

# Histopathologic Technic and Practical Histochemistry

By

R. D. LILLIE, A.B., M.D.

*Medical Director, U.S. Public Health Service; Chief, Pathologic Anatomy Service,  
Clinical Center, National Institutes of Health; and Chief, Laboratory of Pathology  
and Pharmacology, National Institute for Arthritis and Metabolic Diseases*

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“ὅταν δὲ ἔλβῃ ἐκεῖνος, τὸ πνεῦμα τῆς ἀληθείας,  
ὁδηγήσει ὑμᾶς εἰς τὴν ἀλήθειαν πᾶσαν.” Ἰω. xvi-13  
δίδασκε ἡμᾶς, κύριε, γινῶναι ταύτην ἀλήθειαν.

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## Foreword

Since the previous edition was written, there has been a great deal of active investigation of histochemical procedures. I have endeavored to bring selected variants of the newer methods into this book, and to emend them so that the methods may be followed without personal instruction by one who has had previous experience, or a good deal of experimentation to find optimal times, temperatures, pH levels and reagent concentrations. This has entailed in many instances a considerable amount of experimentation in arriving at workable conditions.

I am indebted to Dr. J. H. Peers, Dr. G. Laqueur, Dr. G. Brecher, Dr. B. Highman, Dr. R. W. Mowry, Dr. J. D. Longley, Dr. E. R. Fisher and others of the staff for their suggestions and cooperation in evolving these variants, and to Miss A. Laskey and Mrs. J. Greco Henson, and Mrs. H. Burtner for active help in performance and evaluation of procedures.

I also acknowledge my indebtedness to my predecessors and colleagues from whose works I have borrowed freely. Such of these borrowings as have been taken directly from their original publications are usually so cited in the text, but many have been taken, often in modified form, from other laboratory manuals. These texts are usually cited simply by the author's name, except that in the case of Ehrlich's "Encyklopädie" I have often cited the contributor's name. This last text I have often preferred as a source of those older methods which are still used in unmodified form. The following texts have been thus used, as well as earlier editions of some of them.

### General References

AMER. ASSN., TEXTILE CHEMISTS & COLORISTS: Yearbooks, 1939-1949, Howes, N.Y.

CONN, H. J.: "Biological Stains," 4th and 5th ed., Geneva, N.Y., Biotech., 1940, 1946.

- CONN., H. J., and DARROW, M. A.: "Staining Procedures," Geneva, N.Y., Biotech., 1943-1945, 1947-1949.
- COWDRY, E. V.: "Microscopic Technique in Biology and Medicine," Baltimore, Williams & Wilkins Co., 1943, 1948.
- EHRlich, P.: "Encyklopädie der mikroskopischen Technik," Berlin & Wien, Urban & Schwarzenberg, 1903.
- FEIGL, F.: "Qualitative Analysis by Spot Tests," 3rd ed., Elsevier Publishing Co., New York & Amsterdam, 1946.
- FEIGL, F.: "Chemistry of Specific, Selective and Sensitive Reactions," Acad. Press Inc., Publ. New York, 1949.
- GLICK, D.: "Techniques of Histo- and Cytochemistry," New York, Interscience Publishers, Inc., 1949.
- GOMORI, G.: "Histochemical Staining Methods" in Methods in Medical Research, M. B. Visscher, Ed., Chicago, Year Book Publishers Inc. 1951.
- GOMORI, G.: "Microscopic Histochemistry, Principles and Practice," Univ. Chicago Press, Chicago, 1952.
- GRAY, P. E.: "The Microtome's Formulary and Guide," New York, The Blakiston Company, Inc., 1954.
- HICKINBOTTOM, W. J.: "Reactions of Organic Compounds," 2nd ed., Longmans, Green & Co., London, New York & Toronto, 1948.
- HUECK, W.: "Die pathologische Pigmentierung" in Krehl-Marchands: Handb. d. allg. Path., 1921.
- JONES, RUTH McCLUNG: "McClung's Handbook of Microscopical Technique," New York, Paul B. Hoeber, Inc., 1950.
- KARRER, P.: "Organic Chemistry," 4th ed., Elsevier Publishing Co., New York, Amsterdam, London & Brussels, 1950.
- KRAJIAN, A. A. and GRADWOHL, R. B. H.: "Histopathological Technic," 2nd ed., C. V. Mosby Co., St. Louis, 1952.
- KRAUS, GERLACH, and SCHWEINBURG: "Lyssa bei Mensch und Tier," Berlin & Wien, Urban & Schwarzenberg, 1926.
- LANGE, N. A.: "Handbook of Chemistry," Sandusky, Ohio, Handbook Publishers, Inc., 1944, 1949.
- LANGERON, M.: "Précis de microscopie," 5th ed., Paris, Masson, 1934, 1949.
- LEE, A. B.: "The Microtome's Vade-Mecum," 11th ed., edited by J. B. Gatenby & H. W. Beams, New York, The Blakiston Company, Inc., 1950.
- LILLIE, R. D.: "Histopathologic Technic," Philadelphia, The Blakiston Company, 1948.
- LISON, L.: "Histochemie et Cytochemie Animale," 2nd ed., Paris, Gauthier-Villars, 1953.

- MALLORY, F. B.: "Pathological Technic," Philadelphia, W. B. Saunders Co., 1938.
- McCLUNG, C. E.: "Handbook of Microscopical Technique," New York, Paul B. Hoeber, Inc., 1929.
- McLEAN, R. C. and COOK, W. R. I.: "Plant Science Formulae," London, MacMillan & Co., 1941.
- PEARSE, A. G. E.: "Histochemistry, Theoretical and Applied," Boston, Little Brown & Co., 1953.
- RAMON Y CAJAL, S.: "Histology," translated by M. Fernán-Núñez, Baltimore, Wm. Wood, 1933.
- ROMEIS, B.: "Taschenbuch der mikroskopischen Technik," 13th ed., München & Berlin, R. Oldenbourg, 1932.
- ROMEIS, B.: "Mikroskopische Technik," München, Liebnitz Verl. 1948.
- ROULET, F.: "Methoden der pathologischen Histologie." Wien, Springer, Verl. 1948.
- ROWE, F.: "Colour Index," 1st ed. & suppl. Soc. Dyers & Colourists, Bradford, Yorkshire, 1924 and 1928.
- SCHMORL, G.: "Die pathologisch-histologischen Untersuchung methoden," 15th ed., Leipzig, Vogel, 1928.

R. D. LILLIE

*Bethesda, Md.*  
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