basic histology

2nd
EDITION

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basic histology

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2nd EDITION

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Preface

This book represents the authors' continuing effort to produce a compact text on histology which presents the relevant information necessary for students in the biomedical and biologic fields. The emphasis remains on the biology of the cells as a basis for a better understanding of tissue physiology.

The success of the first edition has been most gratifying. The authors are particularly pleased to have received many suggestions for changes and additions from students and teachers at many institutions in the USA and Europe. Every one of these comments has received our most careful attention, with the result that the second edition represents a substantial revision of the first.

We again acknowledge our debt to all of the men and women whose knowledge and skills contributed much to the success of the first edition.

As the second edition goes to press, we are pleased to be able to announce that translations are planned in Italian, Greek, German, Serbo-Croatian, and Japanese.

–LCJ
–JC

April, 1977

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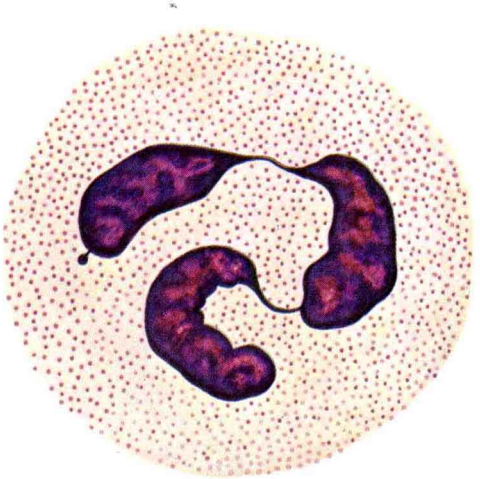
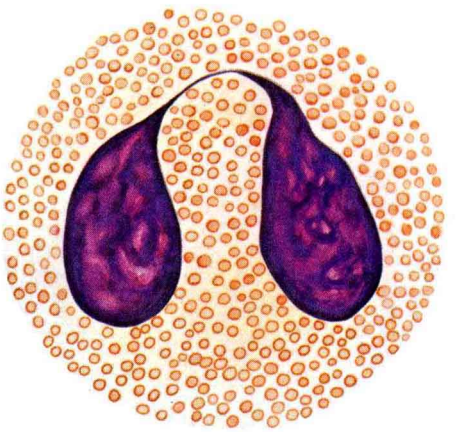


Neutrophilic granulocyte



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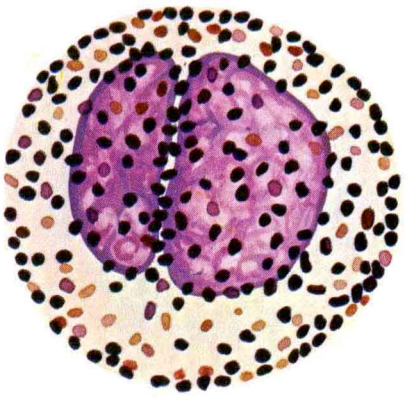

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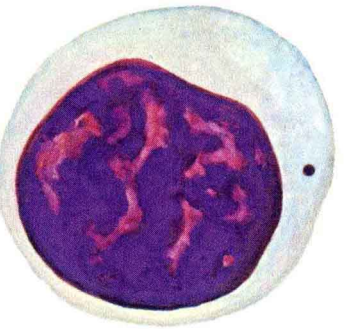

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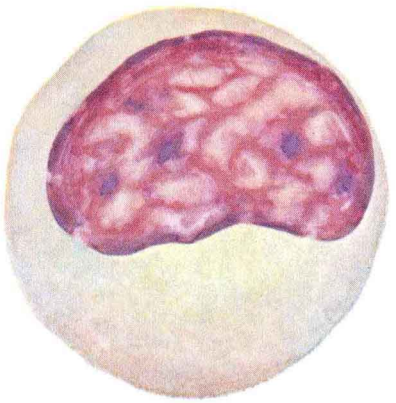
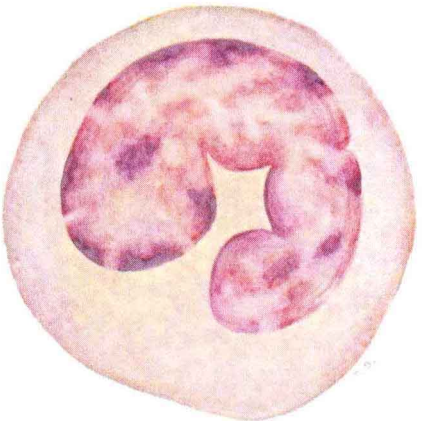
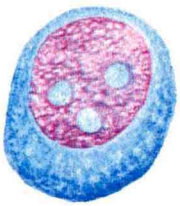


Monocyte

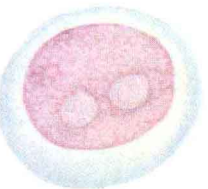


Monocyte

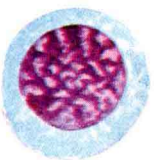
The 5 Types of Human Leukocytes. (See Fig 13–5.)



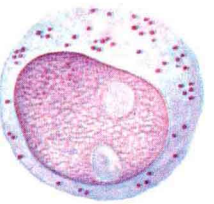
Proerythroblast



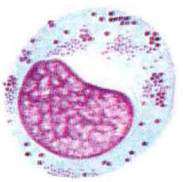
Myeloblast



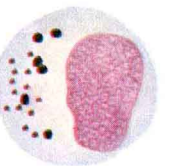
Basophilic erythroblast



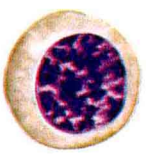
Promyelocyte



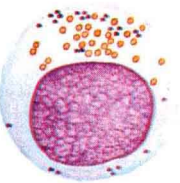
Early neutrophilic myelocyte



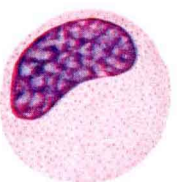
Early basophilic myelocyte



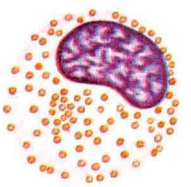
Polychromatophilic erythroblast



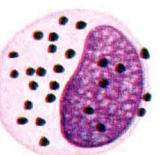
Early eosinophilic myelocyte



Late neutrophilic myelocyte

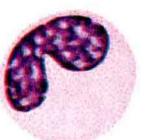


Late eosinophilic myelocyte



Late basophilic myelocyte

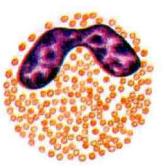
Normoblast



Neutrophilic metamyelocyte

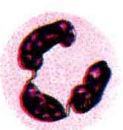
Reticulocyte

Neutrophil with band-shaped nucleus

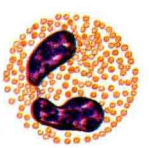


Eosinophilic metamyelocyte

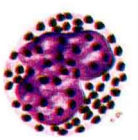
Erythrocyte



Mature neutrophil



Mature eosinophil



Mature basophil

Stages of Development of Erythrocytes and Granulocytes. (See Fig 14–5.)

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Table of Contents

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Methods of Study

Familiarity with the tools and methods of any branch of science is essential for proper understanding of the subject. Some of the more common methods used to study cells and tissues and the principles involved in these methods will be reviewed here: units of measurement, preparation of tissues for examination, optical microscopy, phase contrast microscopy, polarizing microscopy, electron microscopy, radioautography, examination of living cells and tissues, differential centrifugation, and problems in interpretation of tissue sections.

The most important units of measurement used in histology are given in Table 1–1. At a recent international conference, it was recommended that the *Ångström unit* (Å; 10^{-10} meter) be abandoned in favor of the *nanometer* (nm, 10^{-9} meter) and that the nanometer be used in place of the *millimicron* (mμ, 10^{-9} meter). In this book, the nanometer will be used in place of the Ångström unit (1 nm = 10 Å). The *micron* (μ) is now called a *micrometer* (μm), with the value (10^{-6} meter) unchanged.

Table 1–1. Units of measurement used in light and electron microscopy.*

SI Unit*	Symbol and Value
Micron (micrometer)	μ (μm) = 0.001 mm, 10^{-6} m
Millimicron (nanometer)	mμ (nm) = 0.001 μm, 10^{-9} m
Ångström	Å = 0.1 nm, 10^{-10} m

*The preferred SI *(Système International)* units (in parentheses) will be used throughout this book.

PREPARATION OF TISSUES FOR MICROSCOPIC EXAMINATION

The most common procedure used in the study of tissues is the preparation of permanent histologic slides that can be studied with the aid of the optical microscope. Under the optical microscope, tissues are examined by transillumination. Since tissues and organs are usually too thick for transillumination, technics have been developed for obtaining thin, translucent sections. In some cases, very thin layers of tissues or transparent membranes of living animals (eg, the mesentery, the tail of a tadpole, the wall of a hamster's cheek pouch) can be observed in the microscope. In such instances, it is possible to study these structures for long periods and under varying physiologic or experimental conditions. If a permanent slide preparation is desired, small fragments of these thin structures can be fixed, spread on a glass slide, stained and mounted with resin, and examined under the microscope. In most cases, however, tissues must be sliced into thin sections before they can be examined. These sections are cut by precision fine cutting instruments called *microtomes,* and the organ or tissue must be prepared and fixed before the section is made. (See Table 1–2.)

The ideal microscope tissue preparation would of course be perfectly treated with suitable chemicals so that the tissue on the slide would have the same structure and chemical composition as it has in the body. This is sometimes possible but, as a practical matter, seldom feasible, and artifacts resulting from the preparation process are almost always present.

Table 1–2. Stages through which the tissues must pass before paraffin impregnation. (The next steps are microtome sectioning, staining, and mounting.)

Stage	Purpose	Duration
1. Fixation in simple or compound fixatives (Bouin's, Zenker's formalin)	To preserve tissue morphology and chemical composition	About 12 hours, according to the fixative and the size of the piece of tissue
2. Dehydration in graded concentrated ethyl alcohol (70% up to 100% alcohol)	To remove cell water	6–24 hours
3. Clearing in benzene, xylene, or toluene	To impregnate the tissues with a paraffin solvent	1–6 hours
4. Embedding in melted paraffin at 58–60 °C	Paraffin penetrates all intercellular spaces and even into the cells, making the tissues more resistant to sectioning	½–6 hours

Fixation

In order to avoid tissue digestion by enzymes (autolysis) or bacteria and to preserve physical structure, pieces of organs should be promptly and adequately treated as soon as removed from the animal's body. This treatment–*fixation*–usually consists of submerging the tissues in chemical substances in order to preserve as much as possible of their morphologic and chemical characteristics.

The chemical substances used to fix tissues are called *fixatives.* Some fixatives (eg, mercuric chloride, picric acid) promote the precipitation or clumping of proteins. Others (eg, formalin, glutaraldehyde) promote coagulation but not coarse precipitation of proteins. All fixatives have both desirable and undesirable effects. The goal of combining the desirable effects and minimizing the undesirable ones has led to the development of several mixtures. The most commonly used mixtures are *Bouin's fluid,* composed of picric acid, formalin (a saturated solution–37% by weight of formaldehyde gas in water), acetic acid, and water; and *Zenker's formalin (Helly's fluid),* containing formaldehyde, potassium dichromate, mercuric chloride, and water. The simple fixatives most commonly used are a 10% solution of formalin in saline and a 2–6% solution of buffered glutaraldehyde.

The chemistry of the process involved in fixation is complex and not well understood. However, formaldehyde and glutaraldehyde are known to react with the amine groups (NH_2) of tissue amino acids. In the case of glutaraldehyde, the fixing action is reinforced by the fact that it is a dialdehyde and can form stabilizing bonds between protein molecules. For electron microscopic fixation, buffered glutaraldehyde is often used alone or in combination with osmium tetroxide.

Embedding

In order to be able to obtain thin sections with the microtome, tissues must be infiltrated after fixation with a substance that will impart a firm consistency necessary for cutting. This can be gelatin, celloidin, paraffin, resins, or other plastic materials.

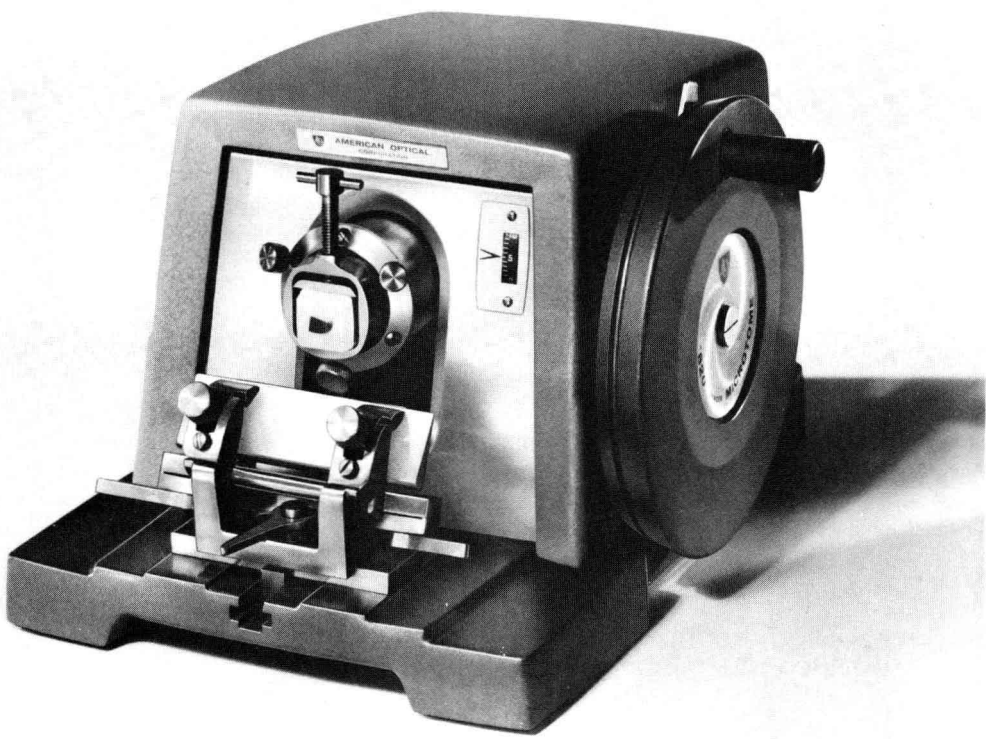

Figure 1–1. Microtome for paraffin-embedded tissues. Rotation of the drive wheel–seen with a handle on the right side of the instrument–moves the tissue block holder up and down. Each turn of the drive wheel advances the specimen holder 3–8 μm, and the block strikes the knife edge, cutting the sections. The sticky paraffin sections adhere to each other, producing a ribbon which is collected and fixed on a slide. (Courtesy of American Optical Corp.)

Paraffin is used routinely for light microscopy; resins of the epoxy type (Epon or Araldite) are more commonly employed for electron microscopy.

The process of embedding or tissue impregnation is usually preceded by 2 main steps: *dehydration* and *clearing*. The water of the fragments to be embedded is first extracted by bathing successively in a graded series of mixtures of ethanol with water (usually from 70% to 100% ethanol). The ethanol is then replaced by a lipid solvent. (In paraffin embedding, the solvent used is xylene or benzene.) As the tissues become impregnated with the solvent, they usually become transparent in a step called *clearing*. Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven, usually at 58–60 °C. The heat causes the solvent to evaporate, and the space becomes filled with paraffin. This is the infiltration or embedding procedure.

The small blocks of paraffin containing the tissues are then sectioned by the steel blade of the microtome to a thickness of 3–8 μm* (Fig 1–1). The sections are laid out on warm water and transferred to glass slides. For electron microscopy, much thinner sections are necessary (0.02–0.1 μm); embedding is therefore performed in a hard epoxy plastic. The blocks thus obtained are so hard that glass or diamond knives are usually necessary to section them.

Immersion of tissues in lipid solvents such as benzene or xylene dissolves the tissue lipids, which is an undesirable effect when these compounds are studied. To prevent this, a *freezing microtome* has been devised in which the tissues are hardened at low temperatures in order to permit sectioning. The freezing microtome—and its more elaborate and efficient successor, the *cryostat*—permit sections to be obtained quickly without going through the embedding procedure described above. They are often used in hospitals, for they allow rapid study of pathologic specimens during surgical procedures. They are also effective in the histochemical study of very sensitive enzymes or small molecules, since freezing does not inactivate enzymes and hinders the diffusion of small molecules.

Staining

With few exceptions, most tissues are colorless, so that observing them unstained in the optical microscope is difficult. Methods of staining tissues have therefore been devised that not only make various tissue components conspicuous but also permit distinctions to be made between them. This is done by using mixtures of dyes which stain tissue components more or less selectively. In histology, most dyes behave like acidic or basic compounds and have a tendency to form electrostatic (salt) linkages with ionizable radicals of the tissues. Tissue components that stain more readily with basic dyes are termed *basophilic;* those with an affinity for acid dyes are termed *acidophilic.*

Examples of basic dyes are toluidine blue and methylene blue. Hematoxylin behaves in the manner of a basic dye, ie, it stains the tissues basophilically. The main tissue components that ionize and react with basic dyes do so because of acids in their composition (nucleoproteins and acid mucopolysaccharides). Acid dyes (eg, orange G, eosin, acid fuchsin) stain mostly the basic components present in cytoplasmic proteins. The basic or acid character of a dye usually explains the staining reaction on a chemical basis, but a physical basis is sometimes also present.

Of all dyes, the combination of hematoxylin and eosin (H&E) is most commonly used. Many other dyes are used in different histologic procedures; it must be stated, however, that, although they are very useful in visualizing the different tissue components, they usually provide no insight into the chemical nature of the tissue being studied.

Besides tissue staining with dyes, impregnation with such metals as silver and gold is a much used technic, especially in the study of the nervous system.

Table 1–3 summarizes staining and impregnation technics used in preparing microscope slides.

*For investigative work this may vary from 1–20 μm.

THE OPTICAL MICROSCOPE

With the optical microscope, stained preparations are usually examined by transillumination. The microscope is composed of both mechanical and optical parts. The mechanical components are illustrated in Fig 1–2. The optical components consist of 3 systems of lenses: condenser, objective, and ocular. The *condenser* projects a cone of light to illuminate the object to be observed. (The role of the condenser is usually underestimated because it does not contribute to the

Table 1–3. Examples of staining technics commonly used in histology.

Technics	Components	Nucleus	Cytoplasm	Collagen	Elastic Fibers	Reticular Fibers
H&E	Hematoxylin and eosin	Blue	Pink	Pink	Irregular	...
Masson's trichrome	Iron hematoxylin, acid fuchsin, Ponceau 2R, light green	Black	Red	Green	...	Green
Weigert's elastic stain	Resorcin and fuchsin, HCl, hematoxylin, Ponceau's picric acid, glacial acetic acid	Gray	Yellow	Red	Black	...
Silver impregnation for reticular fibers	Silver salt solution	...	...	Dark brown	...	Black

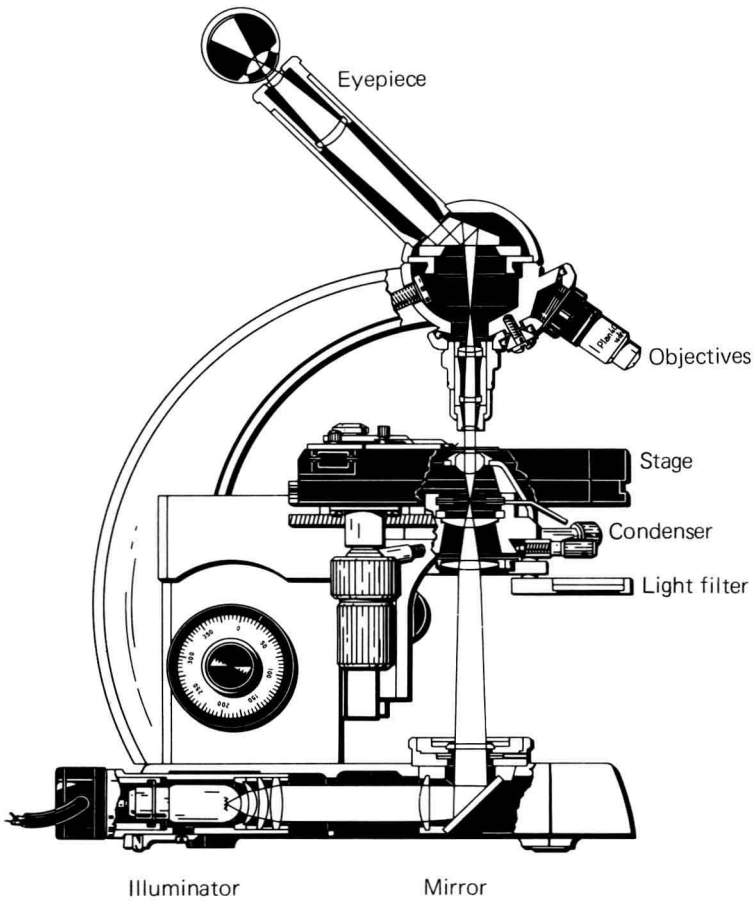

Figure 1–2. Schematic drawing of a student's light microscope showing its main components and the pathway of light from the source (substage lamp) to the eye of the observer. (Courtesy of Carl Zeiss Co.)

magnification; however, its proper use influences the quality of the image observed.) The *objective* lens enlarges the object and projects its image in the direction of the ocular lens. The *ocular* lens further amplifies this image and projects it onto the viewer's retina or onto a screen or photographic plate. The degree of total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses.

Resolution

The critical factor in obtaining a good image with the microscope is the resolution, which is the smallest distance between 2 particles that can be distinguished from each other. For example, 2 particles will appear distinct if they are separated by a distance of 0.3 μm and the microscope has a resolution factor of 0.2 μm. However, if the same particles are examined with a microscope that has a resolution factor of only 0.5 μm, they will appear as a single point. The resolving power of the best optical microscopes is approximately 0.2 μm.

The quality of an image—its clarity and richness in detail—depends on the microscope's resolving power. The *magnification* is independent of its resolving power and is only of value when accompanied by a high resolution capacity. The resolving power of a microscope depends mainly on its objective lens. The ocular lens only enlarges the image obtained by the objective; it does not improve resolution. Thus, high magnification with low resolution gives blurred images of little value.

Numerical Aperture

One of the main characteristics of an objective lens is its numerical aperture (NA), for resolution is a function of NA and of the light wavelength employed (Fig 1–3). NA can be defined as the smallest refractive index (n)* observed between the microscopic preparation and the objective multiplied by the sine of the semiangle of aperture of the lens (μ): NA = n X sine μ (Fig 1–3).

*The refractive index is a measure of the optical density of an object. A light wave traverses an object readily or otherwise depending on the object's optical density.

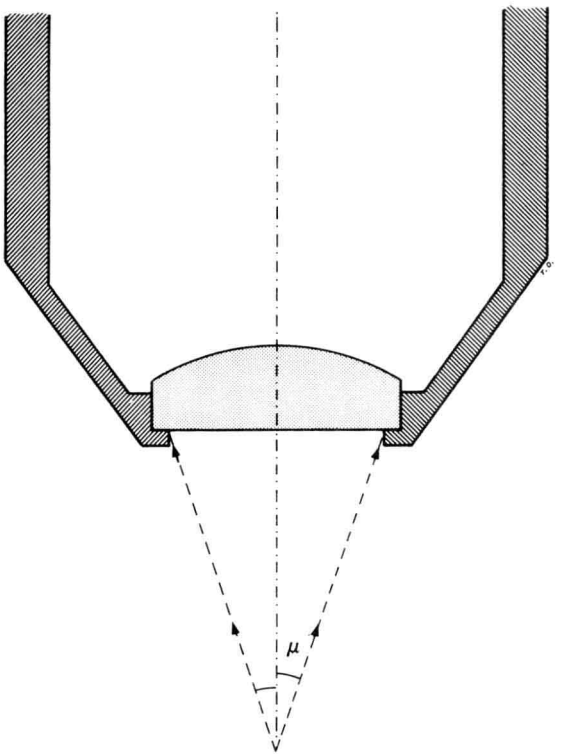

Figure 1–3. Drawing of the light beam which enters the objective lens to show the semiangle of aperture (μ) from which the numerical aperture can be calculated.

The resolution of an objective can be defined by the equation:

$$R = \frac{K \times \lambda}{NA}$$

where K is a constant of 0.61 and λ is the wavelength. Resolution is directly proportionate to the wavelength used and inversely proportionate to the NA. To calculate the resolution when working with white light, a wavelength of 0.55 μm is most often used. This corresponds to yellowish-green, a color to which the human eye is very sensitive. Fig 1–4 is an example of the importance of resolution in microscopy.

An objective lens system often has several numbers engraved on it (Fig 1–5). The first number (upper left) refers to the enlargement; to its right is the NA. The number on the left in the second line is the tube length in millimeters; the number on the right indicates the thickness (in millimeters) of the coverslip for which the objective is corrected. The thickness of the coverslip is important in dry field examination, but when oil immersion is used the oil equalizes the refractive index of the light path between the coverslip and the objective, and the thickness between the usual limits of the coverslip becomes irrelevant.

Objective & Ocular Lenses

Objective and ocular lenses are formed by systems of lenses put together in order to achieve partial correction of their individual defects (aberrations). Although a perfect lens system has not been developed, it is possible to devise objective lenses with increasing optical perfection.

Three common aberrations are as follows:

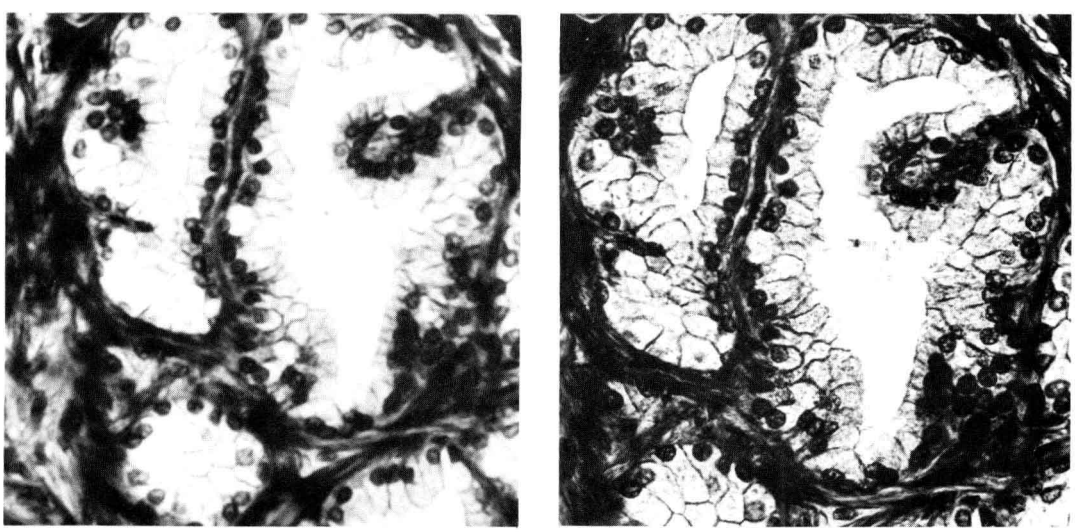

Figure 1–4. Photomicrographs of the same microscopic field at the same magnification (X 350) but with objectives of different numerical apertures (NA). The photomicrograph on the left was made with an objective of NA = 0.22; the one on the right was made with an objective of NA = 1.0. Dog prostate gland stained by Masson's trichrome stain. Observe that the picture at right (NA = 1.0) shows more detail and is sharper than the one on the left.

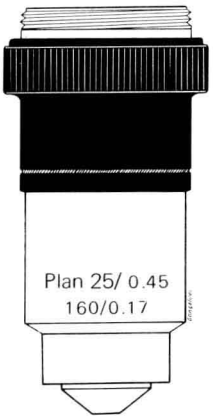

Figure 1–5. Drawing of an objective with the following characteristics: magnification X 25, NA = 0.45, planachromatic, corrected for 160 mm tube and for 0.17 mm coverslips.

A. Chromatic Aberration: This type of aberration occurs because spherical lenses bring light of shorter wavelength into focus closer to the retina than light of longer wavelength. Consequently, several slightly separate images of the object are formed and details are blurred. In the achromatic lens system, this aberration is corrected to a large extent.

B. Spherical Aberration: In spherical aberration, the quality of the image is hindered because the optical properties of the center of a lens are somewhat different from those of its periphery. In apochromatic objective lens systems, complete correction of chromatic and spherical aberrations has been achieved.

C. Curvature of Field: Lenses with this aberration produce an image in which the central field is in focus while the peripheral field is out of focus or vice versa. Planar lenses are corrected to provide "flat field" focus, in which the entire field is in focus.

PHASE CONTRAST MICROSCOPY

Unstained biologic specimens are usually transparent and are difficult to see in detail since all parts of the specimen have almost the same optical density. Consequently, another form of microscopy—*phase contrast microscopy*—has been developed which produces in vivo visible images from transparent objects (Fig 1–6).

Phase contrast microscopy is based on the fact that light passing through media with different refractive indexes slows down and changes direction. This forms phase differences between 2 adjoining regions. These phase differences are—by means of a special optical system—transformed into differences of light intensity so that the image becomes visible (Fig 1–6). The examination of fresh tissue or living cells has been facilitated by the development of phase contrast microscopy.

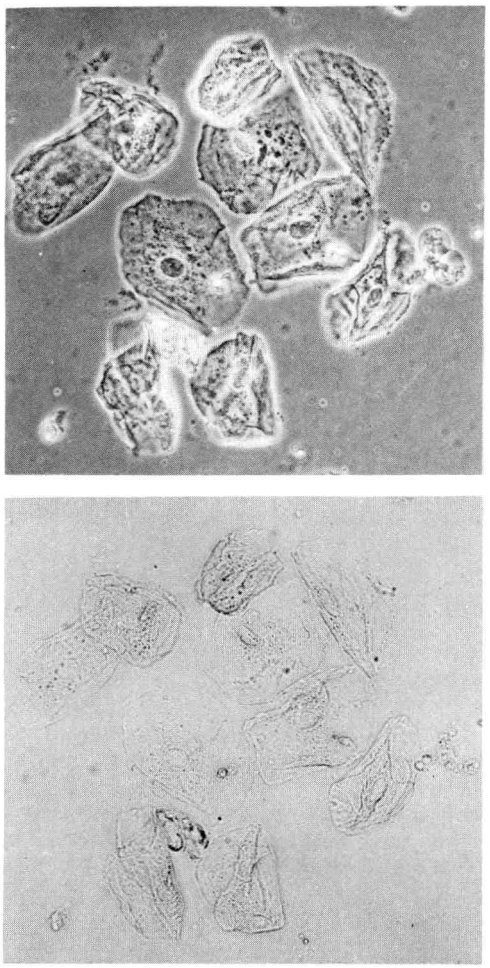

Figure 1–6. Desquamated cells from the oral mucosa. (Unstained fresh preparation.) The top photomicrograph was taken with the phase contrast microscope; the bottom photomicrograph with the standard light microscope. X 300.

THE POLARIZING MICROSCOPE

When light passes through certain substances or body tissues, it divides in a way that produces 2 light rays from one. This is called *polarization.* It occurs with substances whose atoms have a periodic arrangement. Whether or not this arrangement is apparent, these substances are *crystalline (birefringent).* Substances that do not belong to the crystalline group are *amorphous (monorefringent).*

The velocity with which light travels through amorphous substances is always the same regardless of the direction. Therefore, the substance has only one refractive index. In crystalline substances, light velocity changes according to the direction of propagation; from one light ray, 2 refracted rays result. They are polarized rectilinearly, ie, the direction of light vibration follows a determinate direction.

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