TEXT-BOOK OF HISTOLOGY

BREMER
WEATHERFORD

Sixth Edition

A TEXT-BOOK of HISTOLOGY

ARRANGED UPON AN EMBRYOLOGICAL BASIS

BY

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PREFACE TO THE SIXTH EDITION

This is a presentation of the sixth edition of A Text-Book of Histology arranged upon an Embryological Basis by Frederic T. Lewis, which was originally a free translation of Phillip Stöhr's widely used *Lehrbuch der Histologie*. The second edition was changed materially and numerous citations and references to original papers added. The third, fourth and fifth editions were extensively rewritten by J. Lewis Bremer. Recent advances in histology have necessitated a further rewriting but the spirit and form of the previous editions have been adhered to, with emphasis upon development as essential for the understanding of structure and function. The book is a digest of a vast and growing literature, requiring consultation of both the classical and more recently published investigations with selected references to more than seven hundred of them. Of the five hundred and ninety-eight illustrations, three hundred and two are new to this edition; each chosen as a visual explanation of the text.

I am greatly indebted to my colleagues in the Department of Anatomy; Dr. F. T. Lewis and Dr. J. L. Bremer, the former editors of this book and to Dr. G. B. Wislocki, each of whom has been extremely helpful. I am particularly appreciative of the permission given by my former teacher, Professor Wilhelm von Möllendorff, Zürich, to use illustrations from the several works under his editorship. Dr. S. B. Wolbach, Dr. A. B. Dawson and Dr. Shields Warren, all members in other departments of Harvard University have been most generous in giving me either preparations from their collections or lending original drawings used in illustrating their works. Acknowledgment is made to the several authors and publishers who have allowed me the use of illustrations from their publications. Many of the new figures have been meticulously drawn by Miss Etta Piotti from original preparations and others redrawn from illustrations in the literature by either Miss Piotti or Miss Evelyn Glidden. Mr. Leo Talbert and Mr. Irving F. Rider are responsible for the excellent photography.

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PREFACE TO THE FIFTH EDITION

The increasing vitality of the embryological and histological sciences within the last few years seems to require a rather radical revision of this textbook with the incorporation of much new material. More emphasis has been given to the normal functional changes in the various cells and to their activities in the living state, as correlated with the usual histological picture. The newer conceptions of the various hormones have been briefly included, and because of their importance the section on the endocrine glands has been advanced to a position ahead of the sections dealing with those organs in which their actions are best recognized. In general, however, the book follows the same plan as in former editions, and emphasis is still laid more on development and the resulting form than on function, except as the latter helps to explain the morphology.

No modern Textbook of Histology can hope to maintain reasonable dimensions if it is to enter exhaustively into the known details of the present subdivisions of the subject; larger handbooks are available for this purpose. To supply the deficiencies, I have greatly enlarged the bibliography of pertinent references, so that the interested student, with access to a good library, may search for further details in the original publications. This is to his advantage, for there he will find that the literature not only records essentially new discoveries, but also, especially in recent years, just as often tends to question formerly accepted ideas. New technical methods of approach to old problems alter the picture. Even slight familiarity with the literature will disclose the active vitality of modern anatomical science.

Many of my colleagues have helped me in this edition. Professor H. L. Weatherford has written the short section of 'the use of stains,' which replaces the former one on 'microscopic technique.' Professor George R. Minot has provided the new drawing of 'blood,' and Dr. Henry Jackson, Jr., has, himself, drawn the 'bone marrow' and aided in writing that part of the text. To Professor G. B. Wislocki and to Drs. A. L. Grafflin, T. L. Terry, and M. J. Eisenberg I am indebted for advice and assistance in writing of the hypophysis, kidney, eye, and teeth, and for many original illustrations supplied by them. I am indeed fortunate in having such a group at hand to call on, and I hereby thank them all gratefully. Other new illustrations for the book are borrowed from various publications, or are original; devised by me and drawn by Miss Piotti, Miss Cabot or Mr. Schumann. I thank them also for their patience and accuracy.

J. L. Bremer.

Harvard Medical School

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MICROSCOPIC ANATOMY

Microscopy

Microscopic anatomy is the study of the minute structure of animals and plants, at present for general purposes limited by the resolving powers of the compound microscope. Of its two larger subdivisions, cytology[1] pertains to individual cells—their origin, structure, and functions; while histology[2] is concerned with the organization of aggregates of cells and the intercellular materials into tissues. The study of the several fundamental tissues is called general histology and that of the organs—special histology or organology.

DEVELOPMENT OF THE MICROSCOPE

The origin of the compound microscope has been a subject of no little speculation and controversy since a number of claimants have sought the credit for its invention. It is difficult to evaluate all these claims because the early history of the microscope and of the telescope parallel one another. Presumably the earliest compound microscope was made about 1590 by Zacharias Janssen, with the help of his father Hans, a spectacle-maker in Middleburg, the ancient capital of Zeeland in Holland.

Pierre Borel in his '*De vero telescopii inventore cum brevi omnium conspiciliorum historia. Accessit etiam Centuria observationum microscopicarum.*' Hag. Comitum (1655) included among other documents, a letter from Willem Boreel, the Dutch envoy to France, who wrote that as a child in Middelburg he knew Zacharias very well and had often heard it said that he and his father invented the microscope. He also stated in the letter that one of the early instruments was presented to Prince Maurice, the commander of the Dutch forces and a later one to Albert, Archduke of Austria, the governor of Holland. While in England in 1619, Boreel was shown this microscope which the Archduke had given to the Dutchman, Cornelius Drebbel, mathematician to King James I.

Professor P. Harting[3] stated that according to the Middelburg parish-register Zacharias was born in 1577 and would therefore have been only thirteen years old in

[1] The name *cytology* (cytologie) was probably introduced by J. B. Carnoy, 'La Biologie Cellulaire,' Lierre, 1884.

[2] The term *histology* was introduced by C. Mayer, 'Ueber Histologie und eine neue Eintheilung der Gewebe des menschlichen Körpers.,' Bonn, 1819.

"Histology is the study of the texture of the so-called fundamental systems or tissues of the animal body, and of the origins and laws of their normal and abnormal development" (trans., p. 20). Carl Friedrich Heusinger, 'System der Histologie. Erster Theil. Histographie,' Eisenach, 1822.

[3] HARTING, 1859.

1590. It seems highly probable then that he must have accidently placed two convex lenses in the proper relative position in making his microscope. In 1866 two old optical instruments were given to the Museum of the Zeeland Scientific Society, both of them having been in the donor's family for several generations and traditionally believed to be early forms of inventions by Zacharias Janssen. Professor Harting examined these instruments and identified one as a compound microscope and the other as a telescope. The microscope was crudely made of tinned-iron plates soldered together into three tubes—an outer one into which slide two smaller tubes fitted with lenses. When closed the magnification was about three times and when withdrawn about nine times. This instrument is much more primitive than the one presented to the Archduke Albert, which according to Borcel, was an upright affair of gilt brass supported upon three dolphins. As the Archduke came to Zeeland in 1605 it is assumed that the instrument given him was of about that date. Professor Harting referring to the 'Middelburg microscope' wrote (translation)—"In spite of the fact that all direct proofs fail, I consider it by no means improbable that the tradition is true and that this instrument is one of the oldest microscopes, which by Hans and Zacharias Janssen or by the first alone were manufactured long before they brought to a finish instruments more accurate and optically more complete which were destined for Prince Maurice and Archduke Albert." John Mayall Jr. inspected these same instruments left to the Museum and wrote (Journal Royal Microscopic Society, 1889, page 164) that he would "unhesitatingly affirm the microscope to be older than the so-called Galileo microscopes," still extant in Florence.

While the microscope was evidently invented in Holland it was developed and named in Italy. Galileo writing in 1610, said that he had learned of an ocular instrument made by a certain Dutchman, by means of which an object could be made to appear distant or near and that this information stimulated him to apply himself to the problem and arrive at its solution by reasoning. These first crude instruments devised by Galileo were apparently not microscopes but telescopes. It seems evident that he did not become acquainted with the compound microscope until instruments copied by Drebbel from Zacharias reached Rome in 1622.

Within the next two years Galileo presented microscopes to a number of his friends. Portions of letters accompanying the gifts are reproduced by Govi[1]. One of these, dated September 23, 1624 was addressed to Federigo Cesi, the founder and head of the society of scholars—the Accademia dei Lincei, of which Galileo himself was a member. During the years following, this society did much to encourage microscopic studies.

Francesco Selluti, also a member of the Accademia, published in 1625 probably the first illustrations made from microscopic observations on bees which he curiously interpolated (p. 52) in his Italian translation of the poems of Persius (1630). The papal physician, Giovanni Faber in a letter to Cesi dated April 13, 1625, suggested the name '*microscopio*' since the Accademia had already coined the name '*telescopio*.' The versatile Jesuit, Athanasius Kircher ('Ars Magna Lucia et Umbrae'—Rome, 1646) aside from his many speculations told how microscopes should be made and urged those using them to procure the best of instruments.

Tremendous progress was made in the development of the microscope during the second half of the seventeenth century: the instrument apparently being received with mixed enthusiasm. Robert Hooke[2] wrote in his 'Micrographia'—"my faithful *Mercury*, my Microscope" while the diarist Samuel Pepys, (August 13, 1664) after paying five pounds ten shillings for a microscope considered it "a great price, but a most curious bauble it is." Although there is no record that Pepys ever saw anything with his micro-

[2] Govi, 1888. [1] Hooke, 1665.

scope, his contemporaries did. Marcello Malpighi in Italy, Antonie van Leeuwenhoek and Jan Swammerdam in Holland, and Robert Hooke and Nehemiah Grew in England made such fundamental discoveries with their instruments, that this period has been referred to as the "golden age of microscopy." Leeuwenhoek especially skillful in lens grinding made his own microscopes and a catalogue drawn up for the auction of his effects on May 29, 1747 listed 247 completed instruments and 172 mounted lenses. There were three lenses made of quartz but there is no evidence according to Dobell that he ground lenses from diamonds as has sometimes been reported. Professor Harting examined the Leeuwenhoek microscope in Utrecht and found it to have a magnifying power of no less than 270 diameters.

One of the greatest defects of these early microscopes was chromatic aberration—an object placed under the lens would shimmer with all the colors of the spectrum. Leading physicists and mathematicians worked on the problem and in 1757 John Dolland, an English mechanician, using the calculations of Chester More Hall (1722) made the first achromatic glass. It was, however, some time before this glass was used for making lenses for microscopes. Amici of Modena equipped his microscopes with achromatic lenses in 1816 and Charles Chevalier,[1] the Paris maker in 1824.

Perhaps the first microscope made in America was produced by Edward Bromfield,[2] a graduate of Harvard in the class of 1742. A portrait of Bromfield painted in 1745 and now hanging in the anatomical laboratory at Harvard shows him with his microscope which was an upright instrument supported by three metal scrolls. The Reverend Thomas Prince, writing in the American Magazine, Boston for November 30, 1746 tells of Bromfield and his skill in microscopy and lens making.

In 1847, the year following the establishment in Germany of the firm of Carl Zeiss, Charles A. Spencer began making microscopes commercially at Canastota, New York. His lenses were awarded highest honors at the Paris exhibition of 1878 when shown in competition with those of the best makers of Europe.

During the second half of the nineteenth century a number of manufacturers began making and improving upon microscopes in England, on the continent and in America. Many of their improvements were reported in the Journal of the Royal Microscopical Society, founded in 1839, which has been responsible for fostering standards such as the 'Society screw-thread' permitting an interchange of optical equipment from one instrument to another. The Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopsche Technik established in 1884 has also been a medium for publication of improvements in microscopes and methods for microscopy.

For further reading on the history of the microscope see: SINGER, 1914. Notes on the early history of microscopy, Proc. Roy. Soc. Med., vol. 7, pt. 2. Sect. Hist. Med., pp. 247–279. DISNEY, HILL AND BAKER, 1928. Origin and Development of the Microscope. The Royal Microscopical Society, London. CLAY AND COURT, 1932. The history of the microscope, compiled from original instruments and documents, up to the introduction of the achromatic microscope. Charles Griffin and Company, Limited, London. DOBELL, 1932. Antony van Leeuwenhoek and his "little animals." Harcourt, Brace and Company, New York. WOODRUFF, L. L., 1940, Microscopy before the Nineteenth Century, Biological Symposia, Vol. I, pp. 5–36.

USE OF THE MICROSCOPE

Within the last years most of the makers of microscopes have evolved a type suitable for students' use, provided with oculars of two

[1] CHEVALIER, 1839. [2] HAGEN, 1870.

degrees of magnification and with three objectives, 'low power' (16 mm.), 'high dry' (4 mm.), and 'oil immersion' (2 mm.). The figures indicate the distance in millimeters of the objective from the section when the specimen is in focus. Such microscopes also have coarse and fine adjustment for regulating the position of the optical apparatus, and beneath the stage a mirror, one side plane, one concave, for directing the light, and an iris diaphragm and Abbé condenser for regulating the amount of

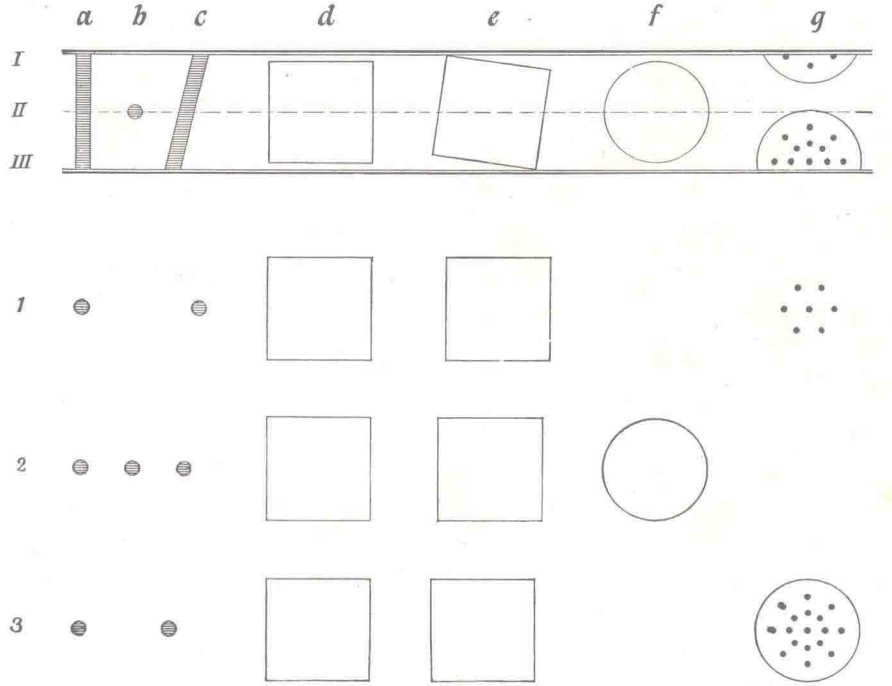

FIG. 1.—DIAGRAM TO DEMONSTRATE THE DIFFERENT APPEARANCES OF VARIOUS STRUCTURES IN A SECTION WHEN SEEN AT DIFFERENT FOCAL PLANES.

The upper row represents a side view of the section, between the coverglass at *I* and the glass slide at *III*. Below are three views such as would be seen through the microscope: 1, (across both pages) when the focal plane is at or just below the coverglass (*I*); 2, at the focal plane *II*; 3, just above the slide (*III*).

light and focussing it on the specimen through the hole in the stage. Since the condenser is designed to transmit parallel light rays, the plane side of the mirror should be used unless disconcerting images of window bar or electric light filaments appear, when the concave side may be substituted. The iris diaphragm acts as in a photographic camera, the smaller the aperture the sharper the picture until a limit is reached on account of lack of light. This limit changes with different specimens, and the best result must be sought for each. For distinguishing color in the section, a wider aperture is often useful.

Always examine a specimen first with a low power objective and then with a high power. In focusing the microscope, have the objective drawn

away from the slide and focus down. This should be done *cautiously*, with a portion of the specimen actually beneath the lens; if there are only cover glass and balsam there, the objective will probably be driven down upon the slide. Unless one is sure that stained tissue is in the field, the slide should be moved back and forth as the objective is being lowered.

In the use of the ordinary microscope and of the usual histological or embryological slide one is in the condition of a one-eyed individual

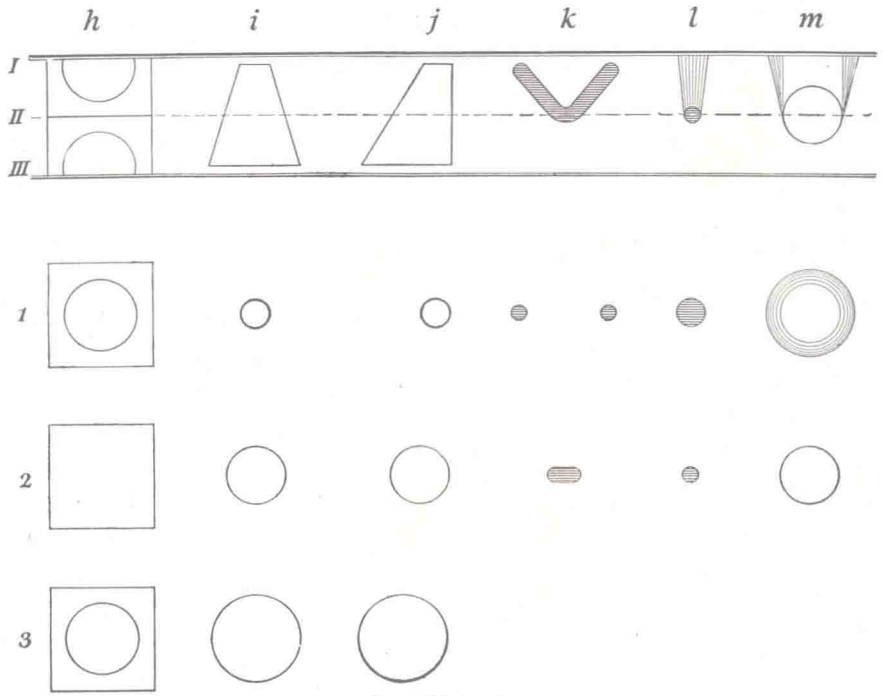

FIG. 1 (*Continued*).

a, vertical rod; **b**, granule; **c**, slanting rod; **d**, hollow cube; **e**, tilted hollow cube; **f**, hollow sphere; **g**, two spheres containing granules, only partly contained in the section; **h**, two spheres within hollow cubes; **i**, truncated hollow cone; **j**, tilted cone; **k**, bent rod; **l**, refractive granule; **m**, refractive sphere (e.g. an air bubble).

looking at a transparent stained-glass window. The perspective shown by a two-eyed vision and by the shadows on a moulded surface is lacking, and must be replaced, especially when one is using the higher objectives, by the careful manipulation of the fine adjustment. The focal plane, *i.e.* the level at which the clearest image is given, is very narrow with the highest powers, so that even in the thinnest sections all parts, from top to bottom, are not in focus at the same time. The constant use of the fine adjustment, changing the focal plane from one level to another in the specimen, and thus allowing a perfect view of all parts of it in the field, distinguishes an experienced microscopist from a beginner. This is very important, because the eye, which always attempts to bring objects in

focus, will strive in vain to do what the hand can do readily. Eye strain can be avoided by the continual use of the fine adjustment.

Monocular vision is fortunately greatly aided by a narrow focal plane. If one of two objects in a section is in clear focus and the other near it looks hazy, but becomes clear as the focus is lowered, the relative position of the two is readily understood. A rod-shaped structure running vertically through a specimen, from top to bottom, will be in focus at all levels of the focal plane, though appearing always in cross section as a round dot (Fig. 1, a). A spherical granule, on the other hand, though resembling the rod at a certain level, will disappear at higher or lower levels (b). A slanting rod will be seen at different positions in relation to other objects in the slide and will seem to move as the focal plane is changed (c appears as a round dot which moves toward a as the objective is lowered). A horizontally placed rod will of course appear as such, but only at one position of the fine adjustment. Thus to the high powers of the microscope the thinnest sections have the third dimension, depth, which can be investigated.

The use of transmitted light as in a transparency also entails certain limitations which must be understood in order to make full use of the usual microscopic specimens. Only those structures which partially block the light rays are distinctly visible, and to block the rays objects must have a certain density or thickness or both. To the naked eye a glass slide held to the light may be invisible, in that one can see through it; but the same slide turned edgewise is readily seen, because the depth of material is now greater. On the other hand an ink spot on the glass slide is visible because of its density of color.

Transferring these ideas to microscopic sections, we can understand that many structures actually present in a specimen may be invisible because we can see through them. Thus the thin top and bottom walls of a hollow cube might be invisible, while the perpendicular sides would be plainly seen (d). At a certain point in the tilting of a thin plane from the horizontal to the vertical position one passes from invisibility to visibility. Tilted planes which are vertical enough to be visible will appear to move, as the plane of focus is altered, just as a tilted rod seems to move (e). If a plane, seen as a line, does not change its position on focussing, it must be perpendicular to the surface of the specimen.

The same principle holds for a hollow sphere. The more horizontal top and bottom are transparent, while the more perpendicular parts of the sides are opaque. The sphere thus appears as a circle (f). If only the top or bottom of a sphere is present in a section (the remainder having been sliced away), its presence may be revealed only by its contents (g).

Sections are made as transparent as possible by being infiltrated with oil or balsam, which has nearly the same index of refraction as the glass

of the slide or cover-glass. Air has a different index, so that any light ray not absolutely in the line of vision will be bent in coming from the specimen to the objective of the microscope, and deliver a distorted image to the eye. Since the rays from the Abbé condenser are nearly parallel, this distortion is slight and negligible for the low power and even for the high-dry objective; but with the highest power even this distortion is rectified by a drop of oil between cover-glass and objective, immersing the lens in oil of the proper index of refraction. This oil should always be removed from the lens, after use, by gentle wiping with lens paper (a fresh piece each time, as the oil is apt to collect dust which might scratch the lens surface).

Opaque objects in transparent specimens will stop the light and therefore appear dark. Refractile objects, which have the property of bending the light rays, will show a dark contour, increasing in size at higher focus (l). If also opaque, such a refractive object will simply appear to grow larger, but if itself transparent, though refractive (like a bubble of air in oil), it may change from dark to light at varying focal planes (m). Partly refractive structures, when stained, may throw their color above them, though themselves out of focus and invisible at a given level. Too deep a stain often spoils a specimen for the observation of delicate details.

In Figs. 1 and 1 (cont.) the preceding paragraphs have been summarized in a diagram. The student should continually remember the third dimension, depth, in studying any microscopic section.

In 'dark-field illumination' a powerful light is thrown on the specimen obliquely, while light from below is shut off. A bright slanting beam of sunlight reveals dust particles in the air, which are otherwise invisible. By the same principle the dark-field microscope shows granules and other structures of ultra-microscopic size. The points of reflected light are seen rather than the actual granules. The method reveals their presence and any motion they may have, without indicating much as to their actual size or character. It is of use chiefly for the study of living tissues.

METHODS FOR STUDYING CELLS AND TISSUES

When cells and tissues are removed from the body they are either colorless or only faintly tinged. Living cells and bits of tissues may be studied under the microscope mounted in their own fluids, in blood plasma, in amniotic fluid or in physiologically balanced salt solutions. It is, however, not always possible to make out details in the way of structure in these preparations because the refractive indices of the different constituents are so nearly the same. The addition of dilute solutions of different dyes such as methylene blue, crystal violet, Bismarck brown and methyl green to the mounting medium stains certain of the

cellular constituents, at the same time allowing the cells and tissues to be studied in a comparatively fresh condition. Other dyes of relatively low

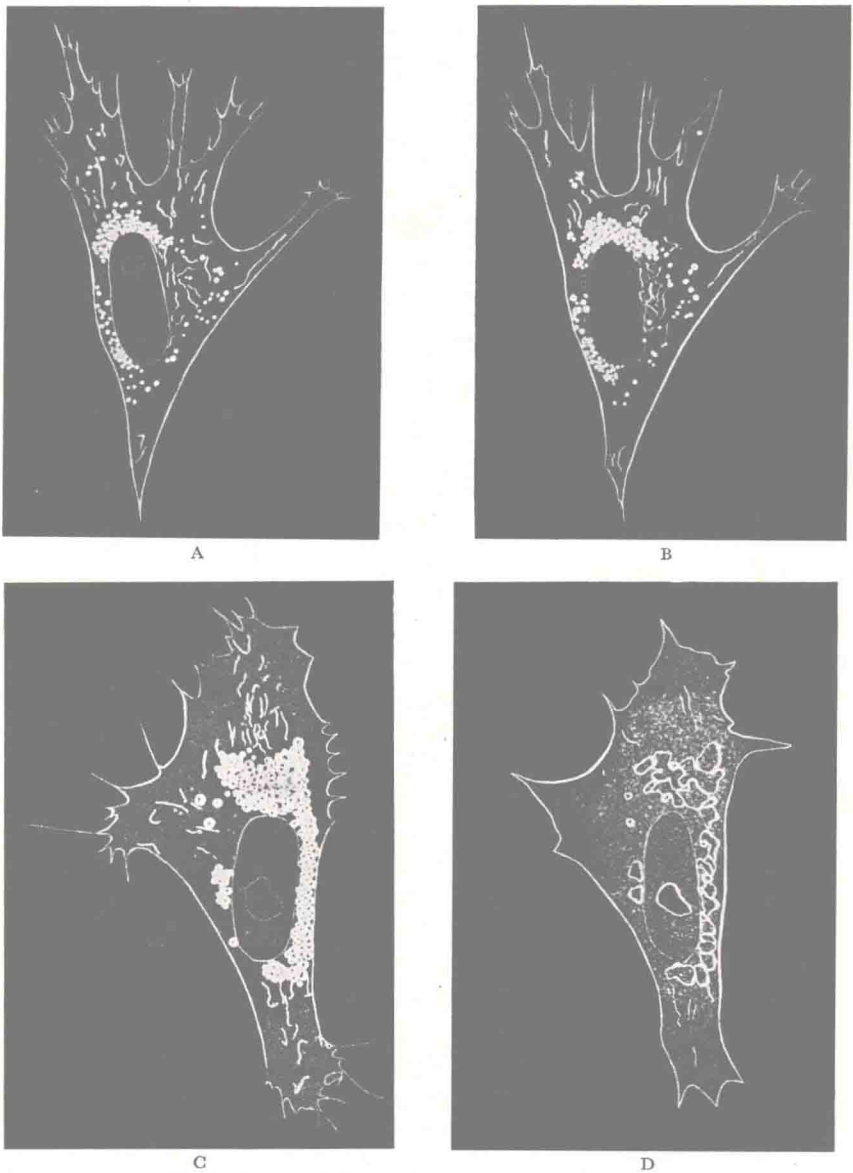

FIG. 2.—CONNECTIVE TISSUE CELLS BEFORE AND AFTER FIXATION.

A, normal living cell; **B,** after fixation by 2% osmic acid-shows little alteration of form and internal structure; **C,** normal living cell; **D,** after fixation by corrosive sublimate. Coalesence of fat globules and loss of the finer processes at the surface. (Strangeways and Canti.)

toxicity—as neutral red and Janus green B. in very dilute solutions applied directly or injected intravenously, have been widely used for

demonstrating more or less selectively certain granules and other formations in the cytoplasm. All these preparations are only temporary.

The development of special methods for studying living cells and tissues has opened up whole new fields of investigation in experimental cytology and histology. Knowledge of the physical structure and the chemistry of living matter has been advanced by micromanipulative methods which allow cells to be stimulated, injected and dissected under the microscope. By tissue culture or explantation living cells and tissues and even small embryos may be grown in suitable media outside the body and their growth and differentiation studied. Also living tissue may be transplanted elsewhere in the body and in successful 'takes' continue to grow: transplantations into the anterior chamber of the eye are particularly useful in studying the growth of blood vessels. Vascular growth and tissue reactions may be observed over long periods, even months, through transparent windows inserted into the rabbit's ear.

Permanent preparations are obtained after fixation, dehydration and embedding in a suitable medium as celloidin or paraffin and sectioning on a microtome. Fixation arrests post-mortem changes in the cell and renders insoluble many of its constituents. Generally the ideal in choosing a method of fixation is one to maintain as life-like appearance as possible, although this may be sacrificed if the observer wants to bring out prominently some particular structure. Dehydration performed after washing out of the fixation fluid, is usually accomplished by successive treatments with alcohols of increasing strengths. Embedding is the infiltration of a tissue with a suitable medium which when solidified holds it together and allows it to be sectioned. In preparation for embedding in paraffin, the alcohol is replaced by some liquid with which it is miscible and the paraffin soluble—chloroform, benzol, oil of cedarwood, etc.

Sections may be stained, or in some instances the entire block of tissue is stained before cutting. In the process of staining, solutions of dyes cause the constituents of the tissues to display different intensities of color since some have an affinity for one dye and not for another. These permanent preparations while valuable for the study of cellular relations and structural details do not always permit a full appreciation of cellular function and when studied exclusively, a student not infrequently carries away ideas of cells and tissues as pictures in terms of fixation and staining.

For those interested in microscopic technique and the methods employed in making the preparations described in this book, manuals are to be found in the laboratory or library. Standard manuals in English are those of Bensley, Guyer, Lee, Mallory, and McClung. The files of Stain Technology contain much information on the history and development of stains and staining, besides recent advances in these subjects.