Basic Techniques for Transmission Electron Microscopy

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Preface

Transmission electron microscopy has been making profound contributions to the solution of problems in biology and medicine in that the transmission electron microscope gives us the hope of direct examination of biological structures at the atomic level. Individual molecules and their components can be identified—thus the emergence of "molecular microscopy." Such information is of vital importance in understanding the correlation between structure and function at the cellular level. Since methodology is a major constraint in obtaining more detailed and accurate information on cell ultrastructure, the problems of biological specimen preparation need to be attacked with the same converging intensity as that afforded by the improvement of resolving power.

In the preface of my book, "Fixation for Electron Microscopy" (Academic Press, Inc., 1981), I expressed my wish to continue compiling information that might help to improve the quality of specimen preservation as well as the interpretation of the fine structure. This wish is partly fulfilled by publishing the present volume, which covers a vast variety of techniques and alternative approaches used for studying morphological and cytochemical aspects of specimens. Much of this volume is a compilation of methods scattered throughout a large number of scientific journals, and should therefore save readers valuable time in searching through journals for the desired method and/or in contacting various laboratories.

To minimize artifacts and to obtain the required information, each type of specimen requires special, optimal processing. The precise parameters of processing a wide variety of specimens are presented in Chapter 7. It is inappropri-

ate to present only the best variation of a basic method, since for a particular study one variation may give a better result than another; for example, the variation for the preservation of yeast cell wall differs from that ideal for preserving yeast mitotic spindle. Furthermore, the use of a superior variation may not be feasible in a certain laboratory because of the unavailability of a required piece of equipment, lack of funds, or lack of expertise. Variations on methods are therefore given so that readers can choose the most suitable approach to achieve their specific objectives.

Generally, no attempt was made to explain the theory or principles that govern preparatory procedures, because the primary objective of this volume is to present procedures in a step-by-step fashion. Other books (e.g., "Principles and Techniques of Electron Microscopy: Biological Applications," 2nd ed., International Pub., Berkeley Heights, New Jersey) by this author present the principles governing preparatory procedures. Original source references are provided throughout the text so that the reader can obtain additional information.

It is almost impossible to personally test each of the methods included. I am hopeful that the methods presented in the text will be successful. However, preparatory methods are subject to modification depending on the objective of the study and available facilities. In order to achieve the best results, it is imperative that an attempt be made to optimize even a basic method.

Preparatory procedures for both eukaryotic and prokaryotic groups are presented. Optimal preparatory requirements for these two groups differ from each other. Since plant specimens frequently present special problems (cell wall and large vacuoles), optimal protocols for these specimens differ from those used for animal specimens. Special preparatory methods for plant specimens as well as for viruses are also presented. The processing of uncommon specimens and the solution of unusual, individual problems are included.

Electron microscopy is playing an increasingly crucial role in the confirmation of human diseases; important diagnostic information is provided by electron microscopy. Accordingly, I have attempted to include techniques useful for clinical medicine. Presently, diagnostic information can be obtained by electron microscopy within three hours after a biopsy or collection of a surgical specimen. I have not attempted to present every preparatory procedure used in diagnostic electron microscopy, but have focused instead on well-established, routinely used techniques (for example, negative staining for viral diagnosis).

This book departs from tradition in that books on methodology present only the contemporary consensus of knowledge. Here relatively new methods are also presented, provided that they show potential usefulness. Some of these methods are in the developmental stage, and will probably be refined and standardized; all are restricted in their scope to the preparation of specimens for transmission electron microscopy. Because of the availability of an enormous number and variety of techniques for transmission electron microscopy, limited available

space did not allow the inclusion of techniques for scanning electron microscopy. Esoteric techniques that require very expensive instruments are also excluded because of space limitations.

This book is intended for students, technicians, teachers, and research scientists in biology and medicine, and is essentially a laboratory handbook that can be used in formal courses and by individuals. I hope that it will yield practical advice to beginners who are learning the preparatory techniques as well as to experienced and busy scientists who cannot afford to spend time searching for procedures in literature. It is suggested that the entire procedure be read and necessary solutions prepared prior to undertaking the processing. An exhaustive list of references with complete titles is provided, as is a detailed subject index.

Because this is a book on methodology, many techniques are included that were extracted and synthesized from scientific journals, personal communications, and laboratory testing. A vast majority of the methods presented were checked for accuracy and updated by their originator. Numerous scientists were very kind to allow me the use of their illustrations, for which I am most grateful. I am also thankful to the publishers of various scientific journals who were more than prompt in granting me permission for reproducing the illustrations.

This volume could not have been completed without the help, encouragement, and inspiration that were graciously extended to me by a large number of very able scientists.

M. A. Hayat

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