Ultrastructural Plant Cytology

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ULTRASTRUCTURAL PLANT CYTOLOGY

with an Introduction to Molecular Biology

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Foreword

This monograph on 'Ultrastructural Plant Cytology' is an extension of a part of the second edition of 'Submicroscopic Morphology of Protoplasm', edited by the senior author in 1953. At that time, electron microscopy was still in its infancy, whereas it has now developed into a decisive tool in cytological research, giving us an insight into the world of ultrastructures. The methods then in use for observing the fine structure of cells, such as ultra- and polarisation microscopy, have now receded into the background, and whereas formerly structures invisible under ordinary light microscopes had to be inferred by indirect means, today they may be represented directly. As a result, a large volume of data has been collected in the course of a few years, so that the whole field of ultrastructural morphology as a branch of general cytology can no longer be encompassed in a manageable textbook. For this reason, we have decided to restrict the description of the ultrastructures and the molecular biology aspects of cytology to the plant cells, commencing with a brief description of the general morphology of biogenic molecules. Despite this limitation, a full account of current methods on the new direction of research has had to be omitted; the basic principles will be found in the 1953 edition, which is still available.

We hope that this monograph of plant cytology brought up to date in the light of the present state of our knowledge will, besides being of assistance to biologists, physiologists, and biochemists, also reveal something of the close liaison existing between the spectacular achievements of organic chemistry and the classical realisations of microscopy to the more technically interested students of agronomy and forestry.

We wish to express our thanks to Dr. Elsa Häusermann for her help with the manuscript, to Ruth Rickenbacher for managing the bibliography, and to Mr. H. Eggmann, Dipl. Sc. Nat., for preparing the drawings.

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PART II

Ultrastructural Morphology

Ultrastructural cytology is based on molecular biology (Astbury, 1961). Its morphological branch encompasses the spatial prerequisites for the co- and interpenetration of the biochemical processes occurring in the cell, and thus provides the basis for cellular physiology. Just as human physiology was only able to develop to its present level after exhaustive clarification of the histological structure of the organs of the body, so cellular physiology will expand to an unprecedented extent once the cellular ultrastructures have been finally resolved.

The morphological sciences describe the spatial relationships of the structural elements. As shown in Table I they form a hierarchical system, since its components may be of very different dimensions. According to the nature of the units being studied, different instruments are required for their resolution. It will thus be seen that ultrastructural cytology lies between classical microscopic cytology and structural chemistry. While structural chemistry describes the interrelationships of certain radicals (methyl, hydroxyl, and aldehyde groups, for example) in organic molecules, and macromolecular chemistry the linking of such micromolecules to high-polymer macromolecules, ultrastructural morphology is concerned with the arrangements of these molecules forming particles, elementary fibrils, helices, or lamellae invisible under the light microscope, and with the associations of such structures in all kinds of tissues. In the chemistry of proteins, the order of succession of the micromolecular monomers along the high polymer molecular chain is designated as a primary structure, the shape of the chains as a secondary structure, the formation of globular or fibrillar particles as a tertiary structure, and the

TABLE I

MORPHOLOGY

Morphological hierarchy		Instruments of research	Scales	Order of magnitude
organs	organography	eye, magnifying glass	millimetre scale	> 0.1 mm
tissues	histology	light microscope	micron scale	> 1 μ
cells	cytology	immersion, phase contrast, and ultra-violet microscope	wavelengths of light	> 0.1 μ
ultrastructures	ultrastructural morphology	electron microscope	millimicron scale	< 100 Å
molecular structures	structural chemistry	X-rays	wavelengths of X-rays	> 1 Å

incorporation of such particles into associations as a quaternary structure (see p. 57). Whilst the primary and secondary arrangements can be taken as belonging to the sphere of structural chemistry, the tertiary and quaternary structures belong to the field of ultrastructures.

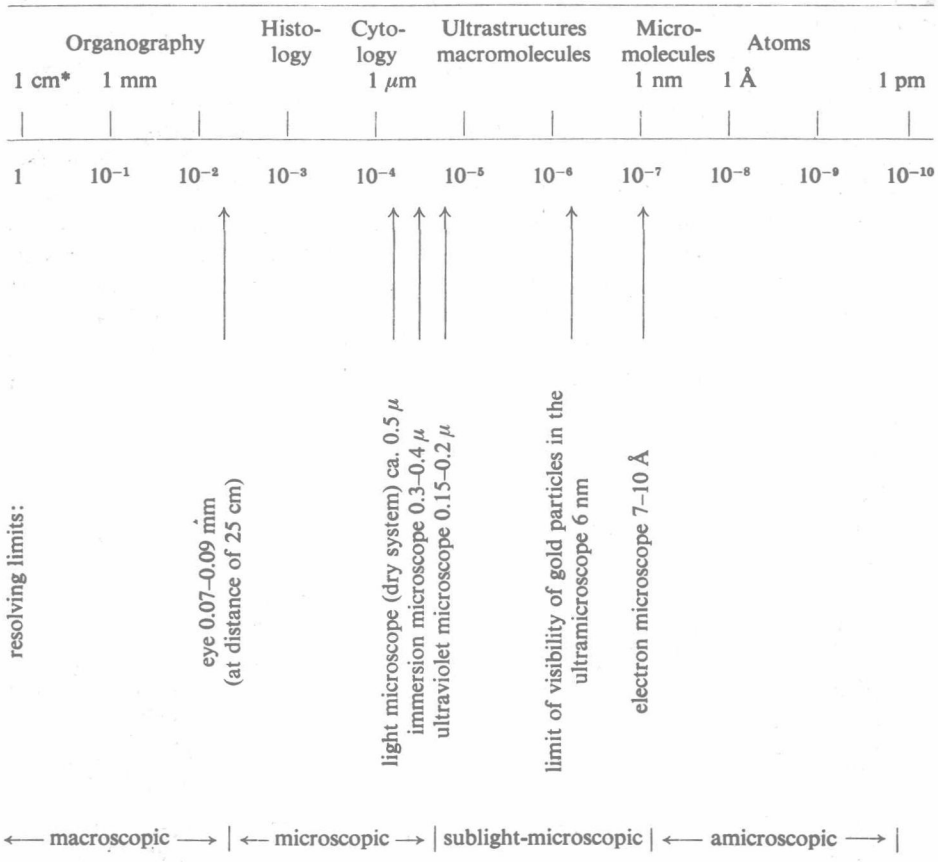
We can thus see how molecular biology joins the former 'inimical brothers' of histological morphology and physiological chemistry in fruitful cooperation. There was a time when the two sciences hardly understood each other: the biochemist spoke sarcastically of the histologist as a stamp collector because his purpose was to prepare and assemble flawless mounts, whilst the histologist regarded the biochemist as a poor biologist making a homogenate of all the various cell constituents and drawing whimsical conclusions from the analysis of this awful mixture. Morphology now knows that, in the last resort, its structural units are macromolecules, and the biochemist is aware that the cycles discovered by him from numerous reaction equilibria can function only when the enzymes are placed in series of well-ordered structures. Thus the old animosity between the morphological and chemical sciences is surmounted and forgotten by the discoveries in the field of ultrastructures.

The size of the structural units considered in morphology is distinguished according to their dimensions as macroscopic, microscopic, sublight-microscopic and amicroscopic. The limitation of these domains is provided by the resolving power of the eye (about 0.09 mm), of the light microscope (about 0.3 μ) and of the electron microscope (about 10 Å). If the measurements are expressed in nm, a proportion of 90 000:300:1 is obtained, showing that light microscopy contributed a threehundred-fold extension of what was observable with the naked eye, and that a similar extension was again achieved with the discovery of the electron microscope.

The measurements of length normally used in morphology are given logarithmically in Table II. As presented here, the microscopic and sublight-microscopic (ultrastructural) fields are equally large. It is recognised that organographical objects are macroscopic and that both histological and cytological structural units are microscopic. Ultrastructures and macromolecules are invisible under the light microscope and are consequently sublight-microscopic, but they are capable of resolution and portrayal under the electron microscope. Micromolecules (molecular weight < 500) and atoms cannot be seen at present even with the electron microscope. They are amicroscopic, but, unlike the case of the light microscope, the ultimate limits of resolution for the electron microscope have not as yet been reached. In 1940 the limit was situated at about 50 Å, and has since been improved to less than 10 Å. As a result, the ultrastructural domain has been considerably extended beyond the region of the smallest colloidal particles, which are what the gold particles with a diameter of about 60 Å detected under the ultramicroscope by Zsigmondy (1925) are held to be. At the beginning of electron microscopy, the sublight-microscopic region was almost congruent with the region of the colloidal particles, whereas today hemicolloid particles can also be shown. Whilst, therefore, the upper limit of the ultrastructural field is fixed by the resolution limit of the light microscope, the lower limit is variable. For this reason, and because ultrastructures have become portrayable under the electron microscope, the original term 'submicroscopic' morphology has been criticised and replaced by the new

TABLE II

THE DOMAINS OF MORPHOLOGY



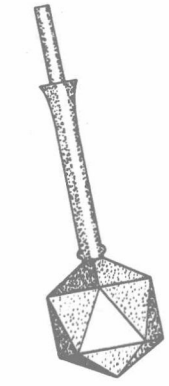
* In recent times, by international convention, the scale of lengths is no longer based on the centimetre (cm) but on the metre (m). 10^{-3} m = 1 millimetre (mm), 10^{-6} m = 1 micrometre (μm; abbrev. micron, μ), 10^{-9} m = 1 nanometre (nm; formerly millimicron mμ) and 10^{-12} m = 1 picometre (pm). The Ångström unit (Å) = 10^{-8} cm or 10^{-10} m, which was introduced by crystallographers and is generally used in electron microscopy, has now been dropped. Since ultrastructure research has not as yet endorsed this innovation, in the present book we thought it best to adhere to the traditional scale.

term 'ultrastructural' morphology. However, this term is not necessarily an improvement, because it is taken from the terminology of colloidal chemistry (e.g. ultracentrifuge, ultrafiltration, ultramicroscope) which uses the prefix 'ultra' to designate particle sizes which are beyond the resolving power of the light microscope. As a matter of fact the term 'submicroscopic' meant much the same thing. The objections of the electron microscopist can be met by defining the term more precisely by the use of the word 'sublight-microscopic'.

It is of great importance that we should start with a correct idea of the dimensions in the ultrastructural domain. The electron microscope, capable of

more than 100,000-fold magnification, is nowadays able of producing images that frequently leave us unaware of the excessively small units observed, or of how far we have already advanced into the realm of molecular dimensions. The transition, therefore, from objects visible under the light microscope to ultrastructural objects is presented in Table III in a sliding scale of particle sizes. On this scale, however, only particles up to the size of the T2 coli phages can be given; red blood cells, which are a hundred times larger, would show a diameter of 3.75 m.

In earlier times, the invisible sublight-microscopic particles were characterised by their particle weight as determined in the ultracentrifuge, and these data are given under the heading of 'molecular weight' in Table III. It is known that the so-called large viruses possess molecular weights of more than 2 billion, with particle diameters (210–320 nm) which are larger than the resolution capacity of the ultraviolet microscope (150 nm). The small viruses with particle weights of the order of 10 million and diameters of about 10–20 nm are no larger than the largest globular protein and carbohydrate macromolecules. Both viruses and haemocyanin



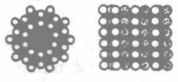


TABLE III

PARTICLE SIZES

Object	Molecular weight	Diameter in nm	Authors
1. Red blood cell		7500	
2. *Escherichia coli*		3000 × 6000	
3. Rickettsia		300	
4. Smallpox virus	$> 2 \times 10^9$	230 — 320	Peters (1960)
5. Coli-phage T2	210×10^6	65 × 95	Taylor, Epstein and Lauffer (1955) Kellenberger (1961)
6. Coli-phage T7	38×10^6	59 — 65	Davison and Freifelder (1962)
7. Tomato bushy stunt virus	8.9×10^6	30	Hersh and Schachmann (1958)
8. Polio virus	6.8×10^6	28	Schaffer and Schwerdt (1959)
9. Haemocyanin (*Helix pomatia*)	6.6×10^6	30 × 33	Van Bruggen *et al.* (1962)
10. Ribosomes (50 S)	1.85×10^6	14 × 16	Tissière and Watson (1958) Huxley and Zubay (1960)
11. Smallest ultramicroscopically visible gold particle	2.7×10^9	6	Zsigmondy (1925)
12. Haemoglobin	67×10^3	5.5 × 6.4 × 5.0	Perutz *et al.* (1960)
13. Myoglobin	17×10^3	4.3 × 3.5 × 2.3	Kendrew *et al.* (1958)
14. Saccharose	342	0.5 × 1.0	
15. Hydrogen molecule	2	0.2	

or haemoglobin are composed of sub-units (see p. 72), from which arises a further analogy of these objects. The protein macromolecules referred to are sometimes larger and sometimes smaller than the smallest colloidal gold particles seen under Zsigmondy's ultramicroscope. Cane sugar and hydrogen are given as examples of amicroscopic micromolecules; they are so small that they cannot be indicated on the scale we have used.

The smaller the particles become at this level, the less we are permitted to think of their internal morphology as something fixed, for the sub-units, radicals, atomic nuclei, and electrons of which they are composed are constantly in a state of oscillation and rotation. The same thing applies to the structural units themselves, when they are associated in larger groups. The molecular fine-structure of the cell organelles which is considered in this book should not therefore be taken as inflexible; we must be aware that ultrastructures, due to metabolism and growth, are in a state of constant rearrangement.

Growth includes the problem of the ontogenetic development of the cell organelles. It is a great advance that cytological ontogenesis can today be followed under the electron microscope, so that ultrastructural research has been brought from the realm of biophysical science back to pure biology. Whilst previously the only method available for the understanding of an ultrastructure consisted of a static description, starting from its macromolecular building blocks through their associations and ultrastructural arrangements up to the structure visible in the light microscope, today the main task is the dynamic elucidation of their origin from micromolecules engaged in metabolic processes. For this reason a knowledge of molecular morphology must precede the undertaking of this task, and therefore, in the first part of this monograph, the forms of macromolecules and their interactions will be described. This is followed by a detailed description of investigations on the fine structure and development of the cell organelles, as a contribution of cytology to the modern science of molecular biology.

PART I

Molecular Morphology

A. Principles of Molecular Structure

Study of the positions of atoms within molecules is the main object of structural chemistry, which in this respect appears as a morphological science. For example, the familiar tetravalent representation of the carbon atom, or the hexagonal representation of benzene, are morphological illustrations (Fig. A–1). The exact spatial orientation of the bonds and the interatomic distances remained unknown for a long time, and the directions and lengths of valencies were represented in a rather arbitrary manner (see Fig. B–2b). At the present time a large volume of data required for an exact morphological representation has become available, and at least the simpler chemical formulae can be drawn to represent actual three-dimensional molecular models projected on the plane of the paper. The exact knowledge of distances and directions is largely due to X-ray analysis, which allows the measurement of distances of the order of an X-ray wavelength (e.g. 1.54 Å for copper radiation), provided that the distances in question are repeated systematically and behave as a lattice. Such lattices cause interference of the incident X-radiation, and give rise to macroscopic effects which can be recorded photographically. It is therefore this principle of repetition which enables us to explore the morphology of molecular structure; the more regularly the given distances are arranged, the greater the accuracy with which the absolute lengths and directions can be determined. This means that X-rays cannot help us in the study of the morphology of molecules in liquids and gases, although the solutions of certain very large molecules, whose construction itself shows a certain periodicity (e.g. carbon chains), constitute an exception. In such cases the measurements are however associated with a certain degree of uncertainty, since the molecules are not oriented in fixed directions. The most reliable values of interatomic distances, frequently reaching an almost unbelievable precision (up to 0.01 Å), have therefore been determined in crystal lattices.

The usefulness of X-ray analysis is unfortunately rather limited in cytology. Although we must attribute a certain structure to the protoplasm, this is not governed by the principle of repetition to an extent sufficient for X-ray study. Periodicity does play an important part in all living matter, but more with respect to time than to the arrangement in space. A rigidly periodic order in space would presuppose an equilibrium of forces, whilst life is based on movement and on the maintenance of non-equilibria. However, as soon as a chemical substance is withdrawn from the metabolic process, the ordering forces can intervene and form

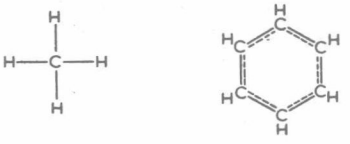


Fig. A–1. Projection of simple molecules on to a plane.