A solution of equal osmolarity to that of the normal bathing fluid of the cell

ANSWER

Answer: C. The fixative solution should be designed so that the death of the cell occurs owing to the action of the fixative itself, while the normal state of **hydration** of the intrinsic cell structures is preserved. These principles have been discussed in relation to the single cell, but they apply equally well to tissues.

The fixative should be dissolved in a solution of neutral salts, so that the total solution is osmotically almost identical to that of the normal bathing fluid of the cell. Fixatives cannot penetrate very deep into closely packed cells.

Even with an adequately prepared fixative solution, poor fixation of tissues can occur, especially in parenchymatous organs such as liver, kidney, and spleen, which consist of many cells packed close together.

QUESTION

Of the following causes for poor fixation, which is the most likely?

A. Too high an osmolarity of the fixative solution
B. Too low an osmolarity of the fixative solution
C. Poor fixation inherent in the nature of the tissue, as in liver, kidney, and spleen
D. Too large a specimen for the penetrating power of the fixative solution and the density of that organ

ANSWER

Answer: D. The center of a large cube of liver, kidney, or spleen is very likely to remain unfixed in the usual formalin-saline fixative solution. This highlights the importance of preliminary trimming of the specimen so that only a small block of tissue is placed in the fixative solution.
