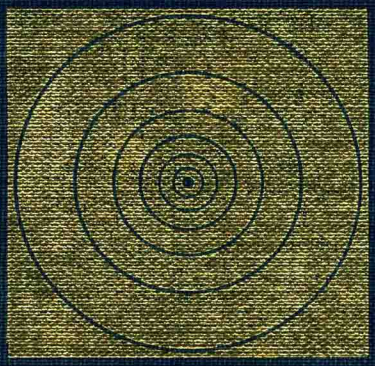
VOLUME 2



DIAGNOSTIC ELECTRON MICROSCOPY

EDITORS: Benjamin F. Trump and Raymond T. Jones

Diagnostic Electron Microscopy

Volume 2

Edited by

Benjamin F. Trump, M.D.
Professor and Chairman

Raymond T. Jones, Ph.D.
Assistant Professor

Department of Pathology
University of Maryland School of Medicine
Baltimore, Maryland

A Wiley Medical Publication
JOHN WILEY & SONS
New York · Chichester · Brisbane · Toronto

Library of Congress Cataloging in Publication Data (Revised)

Main entry under title:

Diagnostic electron microscopy.

(A Wiley medical publication)
Includes index.
1. Diagnosis, Electron microscopic. I. Trump,
Benjamin F. II. Jones, Raymond T. [DNLM:
1. Microscopy, Electron. 2. Diagnosis, Laboratory
—Instrumentation. QH212.E4 D536]
RB43.5.D53 616.07'58 77-12817
ISBN 0-471-89195-9 (v. 1)
0-471-89196-7 (v. 2)

Printed in the United States of America

10 9 8 7 6 5 4 3 2

Diagnostic Electron Microscopy
Volume 2

This volume is dedicated to Dr. Robert E. Stowell. Dr. Stowell, presently Professor of Pathology at the University of California at Davis, was instrumental in developing the role of electron microscopy in pathology. As early as 1955, Dr. Stowell had installed an electron microscope in his Department of Pathology at the University of Kansas. Consequently that laboratory began making significant contributions to normal and abnormal cell biology. These efforts resulted in the stimulation of a large number of individuals, many of them presently in academic pathology, such as myself and my student, Dr. Jones. As a medical student, I can remember Dr. Stowell's intuition that the use of electron microscopy was the next wave of development in pathology. I followed his advice and received his support for training in this area with Dr. H. Stanley Bennet and later with Dr. E. P. Benditt at the University of Washington in Seattle and then returned with Dr. Stowell at the Armed Forces Institute of Pathology for more investigations. It is, of course, through the efforts of men like Robert E. Stowell that pathology continues to advance. His many contributions to pathology education, research, and service will be long remembered. We all stand on the shoulders of giants such as this who have paved the way for present and future developments in the field.

BENJAMIN F. TRUMP

Contributors

Henry A. Azar, M.D., Chief, Laboratory Service, Veterans Administration Hospital, and Professor of Pathology, University of South Florida, College of Medicine, Tampa, Florida

Alex Ferenczy, M.D., Associate Professor of Pathology and Obstetrics and Gynecology, McGill University; Director, Division of Gynecologic Pathology, Jewish General Hospital, Montreal, Quebec, Canada

Ramon L. Font, M.D., Assistant Chief, Department of Ophthalmic Pathology, Armed Forces Institute of Pathology, Clinical Professor of Pathology and Ophthalmology, Georgetown University Medical Center; Washington, D.C.

Julio H. Garcia, M.D., Professor and Director, Anatomic Laboratories; Head, Division of Neuropathology, Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland

Frederick A. Jakobiec, M.D., D.Sc., Instructor in Clinical Ophthalmology, College of Physicians and Surgeons, Columbia University; Director, Algernon B. Reese Laboratory of Ophthalmic Pathology, Edward S. Harkness Eye Institute, New York, New York

Peter W. Lampert, M.D., Department of Pathology, University of California, San Diego, La Jolla, California

Hernando Mena, M.D., Instructor, Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland

Ralph M. Richart, M.D., Professor of Pathology, Columbia University College of Physicians and Surgeons; Director, Division of Obstetric and Gynecologic Pathology, The Sloane Hospital for Women, New York, New York

Sydney S. Schochet, Jr. M.D., Department of Pathology, University of Texas Medical Branch, Galveston, Texas

Kyuichi Tanikawa, M.D., PhD., Professor, Department of Medicine, and Chief of Electron Microscopy Laboratory, Kurume University School of Medicine, Kurume, Japan

Myron Tannenbaum, M.D., Ph.D., Departments of Pathology and Urology, College of Physicians and Surgeons, Columbia University, New York, New York

Marjorie J. Williams, M.B., ChB., Director of Pathology and Allied Sciences, Veterans Administration Central Office, Washington, D.C.

Preface to Volume 1

The term diagnostic electron microscopy implies the utilization of electron microscopy and its associated methods in all of their ramifications for the study of human disease. At the present time, crucial diagnostic information is indeed provided in many cases by electron microscopy, and often information of confirmatory nature or of great educational value to the pathologist and clinician can be obtained. But since the increased amount of information obtainable by electron microscopy (compared with that obtainable by light microscopy) is measured in orders of magnitude, electron microscopical data cannot be judged by, or be fit into, previous concepts based on gross examination and light microscopy. It is probable, then, that we have not yet witnessed the full value of the diagnostic methods of electron microscopy simply because an appropriate data base has not as yet been generated. Therefore, it is important that these methods be used whenever possible, as it will be necessary to define new concepts and classifications of disease.

In the last few years the technology involved in electron microscopy has progressed to the point where methods have become standardized and the instrumentation routine. The quality of instrumentation is now comparable to many of the advanced types of instrumentation present in a well-equipped laboratory. It is possible for any general laboratory to maintain an instrument and associated laboratory staff, which means that the pathologist need no longer be concerned about the difficulty of the techniques and instrumentation. Furthermore, the personnel normally available in the clinical laboratory, such as the electronics technician and the senior medical technologist, are quite capable, with some additional training, of maintaining the instruments.

The field of diagnostic electron microscopy cannot be separated from the larger field of cellular pathobiology. The power of concepts derived from cellular pathobiology and applied to human disease biology cannot be overestimated. Much of the rapid, current progress in this field is due to the essential unity of cell biology in diverse animal and plant species as well as in different organ systems. Information developed on one especially suitable cell type can be rapidly applied, with a minimum of experimentation to other cell types and other organisms leading to much more rapid progression of knowledge. An important concept in this renaissance of general pathology is the correlation between structure and function at the cellular level, which has been made observable through the integration of methods in the fields of microscopy, immunology, biochemistry and physiology. The electron microscope is, of course, a fundamental tool in these investigations because it is at the level of resolution provided by this instrument that most structural correlations with function and metabolism are visible.

Implicit in the development of diagnostic electron microscopy is the study of material from human patients, which makes possible, in addition to diagnosis, studies on the basic biology of human disease. This is currently a very important trend in pathology, and one which needs world-wide support, for it is only by studying human cellular metabolism that we will be able ultimately to improve our understanding of human disease.

This book, the first in our series of treatises on diagnostic electron microscopy, outlines concepts of cellular pathology and laboratory management, and their applications to specific organ systems. Succeeding volumes will concentrate on particular organ systems. We wish to instill in the reader a sense of excitement for the growing field of diagnostic electron microscopy and to convey the urgency of expanding our knowledge of human disease from the equally important perspectives of diagnosis, education, and research.

BENJAMIN F. TRUMP, M.D.
RAYMOND T. JONES, PH.D.

Baltimore, Maryland

Preface to Volume 2

In this volume we shall continue the systematic presentation of diagnostic electron microscopy as it is currently used. The volume begins with a chapter by Dr. Marjorie J. Williams in which she describes the evolution of the diagnostic electron microscopy program in the Veterans Administration. This chapter is an important one, as it provides an excellent data base against which other programs can be compared and illustrates the evolution of the program over the last several years. The program began with three units and has developed into 42 units. The experience in this program, which is the largest organized electron microscopy program in the world, is of great interest to practitioners in the field.

The chapter on the liver by Dr. Tanikawa introduces the use of electron microscopy in liver disease. This is an area that is rapidly increasing in importance as it is becoming evident that many hepatic diseases are impossible to diagnose precisely by light microscopy. Furthermore, new diagnostic entities are being revealed through the use of electron microscopy. There is also an important place in liver disease for estimation of overall hepatic parenchymal cell damage using the electron microscope, and in the future, as morphometric techniques are applied, it should be possible to provide more accurate correlations with liver function tests done on peripheral blood.

In the chapter on hematopoietic and lymphoid systems by Dr. Azar, the many uses of electron microscopy in this field are discussed. In this area the use of the electron microscope is capable both of solving diagnostic problems that cannot be resolved by routine light microscopy and of introducing a greater appreciation of cellular detail, supplementing light microscopy and leading to a better understanding of pathophysiology. Once again, there are numerous examples of diseases of the hematopoietic and lymphoreticular systems which can only be diagnosed in this way. Electron microscopic studies can then be correlated with other studies, including immunofluorescence, and with clinical findings.

The chapter on ocular pathology by Drs. Font and Jakobiec is of interest for diagnosis of diseases of the eye and is also of general interest because of a wide variety of disease processes in conditions that affect this organ. The chapter on the bladder by Dr. Tannenbaum illustrates the application of electron microscopy to an easily accessible human tissue that can be accurately observed by the clinician and the pathologist and that offers the opportunity for careful systematic and sequential studies on the development of human cancer. It is probable that both transmission and scanning electron microscopy, together with cytology, will materially assist in the evolution of knowledge concerning this important human cancer.

In their chapter on gynecology, Drs. Ferenczy and Richart summarize specific ultrastructural features that are of diagnostic help in understanding the his-

togenesis of gynecological disease. There is an almost indefinite variety of neoplastic and non-neoplastic conditions involving this organ system, many are easy to diagnose, but many more are not. Electron microscopy promises to be of substantial help in elucidating the histogenesis of several important neoplasms in this system.

The chapter on the peripheral nerve by Drs. Lampert and Schochet discusses the importance of electron microscopy in morphological studies of nerve biopsies. Many conditions are readily diagnosed in this area which had previously been extremely obscure for the general pathologist. The importance of plastic embedding for even light microscopy of the nerve is brought out. Here, the electron microscope has truly replaced the special stains of the past because of the ease of production and reproducibility of the results.

In the final chapter by Drs. Garcia and Mena, the intricacies of neuropathology of the central nervous system are explored. Obviously, this is a vast subject that could itself occupy volumes; however, in this chapter we are attempting to present some of the more important current applications.

The editors sincerely hope that the users of this book will find it helpful in their studies of ultrastructure, especially as it applies to diagnostic pathology.

Benjamin F. Trump, M.D.
Raymond T. Jones, Ph.D.

Baltimore, Maryland

Contents

1
Diagnostic Electron Microscopy in the Veterans Administration

Marjorie J. Williams, M.B., Ch.B.
Director
The Pathology Service
Veterans Administration Central Office
Washington, D.C.

Diagnostic electron microscopy (EM) in the Veterans Administration (VA) must be viewed as a part of the agency's medical care program and not as an isolated entity. Therefore, a brief description of the scope and organization of the VA's medical care program provides a necessary introduction to the more detailed discussion of the EM program. A summary of medical care in VA facilities is shown in Table 1.

Table 1. Summary of Medical Care in VA Facilities (July 1, 1975 through June 30, 1976)[a]

Facilities	*Beds*	*Patients Treated (Episodes of Care)*
171 Hospitals	93,822	1,178,894
88 Nursing home units	7,585	10,941
18 Domiciliaries	10,152	18,408
215 Outpatient clinics	0	14,223,206 visits

[a] US Senate Committee Print No. 7, 95th Congress, first session: *Veterans Administration Response to the Study of Health Care of American Veterans, September 22, 1977.* US Government Printing Office, Washington DC, 1977

This chapter was prepared by Dr. Williams in her capacity as the Director of the Pathology Service for the Veterans Administration, and its contents therefore remain in the public domain.

Medical care is the responsibility of the Department of Medicine and Surgery (DM&S), which is one of the VA's three major departments. The other two departments are concerned with Veterans Benefits and Data Management. An outline of the DM&S organization as it relates to the EM program is shown in Figure 1. The chief medical director heads DM&S, and the line authority goes from him through the associate deputy chief medical director to the executive councils of the 28 medical districts, the hospital directors, the chiefs of staff, and the chiefs of the various professional services such as the laboratory service in the hospitals. The other organizational elements shown in the figure serve in a staff capacity to the chief medical director.

Diagnostic electron microscopy is one of the VA's some 23 designated special medical services, which also include such modalities as renal dialysis, renal transplant, and cardiopulmonary bypass surgery. These services are supported by specific appropriations and exist only in selected hospitals. The office of the assistant chief medical director for professional services has the responsibility for the nurture, planning, site selection, management, and evaluation of all the special services, and this duty is delegated to the appropriate professional service. For example, diagnostic electron microscopy is delegated to the pathology service in the VA's central office.

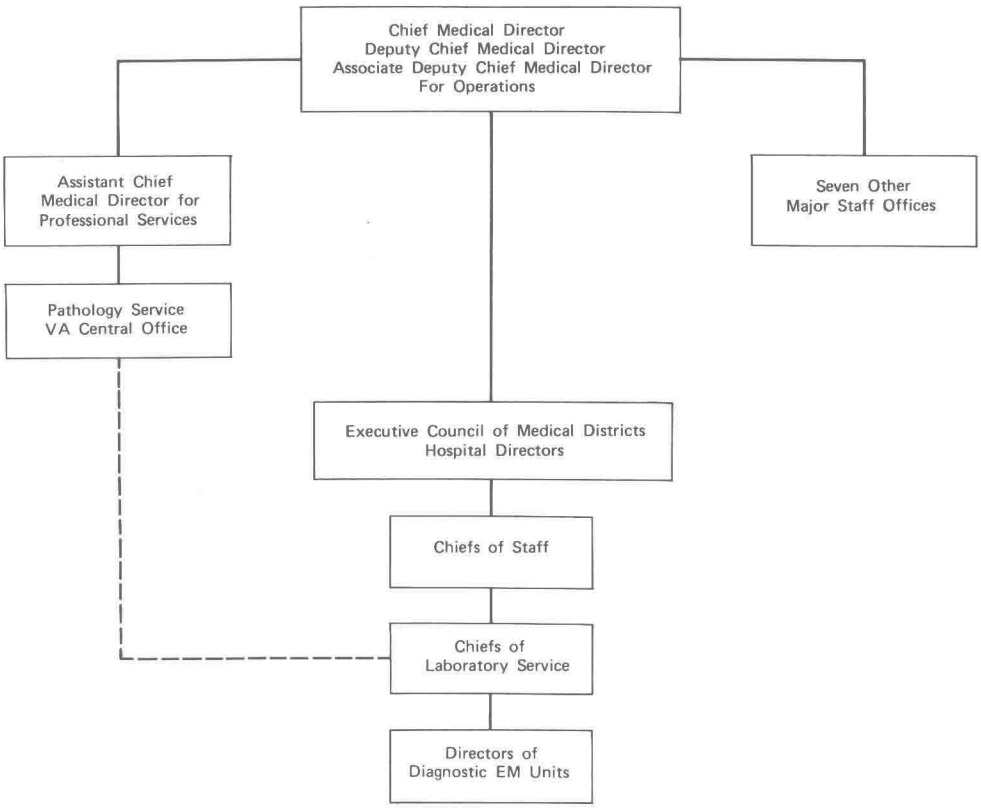


Figure 1. Veterans Administration Department of Medicine and Surgery organization in relation to the diagnostic electron microscopy program.

In the hospitals, the diagnostic EM units are organizationally part of the laboratory services. In some instances, the chief of the laboratory service may also be the director of the local EM program, but more frequently the responsibility is assigned to another pathologist with particular interest and skill in this field.

Another topic that requires brief description is the VA's extensive affiliations with medical schools (there are also affiliations with schools for dentistry and many allied health professions and occupations). There are now affiliate relations between 133 VA hospitals and 103 of the nation's 120 medical schools. Twenty-six percent of the graduate medical education in the United States occurs in VA facilities. Most of the pathology residency training in VA hospitals is conducted through integrated programs with the affiliated medical schools. The diagnostic EM units are all located in hospitals that are affiliated and provide graduate medical education in pathology.

The VA has statutory authority to enter into agreements with affiliated medical schools and other hospitals to share specialized and scarce patient care services. These agreements are established most frequently for the sharing of the special medical services. Sharing agreements for diagnostic electron microscopy are discussed later in detail.

THE VA DIAGNOSTIC EM PROGRAM: ORGANIZATION AND GOALS

The VA program for diagnostic electron microscopy was established in 1966 with the advice and encouragement of the national pathology consultants. It began with the purchase of three instruments and since then has grown to 42 diagnostic EM units dispersed throughout the continental United States and in Puerto Rico.*

The EM program is characterized by centralized planning, site selection, funding, and evaluation, with retention of considerable autonomy by the local unit directors for the daily operations. Details will be presented in this chapter on the management and operational experiences with diagnostic electron microscopy. When the program was begun in 1966, the goals were as follows:

- Provision of a relatively new and specialized modality for the enhancement of histopathological diagnosis.
- Resolution of certain diagnostic problems that could not be solved by light microscopy.

*Locations of VA diagnostic EM units: Albany, NY; Allen Park, MI; Ann Arbor, MI; Baltimore, MD; Birmingham, AL; Boston, MA; Bronx, NY; Charleston, SC; Chicago, IL; Cleveland, OH; Columbia, MO; Dallas, TX; Decatur (Atlanta), GA; Denver, CO; Durham, NC; East Orange, NJ; Gainesville, FL; Hines, IL; Houston, TX; Kansas City, MO; Lexington, KY; Little Rock, AR; Long Beach, CA; Los Angeles, CA; Miami, FL; Minneapolis, MN; New Orleans, LA; New York, NY; Northport, NY; Philadelphia, PA; Pittsburgh, PA; Richmond, VA; Salt Lake City, UT; San Diego, CA; San Francisco, CA; San Juan, PR; Seattle, WA; Shreveport, LA; Tampa, FL; Washington, DC; West Haven, CT; West Roxbury, MA. An additional unit at Madison, WI, will become operational in 1978.

- Affording opportunity for training and experience in ultrastructural pathology.
- Rapid development and application of research in ultrastructure to diagnostic pathology.
- Strengthening pathology graduate medical education programs.

Some 10 years later these goals are still valid. They have been expanded, however, in line with the thrust for use of specialized resources on a regional basis, to include EM support for other VA hospitals in the same medical district.

There are also in certain VA hospitals EM facilities that are part of the research and not the pathology program. The research facilities are funded separately from the diagnostic units, and their selection and evaluation are handled through different channels. A limited number of diagnostic EM studies are carried out on research equipment in some hospitals that do not have diagnostic units, but information about such examinations is not collected by the pathology service in central office. In addition, some specimens are referred elsewhere, frequently to an affiliated medical school, from VA hospitals lacking EM resources. Therefore, the present 42 diagnostic EM units do not meet all the VA's needs for ultrastructural study in support of diagnosis. The number of specimens referred within the VA from one hospital to another is not yet large but should increase as regionalization gains momentum.

VA Electron Microscopy Advisory Group

The VA electron microscopy advisory group was established in 1970 and usually has six members, all of whom are pathologists with experience and interest in electron microscopy. Currently, three members are VA pathologists and three are full-time university faculty members. The present chairman of the group is Dr. Benjamin H. Spargo, professor of pathology at the University of Chicago, and his predecessor was Dr. John R. Carter, professor and director of the Institute of Pathology at the Case Western Reserve University.

The group meets at least once each year with the pathology service at the VA central office in Washington and assists in the review of applications for new EM programs and in the evaluation of existing programs. When a site visit is considered necessary to obtain additional information, one or more of the group are members of the visiting team. At the regular meetings, there are thoughtful, analytical discussions of the VA diagnostic EM program as a whole that may lead to suggestions for changes in direction or emphasis. The fresh insights into the diagnostic electron microscopy program that this group is able to provide as a result of the breadth of experience and knowledge of its members constitute a major contribution.

Funding of VA EM Programs

The EM programs receive their basic funding from the appropriation that supports the special medical services. The support for each approved program is specifically identified and provides for both the initial and the recurring costs. Initial costs include such items as the purchase of the electron microscope and other necessary equipment and the construction or remodeling of space to ac-

commodate the unit. Recurring costs consist of the salaries for the staff, the supplies, and the service contract for maintenance of the electron microscope. The indirect costs are supported from the general operating budget of the hospital.

The selection of the electron microscope and other equipment is made locally, although the decision must be reviewed and approved in the central office. In general, only high resolution transmission electron microscopes are acquired, but a few instruments that may be used also for scanning have been purchased. The salary allocation is sufficient to support on a full-time basis one pathologist and two technologists. This level of staffing is considered satisfactory for a diagnostic EM unit examining at least 250 specimens annually.

Replacement of existing equipment, acquisition of new capital equipment, and salaries for additional personnel, including secretarial support, must be provided by the hospital through regular budgetary procedures.

Application Procedures to Establish an EM Program

The first diagnostic EM units, particularly in 1966 and 1967, were established in VA hospitals where, in the judgment of the pathology service in the central office, they would be used effectively. In the following two years such judgment was still the prevailing basis of selection but was modified to promote appropriate geographical distribution in the VA. By 1970, there were 22 diagnostic EM units established in laboratory services, and the size of the program was such that more formal selection procedures were considered necessary. The EM advisory group was therefore formed.

A formal application procedure has been developed to collect information in a relatively uniform manner. The application must include specific detailed plans for use of EM in diagnostic pathology and training both at the parent hospital and in the medical district; the anticipated contributions to patient care; the name and curriculum vitae of the proposed program director; and a description of the desired equipment. Statements of endorsement from the chief of the laboratory service, the hospital director, the chairman of the pathology department at the affiliated medical school, and the executive council of the medical district must be appended to the application.

The applications are reviewed by the EM advisory group, and it makes recommendations to the director of the pathology service in the central office. Final decisions on the selection depend on several factors, including a favorable review by the consultants, the suitability of the location of the proposed program in the context of broader VA policies and needs, and the availability of appropriated funds.

VA PERFORMANCE CRITERIA FOR DIAGNOSTIC EM UNITS

In the current era of rising medical costs, considerable attention is being directed toward evaluation of the effectiveness of the newer and more complex modalities. However, useful evaluation requires criteria against which performance

can be measured. Because the VA diagnostic EM units share many common features, such as receiving the same basic funding and serving the same type of patient population, the pathology service in the central office has concluded that a single set of performance criteria can be used to evaluate all the programs.

After considerable deliberation, the following basic performance criterion was established in 1973: a minimum of 250 specimens *accessioned* annually, with occasional downward deviation to not less than 225 accessions when justified by special circumstances. Such circumstances might be a change in the program director or significant problems with equipment. On October 1, 1977, the basic performance criterion was changed to a minimum of 250 specimens *examined* annually, with no downward deviation permitted.

Two other performance criteria have been introduced since 1973 as supplements to the basic criterion. One of these requires that at least 66% of the 250 minimum be patient specimens examined to assist in diagnosis, while the remainder may be studied for research purposes. The other criterion requires that at least 51% of the minimum number of examinations be on specimens from VA patients.

The performance criteria have been well accepted by the directors of the EM units and have proved most helpful in the evaluation process. The various criteria may not be directly applicable elsewhere, but their successful use in the VA suggests that with appropriate modification they may have wider potential.

Evaluation of the EM Programs

The evaluation process measures performance of each EM unit in relation to the criteria and also assesses its overall effectiveness in meeting the goals of the program.

The basis for evaluation is the semiannual report prepared by each program director, using a standardized format. The semiannual reports include the following information:

- The number of specimens accessioned and how many of these were examined by EM, by thick section only, or embedded and retained but not studied during the reporting period.
- The distribution of specimens by source, such as kidney, liver, skin, nervous system, and so forth.
- The names and grades of personnel assigned to the EM unit, with the number of hours per week each spends in the unit.
- The number of written reports issued for inclusion in the patients' medical records.
- The use of immunofluorescence.
- The use of scanning electron microscopy.
- The number, location, and activity of any sharing agreements.
- The training and teaching activities.
- The publications and presentations at professional meetings resulting from EM studies during the reporting period.
- The significant contributions to diagnosis.