METHODS IN CANCER RESEARCH

Volume I

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Preface

Approximately 100 years ago experimental oncology, or cancer research, was initiated. Despite many obstacles, the progress in this field has been steady and, for the most part, consistent with the endeavors of skilled scientists who have made clear and important progress. Our knowledge of experimental systems has grown steadily on the basis of new techniques that have funneled into the cancer problem through a wide variety of disciplines.

The present volumes on methods in cancer research are intended to present a comprehensive survey of available methods for analysis of various biological, morphological, biochemical, therapeutic, and oncogenic phases of cancer research. It is recognized that studies in cancer research are multidisciplinary because of the vast ramifications of the problem and that it is beyond the ability of any one individual to be fully familiar with the methods and implications and results in all of the contributory fields. This may result in limitations on experimental success in particular areas and also in a lack of uniformity of quality throughout various aspects of an experimental series. In addition, it is very frequently difficult and confusing for young individuals entering the field of cancer research to grasp the breadth of methodology by consulting all of the various specialized texts on methods. Accordingly, it is hoped that this compilation, which is more restrictive in its coverage, will have particular value to graduate students, postdoctoral fellows, and young investigators who are interested in the cancer problem.

It will be noted that in many of the chapters the authors not only deal with the specific methods involved with their particular subject but also are concerned with the interpretations and specific applications. These more critical aspects of the areas of methodology are of particular importance in cancer research because there has been unfortunate overextensions of data under many circumstances and frequently serious misinterpretation when workers are unfamiliar with limitations of various procedures.

It is hoped that the very dedicated endeavors of the contributors to produce chapters with a high standard of excellence will be reflected in the future investigations in the cancer field.

HARRIS BUSCH

Houston, Texas
February, 1967

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CHAPTER I

METHODS IN ELECTRON MICROSCOPIC CYTOLOGY

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I. Introduction

Electron microscopic techniques as applied to the study of cancer do not differ in any way from general methods of electron microscopic cytology. The following outline of the techniques has, therefore, no specific bearing on oncology, and deals with today's methods for the ultrastructural analysis of normal as well as malignant cells. There is no difference between electron microscopic techniques for clinical or for fundamental cell research. The same technical problems will be encountered in both types of investigations. Fixation hazards and the extreme minuteness of the samples make interpretation of electron micrographs difficult whether the tissues come from laboratory animals or from patients.

Electron microscope cytologists have the surprising tendency to search for a single and almost universal preparatory technique enabling them to demonstrate all aspects of a large variety of cells. This contrasts sharply with the history of light microscopic cytology. Here, a multitude of fixation and staining techniques has been used, frequently with specific purposes, such as demonstrating cytoplasmic basophilia or mitochondria, and without paying too much attention to the simultaneous demonstration of the other cell organelles. Excellent light microscopic techniques have made possible the discovery and the description of the Golgi apparatus, but other techniques were necessary to stain centrioles. As a result, both structures were rarely seen in the same preparations, and the characteristic localization of the centrioles in the center of the Golgi apparatus was not stressed with great conviction by the light microscope cytologists. This typical localization is obvious for the electron microscopist whose technique has a more synthetic and panoramic character, most of the cell organelles being visualized together.

One should not, however, overemphasize these differences. The light microscopist can perform a variety of staining techniques on the same tissue sample. His synthetic view of the whole cell builds up from a continuous mental superimposition of many different images produced by varied staining methods. In addition, light microscopic techniques are frequently simple and rapid, and valuable data can be harvested after a casual glance at a slide stained in a few minutes. On the other hand, the preparatory techniques for electron microscopy are time consuming, never fully predictable, and depend to a considerable extent upon the chemistry of osmium tetroxide. By relying on a single technique, electron microscopists take an increased risk of being misled by artifacts. This point certainly does not escape the attention of friendly biochemists who rarely resist the temptation to bring it up. One might argue, however, as

to which one is the most artifactual or disruptive method, for a slice of liver to be ground with sand in a mortar or soaked in osmium tetroxide. Nevertheless, it was of considerable importance to consolidate the observations made on osmium-fixed cells by completely different morphological methods. Electron microscopy of aldehyde- or permanganate-fixed tissues and phase microscopy of living cells have now provided adequate controls, confirming the reality of most of the cell ultrastructures first demonstrated after osmium fixation.

The differences between laboratories for light and electron microscopes reside not only in the sophistication of the equipment. Their organization differs in many points, among which the slow pace imposed by electron microscope techniques has to be considered. Large differences in the required budgets and frequently also in the scientific programs are additional reasons to organize laboratories for light and electron microscopes on a relatively independent basis. However, in both cases, the necessary background and the general attitude of the investigators are very similar. Classic microscopic anatomy is the best introduction to electron microscopic cytology. A good understanding of cell fine structures will rarely be attained if the same material is not first observed at the low magnifications of the light microscope. This applies particularly to heterogeneous neoplastic tissues.

Today's electron microscopic cytologists should be more than anatomists equipped with a new powerful lens. Their observations extend our visual perceptions of biological structures to the macromolecular level. Under the light microscope, one sees homogeneous populations of cells forming tissues; under the electron microscope, one sees homogeneous populations of macromolecules and particles forming cell organelles. Interpretations of electron micrographs are proposed more and more in terms of biochemistry, and a significant contribution of electron microscopy is the narrowing of the gap between morphological and biochemical sciences. Electron microscopy must therefore be continuously integrated with other disciplines of cell biology, the ultimate and common goal being the analysis of the morphochemistry of normal and pathological cells.

II. Fixation

The tissue samples prepared for electron microscopy have to be extremely small for three as yet inescapable reasons: (1) the poor penetrating power of the fixatives; (2) the technical difficulty of preparing thin sections larger than a few tenths of a millimeter; and (3) the design of the objective pole pieces of the microscopes, which will not accom-

modate an object of larger dimensions. A real danger exists, therefore, in making sampling mistakes, i.e., to look under the electron microscope at cells which are not exactly those one is attempting to study or are not representative of the cells of the sample. This danger is minimal when studying a homogeneous organ like normal liver, but is of real concern in the study of pathological tissues and especially cancers. The samples must therefore be selected with utmost care by the investigator himself and not by technical aides. Semithin sections will have to be looked at under the light microscope for histological orientation purposes and to make sure that the block actually represents a sample of the cells one intends to study under the electron microscope. The following remarks apply to problems involved in the sampling of solid tissues, blood cells, and tissue cultures.

A. Sampling of Solid Tissues

All efforts should be made to collect tissues from living animals. In the case of human biopsies or surgical specimens, the electron microscopist should be in the operating room to advise the surgeon not to apply unnecessary pressures, not to squeeze, and not to let the tissue dry on a piece of gauze, and to immerse a thin slice of the tissue in fixative as soon as feasible. Occasional successes have been reported on the electron microscopic study of tissues fixed several days post mortem (Ito, 1962), or after long storage in a formaldehyde solution (Ashworth and Stembridge, 1964), indicating that all autopsy material is not necessarily lost for electron microscopy. However, under such conditions, one should certainly not expect the best preservation of fine structural details. In experimental work, several authors recommend initial fixation *in vivo* either by injecting a few drops of fixative into the parenchyma of the organ to be sampled a few minutes later (Pease, 1964) or by perfusing the anesthetized animal with osmium tetroxide (Palay *et al.*, 1962) or formaldehyde (Pease, 1964).

In the case of parenchymatous organs such as liver or spleen, a small slice, about 1 mm thick, is cut with new razor blades, lightly blotted on filter paper if the sample is bloody, and placed in a drop of fixative on a piece of dental wax. The sample is then cut with razor blades, in the drop of fixative, into little cubical pieces of preferably less than 1 mm in size. This dissection can be performed under the microscope to discard pieces of connective tissue or necrotic material. Such cleaning of the sample is possible while the tissue retains its natural colors in a drop of glutaraldehyde but is no longer feasible after it turns black on contact with osmium tetroxide solutions. This can save a considerable amount of time and effort,