BIOLOGICAL ULTRASTRUCTURE

Biological Ultrastructure

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PREFACE

Interest in problems concerning the ultrastructure of biological systems has increased enormously during recent years. The field of biological ultrastructure and the molecular organization of living systems provides common meeting ground for comparing the results from a variety of research fields such as histology, pathology, biochemistry, and biophysics as well as physiology and pharmacology. Although much valuable information on the ultrastructure of biological systems has been collected by classical methods such as polarized light microscopy, especially through the work of W. J. Schmidt, it is with the advent of the modern techniques of X-ray crystallography and electron microscopy that this field has rapidly expanded and produced significant results. For several years many laboratories, notably those of W. T. Astbury and F. O. Schmitt, have been engaged in research in this field and their pioneering contributions have served as a tremendous stimulus to ultrastructural research.

The general biologist nowadays is becoming interested in the precise molecular data given by the physical chemists. In particular, ideas put forward by L. Pauling and his associates have contributed to revolutionary changes in the concepts of the structure of the large molecules of biological interest.

In the classical monographs in this field, such as A. Frey-Wyssling's book on "Submicroscopic Morphology of Protoplasm," a wealth of data is presented from the cytologists' viewpoint. In our text we have attempted to describe biological ultrastructure by beginning with the simple building blocks, progressing continuously to larger molecules, and finally considering the ultrastructure of certain biological systems.

As may be imagined, the field of biological ultrastructure is very extensive and it is impossible to treat adequately the whole field in any introductory work such as this. Therefore the chief aim of our book is to introduce the field of biological ultrastructure to students and research workers in a variety of areas ranging from zoology, botany, and medicine to biochemistry and biophysics. In the text, references have been made only to key works which contain more complete pertinent bibliographies; where such comprehensive works are lacking, we have introduced the most significant recent communications. For the convenience of those who wish to delve further into the field, references containing additional information are listed at the end of each chapter.

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CHAPTER I

From Microscopic Morphology to Molecular Structure

The description of the structure of living material has been one of the main preoccupations of biologists, and the detail of description has followed closely the methods available for direct visualization of structure.

With the discovery of the single optical lens system came the transition from anatomy to histology and cytology, for it was with such simple optical means that Robert Hooke was first able to distinguish and describe cell structures. In the wake of the refinements in methods of optical microscopy flowed a swelling stream of description of finer structure, and there was of course more and more to be described as the detail observed became progressively more minute. The flow of faithfully reported data on the microscopic appearance of living organisms continued steadily through the middle of the 19th century with periodic inspired bursts of correlation by such workers as Schleiden and Schwann who first recognized the fundamental role of the cell, and by Virchow, who extended these concepts to pathology, thus in effect founding cellular pathology.

Despite these general correlations, the great mass of descriptive data formed a predominantly static picture of what was clearly a dynamic structure, and the structures described remained largely unrelated at the functional level although grounds for considerable speculation were provided by detailed studies at successive stages of, for example, growth and differentiation. Furthermore, in order to give contrast to the fine structure that refined microscopic methods were capable of revealing, preparative procedures were devised which involved such treatments as dehydration, sectioning, and staining. In other words, morphology became a static picture of a modified structure. Fixation methods were invented to try to keep the gross structure to as near normal an appearance as possible, but the detail described concerned the much finer structures which could not be examined by other methods. Undoubtedly, artifacts were frequently produced and faithfully recorded by the microscopist as possible characteristic structural features. Thus, the classical morphologist has mapped out in minute detail the somewhat shrunken and distorted landscape of tissue preparations, distinguishing countless structures by shape, size, internal structure, external relationships, and response to staining procedures, and the subsequent task has become to relate this data to living material.

Histological and Cytological Approach

In order to appreciate the significance and the limitations of classical morphology one must consider the physical and chemical factors associated with the procedure of observation. The eye will detect variations in wavelength and intensity of visible light, and the ordinary microscope simply enlarges the image so that such variations are apparent at a high resolution. In studying biological systems by optical methods, a fundamental limitation is the very small variation in light absorption among tissue components. In fresh tissue this may be caused largely by the very high water content, but even when dried, the tissue components tend to show only small density variations. Thus, in the absence of any specific wavelength absorption to introduce color differences, the contrast observed in the ordinary light microscope is usually very low. One method of overcoming this limitation has been the application of staining techniques. This has sought to introduce contrast into the image through the varying affinity for dyes shown by different tissue components. This affinity for dyes may arise from chemical interactions, and recent refinements in cytological techniques have sought to develop such interactions to permit the quantitative estimation of chemical components. Physical adsorption of dyes may also contribute to the color variation, and where both physical and chemical factors are involved it is usually very difficult to separate the two. However, the purely morphological approach sought only to differentiate components and not necessarily to explore their chemical constitution, and any contrast introduced into the image was useful as indicating some kind of variation in structure.

The main drawback, from this purely morphological point of view, was that in order to introduce these stains and prepare the sample for microscopic investigation it was in most cases necessary to fix, dehydrate, and section the biological material. Through fixation it was hoped to limit the modification of morphological structure consequent upon removing the major component, namely water, and also the modifications which might result from the embedding and sectioning procedures. Numerous methods of fixation were invented, mainly along empirical lines, and the merit of the procedure was judged from the clarity of the picture finally obtained.

Much recent work has sought to establish a chemical basis for various fixation methods through a study of chemical reactions of the various compounds and ions incorporated in the fixative in relation to the types of chemical groups likely to be present in the system for which the particular method of fixation is effective. This is important from the point of view of cytochemistry, and also, as will be discussed later, for biological ultrastruc-

ture, but the data available are as yet too sparse to permit a detailed treatment of fixation methods as practiced in microscopic morphology on the basis of the chemical reactions involved.

In recent years, great emphasis has been laid on the value of the freeze-drying method of fixation for preserving microscopic structure. This is more of a physical method, concentrating on the control of the physical condition in the tissue during the removal of water. It has been found that many of the distortions produced in biological tissues during normal dehydrating result from the shifting of liquid phases through the structure, and it was early realized that many of these distortions could be avoided by immobilizing the water phase by freezing, and removing it by subliming off at low pressure. Experience has shown that in general the best results are obtained by rapid freezing and drying at very high vacuum below a critical low temperature, but the quality of the results is still found to vary appreciably according to the nature of the tissue. These fixation methods are intended to preserve the general morphological structure through the processes of embedding and sectioning and subsequent removal of the embedding medium. At this stage, the contrast in the specimen may be improved by introducing various kinds of organic dyes, metal salts, etc., which react with the morphological components to varying extents. Thus, the affinity of nucleic acids for basic dyes (e.g., methylene blue) facilitates the mapping of structures rich in both pentose and deoxypentose nucleic acids. The further differentiation between these two compounds can be achieved by the Feulgen-staining reaction which is specific for the sugar in the deoxyribose nucleic acid and hence has been used extensively in nuclear and cytogenetical studies.

Acid polysaccharides respond to metachromatic staining, but in a manner less specific than is the case with the Feulgen reaction. However, other auxiliary staining methods contribute to make the identification of polysaccharides a relatively reliable procedure. On the other hand, many commonly used staining procedures such as the hematoxylin-eosin and Ladewig staining methods show little specificity, although they are used extensively to add general contrast through their graded attachment to different cell structures. Such staining techniques have been applied to provide a great wealth of descriptive data on both normal and pathological tissues. Mention should also be made of the so-called impregnation methods commonly used to visualize fibrillar structures. In many such cases it has been found that the intensity of response to the impregnation procedures is governed largely by the physical state of subdivision within the morphological components. Thus, for example, the pathologists differentiate between collagen and precollagen on the basis of deposition of silver, although chemical

studies show that both are collagen, and electron microscope pictures have indicated that the main difference between the two systems is probably in the state of aggregation of the elementary fibrillae.

If these histological data are to be related more specifically to the underlying fundamental processes, then a still greater effort must be made to appreciate in detail the chemical basis of the procedures used, and also to relate these data to the living structure.

Remarkable advances in the study of living tissues have come from developments of techniques such as phase contrast and interference microscopy. The structures in the living tissue introduce phase changes into transmitted radiation, and as the eye is insensitive to phase differences these techniques have been developed to convert such phase variations to intensity variations which will be appreciated by the eye. This has helped to distinguish cellular details in living materials with a resolution comparable to that obtained by classical histological methods, and through the observation of living processes has helped to add a dynamic aspect to microscopic morphology. In numerous cases it has led to confirmation of suggestions derived from earlier histological studies. Undoubtedly the classical histological methods do clearly distinguish real differences in structure, but often through gross distortion, and the individual structures seen may bear little resemblance to the living material.

It was clearly shown by the early work of Abbe and Rayleigh that the resolution of an optical magnifying system is ultimately limited by the wavelength of radiation used. When the theoretical limit with visible radiation was practically achieved it was quite natural to explore the potentialities of shorter wavelength radiation. In the ultraviolet region practical considerations confined the useful wavelength range to above 2500 Å, but even this gave an extension of the limit of resolution by a factor of about two. An added advantage of the ultraviolet microscope was that some cellular components showed specific absorption in this region, thus facilitating a localization of certain materials in the living cells. Below 2000 Å the absorption of radiation by matter becomes very great, necessitating operating *in vacuo*. This limitation persists down through the short wave ultraviolet and soft X-rays, but in coming to the harder X-rays (shorter wavelength) the absorption is considerably reduced, and from this point of view X-rays should be adaptable for microscopy. However, great difficulty has been experienced in providing an optical system for controlling this radiation and effectively exploiting its possibilities for higher resolution, a resolution which could theoretically approach atomic dimensions. It had been observed that similar wavelengths were associated with electrons in motion, and that electrons, by virtue of their charge, could be

readily controlled in magnetic and electrostatic fields. These factors have been rapidly exploited in the development of the electron microscope over the past twenty years. The nature of the image formation with electrons is such that the whole optical path must be enclosed in high vacuum, which precludes the examination of living tissue, at least at high resolution. The resolution of the electron microscope is already approaching atomic dimensions, but from the biological point of view the preservation of structure at this level under the imposed conditions of preparation introduces its own limitations. Owing to the great focal depth of such instruments, high resolution can only be obtained in very thin specimens, and in general it can be said that the thickness of the compound sample should not much exceed the value of the desired linear resolution.

This morphological pathway has now led down to the level of visualization of molecules and their interactions, and it is here that the exact nature of living processes should be made clear in terms of sequences of chemical interactions occurring at specific locations in the morphological picture. This morphological approach to living processes is summarized in Table I.

Chemical Approach

The chemical analysis of biological systems has gone hand in hand with the morphological classification. The main task of early biochemistry was to determine the gross composition of organs and tissues. Analyses for such components as water, protein, lipid, carbohydrate, nucleic acid, and mineral

TABLE I

Dimension	Terminology	Example of structure	Methods of direct observation of single units
0.1 mm (100 μ) and larger	Anatomy	Organs	Eye and simple lenses
100 μ–10 μ	Histology	Tissues	Various types of light microscopy and X-ray microscopy
10 μ–0.2 μ (2000 Å)	Cytology	Cells, bacteria	
2000 Å–10 Å	Submicroscopic morphology (supramolecular structure)	Viruses, cell constituents	Electron microscopy
Below 10 Å	Molecular and atomic structure	Arrangements of atoms	Not directly observable

salt were early carried out on anatomically resolvable components. The most striking fact drawn from these studies was the high water content, water accounting for something of the order of three-quarters of the weight of living material. From this first chemical mapping of tissues, biochemical studies have developed along two main lines. One has sought to characterize chemically the progressively smaller morphological units resolved by the microscopic methods; this line has become the field of histochemistry and cytochemistry. The second has concentrated on the isolation and characterization of biologically important substances. This has led to detailed knowledge of the chemical components present in the biological system which must, ultimately, through their interactions, provide the activity of living material, although it must be emphasized that the interactions are intimately related to their biological environment. The activity of a particular chemical component which is isolated from a large bulk of tissue may be limited to a restricted morphological region; and, in order to focus more precisely the site of action of individual substances, the trend in recent research has been to work with smaller and smaller samples carefully dissected from the gross specimen and also to isolate individual morphologically distinguishable components by physical methods such as ultracentrifugation so that the nature of the substances eventually isolated and characterized may have a more specific functional significance.

These attempts to obtain homogeneous fractions of morphologically identifiable tissue and cell components for chemical studies lend more weight to pure histo- and cytochemical investigations where attempts are made to identify the chemical constituents of a single morphological feature. With the size of sample studied in histochemistry and cytochemistry, the standard methods of chemical analysis are often not applicable, and special analytical techniques have been developed based on staining reactions, microabsorption of radiant energy, autoradiography, microinterferometry, and other specialized forms of microscopy. An outstanding advantage of these methods is that it is possible to study extremely small volumes of material (see Table II), but in these small volumes it is usually necessary for the chemical components to be present in relatively high concentrations. Although the methods are capable of very fine differentiation, the accuracy with which individual chemical components can be quantitatively estimated is usually not comparable with that of the standard chemical analysis methods applied to the larger specimens. With the emphasis on the composition of a single histo- or cytological component, comparative studies on cells and tissues become possible, but when applying these methods to compare different samples one must bear in mind the individual variations and obtain a true representative value by examining a large number of components in each sample.

TABLE II

Linear dimension	Weight	Terminology	
1 cm	1 gram	Conventional biochemistry	
1 mm	1 mg 10^{-3} gram	Microchemistry	
100 μ	1 μg 10^{-6} gram	Histochemistry	Ultramicrochemistry
1 μ	1 $\mu\mu$g (or 1 picogram or 10^{-12} grams)	Cytochemistry	Ultramicrochemistry

The chemical studies thus indicate the nature and properties of the chemical components which are to be associated with the sites distinguished in the morphological picture of living material. However, in order to approach the ultimate site of action of the chemical components, the individual molecules must be organized within the unit defined by the histochemical methods, for the ultimate site of action is the molecule itself, and substances present only in traces according to chemical analysis are nevertheless of great importance at some more specifically located point.

Biological Ultrastructure

Assuming that we now have the chemical components allocated to morphological compartments, the aim of ultrastructural studies is to add the all important water component, and to devise molecular organizations which will account for the physiological properties associated with the structure. To do this it is necessary to start with the molecules themselves, and, considering their charge and space characteristics which can be derived from the great wealth of structural data available on the numerous compounds isolated from biological systems, to fit them into the general parameters derived from the tissues themselves in a manner which may provide the required functional significance.

In general, biological systems cannot be studied in detail by the direct methods of structural analysis, but some structural parameters can often be deduced through a modified approach, and a number of systems are available in which structural features are repeated in a pattern sufficiently regular and extensive to yield more detailed structural information and hence provide reliable data on types of organization which are actually used in biological systems. That we are able to deduce so much from the repetitive systems depends on the fact that here it is possible to apply the methods which have proved useful in crystal analysis. In general, it can be said that these methods give direct information on the dimensions and orientations of molecular components in the structures. In addition to the X-ray diffraction techniques by which it is possible to do complete structural analyses of the simpler inorganic and organic crystals and even of some of

the complex ones such as penicillin, several other techniques can be used to extract structural information from repeating structures. With the larger repeating units of biological systems, the electron microscope is often capable of a direct measurement of structural parameters. It is of course possible to resolve a single unit by this technique, but the identification of the unit is much more reliable, and the measurements much more accurate, when more than one unit can be considered. The orientation of structures can be studied through a number of techniques. The one first applied extensively to biological systems was the polarized optical method. The advent of the polarizing microscope marked the beginning of biological ultrastructure, for it was with its aid that the early German workers, notably Ballentine, were able to pick out the ordered structures, and with the later quantitative measurements, principally by W. J. Schmidt, to make the first detailed suggestions of the molecular organizations in tissues. Polarized light studies will or course detect relatively small amounts of structure such as a single ordered layer, and also they will show up organization of quite large structural units such as micelles, but again it is with the extensive repeating pattern that the most valuable results are obtained. The study of the absorption of various wavelengths of radiation has also yielded valuable information on the orientation of structural components. If the absorption of certain groups or bonds such as are active in infrared and ultraviolet absorption is studied with the specimens oriented at different angles with respect to the direction of the electric vector of polarized radiation, the absorption may vary according to the angle of orientation, and measurements of the dichroic ratio (the ratio of the absorptions measured with the specimen axis parallel to and perpendicular to the electric vector of the polarized radiation) may indicate the orientation of the particular bonds or groups responsible for the absorption. Information derived from any of these methods may be useful in solving the problems of structural organization. Again the dichroic ratio is only appreciable in extensively organized systems, and only occasionally are suitable systems encountered.

Although the repetitive structures may, in general, be the exceptions rather than the rule, such biological model systems can be found for organizations based on each of the major molecular constituents of living material. In most cases these structures are normal tissues in which the units have been repeated in a regular pattern to serve a particular function as in bone, muscle, tendon, and myelin sheath. However, it may be that structures are developed to different extents in different species of living material, and it is usually worth while seeking the ideal system in which to study a particular component. It is also worth while looking into pathological conditions where a particular component often accumulates to an extent that makes

it available for detailed studies which otherwise might not be possible. There is every reason to believe that the individual molecules behave similarly in the various kinds of systems, and structural principles established in the model systems are not likely to be changed fundamentally in the systems which are not available for direct study. The final requirement is to endow this molecular morphology with a dynamic aspect so that the function, and the meaning of renewal and replacement, can be fully understood. In considering this functional aspect, it is the significance of individual molecular organizations that is important, and manifestations of this same function are often to be found in many morphologically distinguishable components which should be discussed simultaneously if the function is to be treated adequately. It is with this purpose in mind, and also because it provides a much more orderly marshalling of the available experimental data on biological ultrastructure, that the material in this book is treated by starting from the individual molecular components and building up to the complex biological organizations in which they may play an important role, rather than taking the morphological picture and filling it up with molecules.

Further Reading

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