HISTOLOGY

Edited by

Roy O. Greep, Ph.D.,

Dean and Professor of Dental Science
Harvard School of Dental Medicine

With

Thirteen Contributors

LONDON
J. & A. CHURCHILL, LTD.
104 GLOUCESTER PLACE
PORTMAN SQUARE
1954

Contributors

RUSSELL BARRNETT, M.D.

Associate in Anatomy, Harvard Medical School

RUSSELL L. CARPENTER, PH.D.

Instructor in Ophthalmology, Harvard Medical School; Professor of Zoology, Tufts University

HELEN WENDLER DEANE, PH.D.

Assistant Professor of Anatomy, Harvard Medical School

EDWARD W. DEMPSEY, PH.D.

Professor and Head of the Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.; formerly Associate Professor of Anatomy, Harvard Medical School

DON W. FAWCETT, M.D.

Assistant Professor of Anatomy, Harvard Medical School

ROY O. GREEP, PH.D.

Professor of Dental Science and Dean of the Harvard School of Dental Medicine

ELIZABETH H. LEDUC, PH.D.

Assistant Professor of Biology, Brown University; formerly Associate in Anatomy, Harvard Medical School

MOSES H. LURIE, D.M.D., M.D.

Assistant Clinical Professor of Otology, Harvard Medical School

GEORGE FISHER ODLAND, M.D.

Assistant in Dermatology, Harvard Medical School; formerly Research Fellow in Anatomy, Harvard Medical School

MARCUS W. SINGER, PH.D.

Associate Professor of Zoology, Cornell University; formerly Assistant Professor of Anatomy, Harvard Medical School

REIDAR FAUSKE SOGNNAES, PH.D., D.M.D.

Charles A. Brackett Professor of Oral Pathology and Associate Dean, Harvard School of Dental Medicine

LEON P. WEISS, M.D.

Instructor in Medicine, State University of New York at Brooklyn; formerly Research Fellow in Anatomy, Harvard Medical School

GEORGE B. WISLOCKI, M.D., SC.D. (HON.)

Hersey Professor of Anatomy, James Stillman Professor of Comparative Anatomy, and Head of the Department of Anatomy, Harvard Medical School

Preface

Originally conceived as a sixth edition of *A Textbook of Histology* by Bremer and Weatherford, when the manuscript for this book reached its final stages, it became quite apparent that this was in reality a new text. It is a text designed for the newer methods and techniques of teaching histology and evidences the impact of new tools and techniques on the study of both living and fixed tissues. Although the authors were free to draw material from the Bremer and Weatherford work, less than half the original illustrations and practically none of the text were retained.

The present work is a joint effort of various persons concerned, at one time or another, with the teaching of microscopic anatomy to medical and dental students at Harvard University. The authors have devoted their writing almost exclusively to the microscopic anatomy of the human body. Although morphology has been the paramount consideration throughout the book, a higher objective has been sought—namely, to present microscopic anatomy in such a way as to emphasize its contribution to the totality of human biology. Form and function are embracing attributes of living organisms, and fixation of tissue is but an effort to obtain the closest idea possible of what the structure is like while living.

An attempt has been made throughout the text to provide information, wherever possible, as to the chemical composition of the substances under study. It is the sincere hope of the authors that this will enable the student not only to see but to understand the nature of cells and tissues. Studies having this objective have been called, among other terms, *histochemistry* or *chemical cytology*. Whatever the name, the goal is a more discriminating analysis of structure. Histochemistry has been introduced into this book through a chapter devoted to the description and interpretation of selected techniques, and by the incorporation of histochemical information at any point where it might contribute materially to the explanation of structure or to the revelation of function.

Throughout the text, the method of presentation has been by straightforward description of definitive anatomy. Embryological considerations have been included, however, when completeness of treatment was deemed better served. There has been no attempt to present a comprehensive treatment of detailed knowledge; rather the authors have strived to restrict their material to a hard core of fundamental facts requisite to an effective

understanding of histology. Where selection of detail has been necessary, the choice has been governed by relevance to concept, or establishment of principle. In treating unsettled issues, and there have been many, an attempt has been made to deal with all facets fairly and to provide for the student's query—"What is your opinion?"—an impartial and critical answer.

Included in this book are many of the fine works of Miss Etta Piotti, medical artist. Her skillful services are acknowledged with deep gratitude. Many of the numerous photomicrographs are the work of Mr. Leo Talbert and Mr. John Pushee. For their mastery of this art, patience, and cooperative assistance, we are sincerely grateful. To our colleagues across the lands who have provided illustrations and to the publishers who have so kindly permitted the reproduction of many useful illustrations, we tender our sincere thanks. For conscientious and painstaking manuscript typing, Mrs. Clyde Irving and Mrs. Jeannette James have our grateful appreciation.

Dr. Alden B. Dawson has read and made suggestions concerning the chapter on cell structure and function, and Dr. Manfred L. Karnovsky criticized the chapter on histochemistry. Dr. Edna Tompkins of the New England Deaconess Hospital laboratories provided supravitally stained blood preparations and otherwise lent skillful assistance in preparing the illustration showing blood cells stained by this technique. Dr. Albert I. Lansing of Washington University supplied illustrations showing aging in arteries and assisted in drafting the description of these changes. Dr. George B. Wislocki's advice and counsel to the Editor were availed of in greater measure than can be told here. To all these people, many thanks.

There was, furthermore, among the contributors much cross-reading of the chapters, and a continuous exchange of suggestions and materials. In addition, many chapters have been kindly read by advanced students in the Department of Anatomy.

In closing, the Editor is especially pleased to express his gratitude to the various contributors to this text. In every instance, there has been a conscious effort to preserve their individual styles of writing, nevertheless, uniformity as regards methodology of approach, introduction to function, reference citations, and general format has been a constant goal. It is a personal pleasure also to acknowledge the friendly cooperation and ready assistance which the publishers have given all of us throughout the preparation of this work.

R. O. G.

Boston, Mass.
Autumn 1953.

Table of Contents

PREFACE vii

1. STRUCTURE OF ORGANISMS 1
Edward W. Dempsey, Ph.D.

2. CELL STRUCTURE AND FUNCTION 10
Helen Wendler Deane, Ph.D.

3. HISTOCHEMISTRY 32
Helen Wendler Deane, Ph.D.

4. EMBRYOLOGICAL ORIGIN OF TISSUES 51
Helen Wendler Deane, Ph.D.

5. EPITHELIUM 63
Helen Wendler Deane, Ph.D.

6. MESENCHYMA AND CONNECTIVE TISSUE 83
Don W. Fawcett, M.D.

7. ADIPOSE TISSUE 113
Don W. Fawcett, M.D.

8. CARTILAGE 124
Don W. Fawcett, M.D.

9. BONE 132
Don W. Fawcett, M.D.

10. MUSCULAR TISSUE 164
Edward W. Dempsey, Ph.D.

11. NERVOUS SYSTEM 194
Marcus Singer, Ph.D.

12. BLOOD VASCULAR SYSTEM 273
Russell Barrnett, M.D.

13. BLOOD 304
Leon P. Weiss, M.D.

14. Development of the Blood Cells and Sites of Blood Cell Production 338
Leon P. Weiss, M.D.

15. Bone Marrow 355
Leon P. Weiss, M.D.

16. Lymphatic System 364
Leon P. Weiss, M.D.

17. Origin of Blood Cells and Their Relationship to Connective Tissue 417
Leon P. Weiss, M.D.

18. Skin and the Epidermal Derivatives 428
George Fisher Odland, M.D.

19. Oral Cavity 458
Reidar Fauske Sognnaes, Ph.D., D.M.D.

20. Respiratory System 512
Elizabeth H. Leduc, Ph.D.

21. Alimentary Tract 540
Helen Wendler Deane, Ph.D.

22. Liver and Gall Bladder 580
Helen Wendler Deane, Ph.D.

23. Pancreas 606
Helen Wendler Deane, Ph.D.

24. Urinary System 620
Roy O. Greep, Ph.D.

25. Female Reproductive System 653
Roy O. Greep, Ph.D.

26. Placentation 704
George B. Wislocki, M.D., Sc.D.

27. Mammary Glands 739
Edward W. Dempsey, Ph.D.

28. Male Reproductive System 748
Roy O. Greep, Ph.D.

29. Hypophysis 784
Roy O. Greep, Ph.D.

30. Pineal Body 806
Roy O. Greep, Ph.D.

31. THYROID GLAND 810
Edward W. Dempsey, Ph.D.
32. PARATHYROID GLANDS 820
Roy O. Greep, Ph.D.
33. ADRENAL GLANDS 825
Roy O. Greep, Ph.D.
34. EYE 844
Russell L. Carpenter, Ph.D.
35. EAR 890
Moses H. Lurie, D.M.D., M.D.
INDEX 919

chapter 1

by Edward W. Dempsey

Structure of Organisms

The structure of organisms is the subject matter of the science of morphology, which is the oldest and perhaps the largest part of biology. Long before written history men were concerned with recognizing different animals by their shapes and sizes. Pictographs, burial practices, and remains from kitchen middens all give testimony to a considerable, if crude, knowledge of the fashion in which animals are made. Ancient writings from Egypt, Arabia, China, and India also testify to man's interest in the structure of living organisms, including man himself.

During the dark ages direct observation and experimentation with natural phenomena nearly ceased. The ancient knowledge, its origin shrouded in obscurity, was preserved during this period in the writings of Aristotle, Galen, and others, and in the relics of earlier civilizations such as the Egyptian heiroglyphs and papyri. During the Renaissance mankind began anew to study and experiment with nature. The turning point at which scholastic dependence upon ancient authority began to give way to the modern scientific method of direct study is usually considered to have occurred with the publication of Vesalius' (1514–1564) studies of the anatomy of man. His principal work, *De Fabrica humani corporis*, was published in 1543.

At first the study of structure was pursued by observation with the naked eye after dissection with simple knives. The word *anatomy* means a cutting or dissection. Soon, however, simple lenses began to be employed as aids in distinguishing fine details and textures. Malpighi employed such lenses in his studies of the spleen, lungs, and kidneys, which were published in 1661–1666. Robert Hooke also used simple magnifying lenses and published, in 1665, a book called *Micrographia* which by its title calls attention to the fact that small structures were being observed. During the period between 1673 and 1716 Leeuwenhoek developed compound lenses which permitted much higher magnifications, and published an amazing series of observations on protozoa, bacteria, spermatozoa, muscle, nerve, and many other structures. Thus, by the beginning of the 18th century morphology

was divided into macroscopic and microscopic parts. These divisions are still recognized today by our courses in gross anatomy and microscopic anatomy.

The development of microscopic anatomy proceeded slowly throughout the 18th century. During the early part of the 19th more rapid progress began. The compound microscope by this time had become highly developed, permitting magnifications and resolutions nearly equal to those of modern instruments. The microscopists of this era, utilizing fresh, teased, or macerated specimens and with free-hand sections, worked out the rudiments of histology (the study of tissues) and the beginnings of cytology (the study of cells). It was during this period that Robert Brown recognized the nucleus, Schleiden and Schwann enunciated the cell theory, Virchow established the field of cellular pathology, and initial insight into the cell was gained from the investigations of Henle, von Mohl, Remak, and Purkinje.

Although adequate microscopes were available in the early 1800's, techniques for preparing tissues were far from ideal. Thick specimens cannot be observed to advantage with the high powers of the microscope, consequently precise and dependable methods for preparing thin sections were desirable. At first free-hand sections were cut with simple knives. Valentin developed a double knife in 1839 which reproducibly cut sections from tissues having an adequate consistency. Ranvier, Altmann, Caldwell, and Minot all took a hand in the development of sectioning techniques, with the result that in 1892 with the introduction of Minot's rotary microtome, sections as thin as one micron could be prepared in favorable instances. Side by side with the development of microtomes went the development of fixing and embedding techniques. These procedures preserved tissues for future study and modified their consistency so that sections could be prepared more readily.

Living tissues and cells are highly transparent, except in occasional instances in which they contain pigmented substances such as the hemoglobin of red blood cells and the melanin granules of skin and hair. Because of this transparency, little can be seen by microscopic examination unless the tissues are treated in a way causing their structural parts to assume different refractive indexes or different abilities to absorb light. The early microscopists used stains occasionally, but real success with staining procedures occurred only in the latter half of the 19th century. This was the period during which the anilin-dye industry was developed. As dependable dyes were introduced, available staining methods became routine. These staining procedures permitted intracellular objects such as nuclei, mitochondria, granules, cell borders, intercellular fibers, etc., to be distinguished with a clarity and reproducibility impossible formerly. Thus, with techniques available for preserving, hardening, embedding, sectioning, and staining tissues, histologists and cytologists were able to use the compound microscope to work out the entire field of the microscopic architecture of tissues and cells.

Modern developments in histology and cytology are projections along the lines already indicated. The microscope extended morphological information to a new dimension of smallness. However, the ordinary compound microscope cannot distinguish objects smaller than about 0.2 microns. The ultraviolet microscope permits approximately twice this magnification. The electron microscope will distinguish objects as small as 50 Ångstrom units (Å = 10^{-8} mm.) and x-ray diffraction apparatus has been used to study the distances separating atoms within a molecule. These instruments are presently being used to extend microscopic architecture to a new order of smallness beyond that possible with the ordinary light microscope. The word *ultrastructure* is frequently applied to the phenomena observed in these new dimensions.

The introduction of staining procedures heralded investigations into the chemical nature of cellular and tissue structures. Being in itself a chemical compound, a stain must unite with a structural component by some kind of a chemical force. Thus, the affinities of tissues for stains provide a tool whereby the chemical nature of anatomical structures can be investigated. Histochemistry and cytochemistry are fields comparable to histology and cytology, in which the primary concern is to define the nature of morphological structures in chemical terms.

Finally, histo- and cytophysiology, as well as histo- and cytopathology should be mentioned. These are fields of investigation in which correlations are sought between morphology and functional activity, and between morphology and disease processes, respectively.

The Preparation of Tissues

Mention has already been made that tissues cannot be studied to advantage unless specially prepared for microscopic examination. There are numberless individual methods, each with specific advantages. The following account is only a brief statement designed to introduce the student to the preparations he will study during this course. The books by Cowdry, Gatenby and Beams, and McClung are recommended as compendia of microscopical methods. A journal, *Stain Technology*, is published monthly and is devoted to new procedures of value in microscopy.

A tissue, the consistency of which is fluid or semi-fluid, may be spread or teased apart into a thin film upon a glass slide. With a little practice blood may be spread into thin films which are ideal for microscopic examination. A cover slip may be placed on the fluid preparation or the film may be stained after drying and then covered. Small bits of loose connective tissue may be spread, by teasing with needles, and similarly examined fresh or after staining. The tissue is best examined when covered with a cover slip and when some fluid, the refractive index of which is near that of the tissue itself, seals the tissue to the glass surfaces. Water, salt solutions, sugar solutions, glycerol, and serum all have their uses in mounting fresh tissues.

Some tissues have a consistency so firm that they cannot be teased into thin films. This firm consistency is usually the result of a dense, supporting meshwork of connective tissue fibers. These may be destroyed by maceration in weak acids, a process similar to the tenderizing of meat. After such maceration, gentle teasing will separate the architectural units so that they may be studied individually. Such procedures allow liver lobules to be separated from one another. Similarly, individual smooth muscle cells may be isolated from the intestinal wall or from the uterus by maceration.

The great majority of microscopical preparations involve cutting the tissue or organ into thin sections which are spread upon a glass slide. Free hand sections of certain dense tissues can be prepared. Cartilage is an example. Bone is sometimes prepared by an analogous technique: a thin segment may be sawed from a large bone, and the segment reduced to the proper thinness by grinding it against an abrasive stone. Most tissues, however, are not sufficiently firm to permit adequate sectioning. This difficulty can be circumvented by freezing the tissue, or by embedding it in a solution of gelatin or some other substance which solidifies to the proper consistency.

The foregoing procedures all involve the use of fresh tissues. Fresh material has many advantages but unfortunately there are also some practical disadvantages. Fresh preparations are seldom permanent, and so cannot be stored for future reference. Moreover, fresh tissues cannot be stained with some of the more delicate cytological stains and are, therefore, not ideal for demonstrating certain stainable structures. For these reasons, fixing and dehydrating procedures were devised.

Fixing fluids act as preservatives, inhibiting autolytic changes and bacterial growth. They also have two other desirable effects: they harden protoplasm somewhat and thereby facilitate the preparation of thin sections, and they increase the affinity of tissues for stains. After fixation, tissues are ordinarily dehydrated by immersion in a graded series of alcohol solutions, ending in absolute alcohol. They are then transferred to xylol or chloroform or some other nonpolar solvent preparatory to infiltration in melted paraffin or some other embedding medium. After infiltration, the paraffin is cooled and solidified into a block containing the embedded tissue, which can then be cut into thin sections on a microtome. Other embedding media, such as celloidin, are sometimes used instead of paraffin.

After the section is cut, it is floated in a drop of water on a glass slide on which a little egg albumin has been spread. The albumin acts as an adhesive, cementing the section immovably to the glass. The drop of water is then evaporated, so that the section settles down onto the glass surface to which it becomes attached. The mounted section is now ready for staining.

Many staining procedures have been devised for special purposes. Some of them are extremely elaborate. For most purposes, however, a basic dye serves as a nuclear stain and the cytoplasm is stained a contrasting color with an acid dye. To apply the dyes, the residual paraffin must first be

removed by placing the mounted section in a bath of xylol. The tissue is then hydrated by running it down to water through a graded series of alcohol solutions. It is then immersed in the stain solutions, after which it is again dehydrated. From absolute alcohol the section is transferred to xylol, oil of origanum, or some other clearing agent, in order to render it transparent. A drop of Canada-balsam solution is then placed on the section, a thin cover glass applied, and the stained section is finally ready for observation.

Stains can also be used as an adjunct for studying living or moribund cells in a fresh, unfixed condition. Most dyes are either so toxic as to kill animals when injected in sufficient amounts for staining, or are destroyed so rapidly in the living animal that the tissues are not stained. However, there is a class of pigmented substances which are taken up by the phagocytic cells of the body and which are not particularly poisonous. These are the *vital* dyes. Trypan blue, carbon particles (India ink), and thorotrast are examples of such vital dyes. These substances exist as colloidal particles, rather than as solutions, and are ingested by and concentrated in certain cells, which are therefore said to be phagocytic.

Certain other dyes, although so toxic as to kill cells, are taken up by the dying cell. The moribund cell is not necessarily killed at once; on the contrary it may survive for some time while stained by the dye. The leucocytes of the blood may be beautifully stained by Janus green, which colors their mitochondria, and by neutral red, which stains their granules. Such *supravitally* stained cells retain their motility for several minutes or even hours. Nerve cells and their processes are frequently demonstrated by supravital staining with methylene blue. Spreads of loose connective tissue and the thin mesenteries of small rodents are ideal preparations for supravital staining. Elastic tissue, collagen, mast cells, fat, and other structures may be brought out if suitable dyes are applied supravitally to the surviving tissue.

The Microscope

Basically, the ordinary light microscope consists of two sets of lenses together with auxiliary optical and mechanical systems for holding and examining objects. An objective lens provides an initial magnification, bringing the image into focus in a plane lying somewhere in the barrel of the microscope. An ocular lens is placed so as to magnify this primary image a second time. Thus, the compound microscope acts as a two-stage magnifying device.

A moment's reflection will indicate that a very bright light must illuminate the object if it is to be perceived after high magnification. The effect of magnification is to spread the light from a small area over a larger one. Light intensity under such conditions follows the law of inverse squares. Thus, if an image is formed at a magnification of 100 diameters, the light intensity will have been decreased $100 \times 100 = 10{,}000$ times. In order to obtain the very

bright illumination necessary for the effective use of the high powers of the microscope, a condensing lens is ordinarily employed beneath the stage of the instrument. The effect of this lens is to concentrate the light from a large area into a very bright beam illuminating the object, thus providing enough light for the inspection of the magnified image.

Modern microscopes are provided with a number of mechanical controls. Coarse and fine adjustments raise and lower the tube containing the objective and ocular lenses. This permits accurate focusing of the instrument so that a sharp image is produced. Mechanical stages are usually provided for holding the slide in place and for moving it smoothly to any desired position. A rack-and-pinion adjustment permits raising or lowering the condenser lens. An iris diaphragm is usually inserted into the condenser assembly. Open, it transmits the maximum amount of light into the instrument, closed, the light intensity is reduced. Finally, the objective lenses can be interchanged quickly by turning the revolving nosepiece.

If a tissue is examined carefully, first with the low-power objectives and then with the higher powers, it will be seen that more and more fine detail becomes visible. This ability to reveal fine detail is called the *resolving power* of the instrument. The resolution possible with lens systems is limited by the nature of light and the optical properties of the lenses. The formula, $R = \frac{\lambda}{2NA}$ describes the behavior of lenses, where R = resolving power in microns, λ = wavelength of light in microns, and NA = numerical aperture of the lens. The numerical aperture is a fixed constant for any given lens, and may be determined by finding the ratio of the diameter of the lens to its focal length. The NA is usually engraved on the barrel of microscope objectives. The lower-power (10X) objective usually has an NA = 0.25, the high dry, 0.65, and the oil immersion, 1.25. Taking the average value for the wavelength of visible light as 0.5 μ, and substituting in the equation above, the resolving power of the 10X objective is found to be 1.0 μ, the high dry, 0.4 μ, and the oil-immersion lens, 0.2 μ. These figures express the size of the smallest detail clearly distinguishable with the lenses.

The Polarizing Microscope. The basic microscope can be modified by the addition of suitable accessories to permit optical analyses of many kinds. The polarizing microscope is one such modification. Many natural objects, among which are certain crystals and fibers, exhibit an optical property known as birefringence or double refraction. These objects have two or more indices of refraction, depending upon the direction of illumination. In biology, the detection of birefringence is quite important, since it is caused by the orientation of particles or micelles too small to be resolvable by even the best lenses. Thus, birefringence permits deductions about the submicroscopic organization of structure too small for direct visualization. An excellent account of the theory and practice of polarization microscopy

has recently been written by Bennett, in the *Handbook of Microscopical Technique* edited by McClung.

In polarizing microscopes, a Nicol prism, or a sheet of Polaroid, is placed in the light path below the condenser. This "polarizer" has the property of converting all light passing through the instrument into plane polarized light, or light vibrating in one optical plane. A similar prism, the "analyzer" is located in the barrel of the microscope above the objective lens. When the analyzer is rotated until its axis is perpendicular to that of the polarizer, no light can pass through the instrument and the field is black. The field remains dark if an isotropic, or singly refractive object, is placed on the stage for examination. A birefringent object, however, appears light on a dark background when examined under these conditions. The anisotropy, or birefringence of muscle fibers is illustrated in Fig. 116. Collagenous fibers, the myelin sheaths of nerves, the rods and cones of the retina, and the lipid droplets of the adrenal cortex are other examples of strongly birefringent objects.

The sign of birefringence is often of use in deducing the ultrastructure of anisotropic objects. An account of this property will be found in the account of polarization optics by Bennett. The sign is determined by the relative magnitude of the indices of refraction as referred to the optical axis of the structure under question. If the sign is negative, the submicroscopic micelles are regarded as being oriented transverse to the long axis of the structure, whereas if positive, the micelles are disposed parallel to the long axis. The myelin of nerve sheaths has a negative sign, indicating that the lipid micelles are oriented at right angles to the nerve fiber. Protein structures such as collagenous fibers have a positive sign, indicating that their ultrastructures are parallel to the fiber axis.

The Darkfield Microscope. Although the resolving power of microscope lenses does not permit accurate visualization of objects smaller than about 0.2 μ, it is sometimes possible to detect the mere presence of such submicroscopic particles. Everyone is familiar with the sight of dust particles dancing in a beam of sunlight entering a darkened room. This phenomenon is known as the Tyndall effect. Particles too small to be seen directly nevertheless glisten when illuminated by a strong, oblique beam of light. This phenomenon is made use of in darkfield microscopy. A special darkfield condenser, constructed so that no light comes through the center of the lens, is usually employed. Thus, the light striking the object comes to it at an angle so oblique to the axis of the microscope that none of it can enter the objective lens. The field is therefore dark. Small particles, even those too small to be seen with transmitted light, will appear as glistening spots when placed in the field of such an instrument.

Darkfield microscopy has proved most useful for examining blood spreads and microincinerated specimens. In the former, the granules of leucocytes

and the tiny droplets of fat called chylomicrons are beautifully displayed, while in the latter, the mineral ash remaining after tissues have been incinerated in a furnace is readily seen. Policard (1942) has summarized the cytological results of 20 years' work employing microincineration.

The Phase-contrast Microscope. In the ordinary microscope, objects are visible because they absorb more light than do their surroundings. Highly transparent structures such as most unstained tissues are not readily visualized. However, such transparent objects may be rendered visible under the proper conditions by virtue of their refraction. A piece of glass is perfectly transparent, yet it is still visible because its index of refraction is different from that of the air surrounding it. If a glass marble is placed in water, its visibility diminishes because the refractive index of the water is nearer that of glass than is the index of air. If the marble is placed in a medium the index of which is identical with that of glass, it disappears. The phase-contrast instrument is a basic microscope equipped with accessory phase-retardation plates which take advantage of the refractive differences between microscopic objects and the medium in which they are immersed.

The refractive index describes the optical density of an object or the speed with which it is traversed by a light wave. Air has a refractive index of approximately 1.0, water about 1.3, and glass about 1.5. Light travels fastest in air, more slowly in water, and slowest in glass. Thus, light waves traversing equal distances through air, water, and glass will not emerge at the same time. The wave through glass will be retarded as compared with that through water, and that through air will be advanced. The waves therefore will emerge out of phase with one another.

Such phase advances or retardations are not detected by the eye, which responds rather to amplitude or intensity differences. The phase-contrast apparatus consists of optical plates placed within the condenser and objective lenses which convert the phase differences into amplitude differences. The effect, therefore, is that differences in refractive index are rendered directly visible. These differences may be increased or decreased by a proper choice of mounting media. If a tissue is mounted in a medium having the same refractive index as the object to be examined, it will not be visible in the phase microscope. Phase microscopy is most successful when examination is conducted in low-index fluids, such as serum, saline solutions, etc. It is apparent, therefore, that the instrument is peculiarly adapted for studying living, fresh tissues in their natural media. Blood, tissue cultures, and fresh tissue spreads and sections are ideal material. Several phase-contrast pictures appear elsewhere in this book; figure 1 is an example.

Microscopes Employing Radiations Other than Light. The microscopes mentioned so far utilize visible light which is passed through lenses to form a magnified image. Light is by no means the only form of energy which can be caused to form images. Infrared, or heat rays, may be imaged by suitable lenses or mirrors, and is occasionally used in microscopy. Because

infrared wavelengths are longer than those of visible light, the resolving power of the infrared microscope is less than that of the ordinary instrument. However, certain chemical substances or groupings selectively absorb infrared rays of specific wavelengths and so an absorption spectrum may reveal the chemical nature of a microscopic object. Ultraviolet light can also be used in microscopy. In this case the short wavelengths of the ultraviolet rays permit higher resolution than that obtained with visible light. This greater resolving power (about twice that of the ordinary microscope, or ca. 0.1 μ) together with the specific ultraviolet absorption of some organic chemicals make the ultraviolet microscope an important research instrument at the present time. Ultraviolet light is also employed in fluorescence microscopy. Many substances have the property of emitting visible light when irradiated by invisible rays. This phenomenon is utilized in fluorescence microscopy. Ultraviolet light is focused on the specimen which glows and can be observed by its emitted fluorescent light.

The electron microscope is another example of an instrument employing invisible radiation. As in all such microscopes, the final image is projected upon a photographic film where development will render it visible. The electron microscope, like the ultraviolet microscope, depends upon energy the wavelength of which is shorter than ordinary light and therefore permits greater magnification. The electron microscopes in current use operate at resolutions down to 50 Å and experimental models have reached 10 Å. Thus, approximately a hundredfold increase in useful magnification has been achieved.

Just as ordinary microscopy requires special methods for preparing sections thin enough to be transparent and staining procedures to enhance the visibility of the microscopic structures, so does electron microscopy require new techniques for preparing tissues for examination. Sections approximately 0.1 μ in thickness must be prepared. This has been accomplished by embedding the tissues in plastics of a suitable consistency, and by cutting sections with glass knives which are considerably sharper than the steel knives formerly employed for ordinary microscopy. Stains enhance contrast in the light microscope; similarly, reaction of the tissues with compounds containing heavy metals such as osmic, phosphomolybdic, and phosphotungstic acids greatly increases the detail visible with the electron microscope.

Electron microscopy is as yet in its infancy. The instrument has been available for approximately a decade. Improvements in its design have been rapid and techniques for preparing tissues have only recently been available. It is safe to say that a rapid development of the electron microscopy of mammalian tissues will occur. Structures smaller than individual macromolecules can now be visualized. Cytology is therefore rapidly approaching the dimensions ordinarily studied by the deductive methods of physics and chemistry.