STRUCTURE and ULTRASTRUCTURE of MICROORGANISMS

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Structure and Ultrastructure of Microorganisms

AN INTRODUCTION TO A
COMPARATIVE SUBSTRUCTURAL
ANATOMY OF CELLULAR ORGANIZATION

by

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Acknowledgments

πάντ ῥεῖ—all is flux—(Heraclitus). Since I first started on this book a flood of publications on the subject has filled the pages of the leading journals, and there seems to be little hope of keeping pace with the ever-increasing volume of new material.

However, this book is not an encyclopedia of work in this field. Only those works which determine the direction of flow are quoted. At one stage complete reconstruction became necessary due to rapid developments in the application of the electron microscope to the study of submicroscopical organization. My thanks are due to the publishers for their patience and co-operation; to Mrs J. Brimley for her expert secretarial help in preparing the manuscrips; to Miss J. M. Allen B.Sc., to whom I am most grateful for help in the reorganization of the book, and to Dr Ewen, the Chief Medical Officer of the East Anglian Hospital Board, and members of his Board, for their continued support of my researches on the structure of bacteria. I would like to acknowledge the technical assistance given by Mrs Crawford and Miss Wettsteni and the help I received from my colleagues at the Strangeways and Cavendish laboratories, and thank all those who read through the sections referring to their own work: Dr J. R. G. Bradfield, Dr D. H. Northcote, Dr M. A. Rudzinska, Miss A. M. Glauert and Dr D. A. Hopwood. My thanks are also due to those who responded so willingly to my request for their original photographs which has made it possible for every illustration to be made from an original print.

Introduction

Progress in the biological branches of science when plotted against time is not represented by a straight and continuous line. The history of science produces ample evidence of the fact that there are sudden rises of the curves, followed by plateaux when no major discoveries are made for some time. The peaks coincide with discoveries made in other branches of science which have provided the biologist with new tools of observation. A good example of this phenomenon is the changing concept of the cell and its structural organization.

Galileo built his first microscope in the early years of the 17th century. In 1664 Robert Hooke produced his beautifully illustrated "Micrographia", including a chapter headed "Schematism and Texture of Plants"; in 1675 Malpighi published his "Anatome Plantarum", preceded by similar studies of the texture of animal tissues ("De Pulmonibus", 1661, and "De Viscerum Structura, 1666"). Nehemias Grew, in 1682, suggested to the Royal Society that a new branch of science should be created which should be called the "Anatomy of Plants".

The next phase began when Euler, who in 1796 published his treatise on dioptrics, gave the biologist a much improved tool of research which led to the construction of the modern compound microscope. In Robert Hooke's atlas the ultimate structural units of the plant tissues were described as "cellulae": compartments serving as containers of the life-maintaining juices. He estimated that one cubic inch of cork contained 1200 million cells. Schwann and Schleiden about 200 years later showed with the help of their new microscope that the cell was "the ultimate unit of structure and function in all live organisms—the primary agent of plant and animal tissues".

At the time that the "Cell Theory" was taking shape the new research tool was applied to the study of "Infusoria" (Ehrenberg, 1839) and Bacteria (Ferdinand Cohn, 1850). Ehrenberg produced his illustrated atlas of the microscopical structure of Protozoa, which is a classic. Ferdinand Cohn presented his first studies on Bacteria using, as he mentioned in his autobiography, one of the first commercial types of microscope manufactured in Vienna, and the only one of its kind used in German universities: an instrument as big as our present electron microscope and very tiresome to operate.

The invention and development of the electron microscope in the twentieth century created a situation which was very similar to that when the microscope was first introduced; the biologist was given a new optical tool of research extending the range of observable structures far beyond the limits imposed by the physics of optics. It is outside the province of this work to discuss the structure or principle of operation of the electron microscope. Leading textbooks should be consulted, cf. V. E. Cosslett's "Electron Microscopy", 1951). Nor is it the purpose of the electron microrecord all publications dealing with the application of the electron microscope to microbiological problems. Sufficient to say that while the invention of the microscope initiated a new branch of science—the Anatomy of Plants—we have today seen a whole conference devoted to Bacterial Anatomy, and microbiologists are becoming more and more interested in the submicroscopical organization of the bacterial cell.

This new line of research is often said to be concerned with ultrastructure or, better, substructure, i.e. structure observable on the submicroscopical level.

The term ultrastructure is somewhat misleading since beyond the ultrastructure is still another structure that is not seen with the eye; as Hippocrates said 2500 years ago, what cannot be seen with the naked eye can be seen with "the eyes of the mind"—meaning the use of indirect methods of observation. Beyond the observable microstructure and ultrastructure is the large field of the molecular structure of the biochemists and biophysicists. In the past, when the microscope was the only tool for observation, there was a wide gap between the two. One of the leading workers in this field recently said: "During the past six years molecular biology has changed from a subject of speculation and uncertainty to an exact science. . . . If the rapid development of molecular biology continues, gaps may gradually be filled and more and more of the fundamental workings of living systems may become understood in terms of the interaction between molecules and known structure." (Perutz, 1958).

The electron microscope has helped to bridge this gap. In fact, a border science is now developing where biochemists and physicists join the biologist, using the electron microscope as a tool for the exploration of this border territory between the observable and the non-observable structure.

But it is not only the morphological field in which the electron microscope today is leading research; we also speak of "biochemical anatomy" with the idea of establishing the "relationship between morphological units underlying biochemical machinery" (Pomerat, 1957). Dubos (1957) called for "more ingenuity and new techniques to recognise within the

live cell biochemical functions localised by certain sites". Stuart Mudd at the same time complained that "many biochemists do not believe in anatomy and many morphologists have no background of biochemistry to pursue profitably the question of biochemical functions in relationship to the sub-cellular structure". These warning words call to mind a similar situation of about one hundred years ago when Claude Bernard drew attention to the inseparable relationship between structure of organs and their function. He held up a peculiarly shaped pair of scissors and pointed out to his audience of students that they could never understand its "forme particulaire" without being told that this pair of scissors was constructed for the special purpose of opening up an access to the pituitary gland. Anatomists have succeeded in demonstrating the adaptation of structure and function as far as the mechanics of the skeleton are concerned; but it is quite a different matter to demonstrate the correlation of substructure and function in the organelles of the cell. However, whether practicable or not it must be the aim of the biologist and electron microscopist to establish this relationship whenever biochemical or enzymatic operations are "structure-bound".

Pantin (1951) and in his address to the International Conference of Zoology, London 1959, underlined the fact that in the living world different species solve the same functional problems in different ways: "the structure of the machinery by which these functions are brought about differs *and different structural solutions of the same basic functional problems are sought and tried out.*" The time has not yet come to present a comprehensive study of a comparative functional anatomy of subcellular organization; this book may serve as an introduction to such a study.

There is a tendency today among microbiologists to show that microorganisms "are as complete organisms as the cells of higher species" (Mudd, 1952). The electron microscope is used to search for bacterial nuclei, chromosomes, mitochondria, microsomes—i.e. for replicas of the organelles which are normal constituents of the "cell". However, in assessing the results of this search we shall see that there is hardly one organelle in the bacterial cell which is a structural replica of a similar functional unit in the cell of higher organisms or that of algae and protozoa. Bacteria are no longer just the smallest living organisms ("die kleinsten Lebewesen", F. Cohn); they are not miniature cells. They are, with regard to sub-structure, nearer to the viruses than to the "cells".

The aim of molecular biology in both viruses and bacteria is the resolution of structure at the molecular level. With existing techniques only the viruses show evidence of sub-unit organization which agree in some detail with results obtained by chemical and physical methods, as explained in the appendix on "Viral Macromolecular Structure", by

Horne and Waterson. The same technique should be applied (and is being applied) to similar problems in bacteria, i.e. the nucleoprotein "granule" (ribosomes) and more recently to bacterial flagella.

The electron microscope already shows the truth of the concepts developed by Aristotle in his "Metaphysics", and expressed by the Renaissance philosophers as "Physis est forma"; each biological structure has its substructure and each substructure has its sub-substructure, and so on, until matter has been replaced by form.

Frey-Wyssling, who was one of the first to become interested in submicroscopical structure, in paraphrasing the old saying of Virchow *"Omnis cellula e cellula"* or Fleming's *"Omnis nucleus e nucleo"*, found an adequate expression to characterize the new situation created by the introduction of the electron microscope: *"Omnis structura e structura."*

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SECTION II: ENZYME CARRYING STRUCTURES

SECTION III: THE CELL ENVELOPES

CHAPTER I

Ultrastructure of the Cell

I. Reconstruction of Classical Concepts

The introduction of electron microscopy as a method of research in biology has given birth to a new branch of cytology, often referred to as *submicroscopical anatomy* of the cell.

Anatomy is the "Science of Organization", i.e. of *structural* organization; the *functional* organization of the living body has to be destroyed, and its machinery dismantled so that each component part or structure can be studied separately.

In studying the submicroscopical anatomy of the cell in ultrathin sections of tissues or of single-cell organisms a similar dismembering technique has to be applied. The cell is also dissected: cut into thin slices of the order of 0.05 μ from which a three-dimensional model may be reconstructed.

It has been said "that basically the problems of tissue fine structure involve the identification and description of the microscopically visible structures within the cell" (Dempsey, 1956). The same structures are studied with two different methods of observation. Description of the structures seen in the electron micrographs of thin sections of the cell is only a preliminary to the more intricate and serious problem of identification.

In describing structures discovered by the use of a new optical tool for observation, pioneers in the field prefer to introduce their findings by names or terms of their own making—where this seems necessary—developing a language which the reader of electron micrographs can understand. Some concepts of classical cytology no longer fit the new findings. Therefore traditional names have been replaced by new descriptive terms, establishing in this way the identity of the newly discovered structures. Although, for example, the ergastoplasm of the cytologists and the endoplasmic reticulum of the electron microscopists refer in general terms to the same structure, the findings of the electron microscopists have led to a completely new concept and it is quite legitimate to introduce a new term for it. In a similar way the microsomes of the

cytologists supposed to be granular bodies at the limit of the resolution of the light microscope have lost their identity and here again new concepts replace old ones.

Anatomy is a descriptive science. It has been suggested for the sake of description of the structural organization of the cell to distinguish between "particulate bodies" and the "matrix" in which they are embedded. The cytoplasm of the cytologist becomes the "ground substance" of the electron microscopist.

"What is left over after removal of all particulate bodies from the cytoplasm is a homogeneous mass as seen in the electron microscope which we call the groundplasma. This matrix consists of a macromolecular mixture, and, in the manner in which its constituents are chemically and morphologically identified as definite macromolecular fractions, the 'groundplasm' will gradually wane into nothing unless it can be proved that it is characterized by an a-microscopical structure which then would have to be cleared up by indirect methods. *As a result of this discussion we find that the term cytoplasm is defined by its negative property of comprising that fraction of the living substance that escapes our morphological analysis*"! (Frey-Wyssling, 1957, p. 71).

The distinction between a groundplasma which shows no structure in the electron microscope and particulate bodies embedded in it, should be regarded only as a proposition. We shall follow this recipe in describing the submicroscopical anatomy of the cell at various levels of organization.

II. Survey Map of the Cell

a. In the metazoan cell, the structure of which will serve as a prototype of the subcellular organization, the main particulate bodies or structures as revealed in thin sections in the electron microscope are shown in Fig. 1. They are:

(i) the *nucleus* bounded by the nuclear envelope;
(ii) the *nucleolus*;
(iii) the *mitochondria*;
(iv) a structural pattern of vesicles and lamellae known as the *endoplasmic reticulum* with granules attached to it known as the *Palade granules*;
(v) the *Golgi apparatus*; and
(vi) various types of inclusions or products of cytoplasmic differentiation.

These particulate bodies are easily identifiable by reference to the microscopical findings; they are embedded in the cytoplasm which at its surface is bounded by a structure which appears in the electron micro-

graphs of thin sections as a membrane—the plasma membrane of the cytologist.

b. The protozoan cell shows a structural organization which can be recognized as a special form of the basic design, although, as each in-

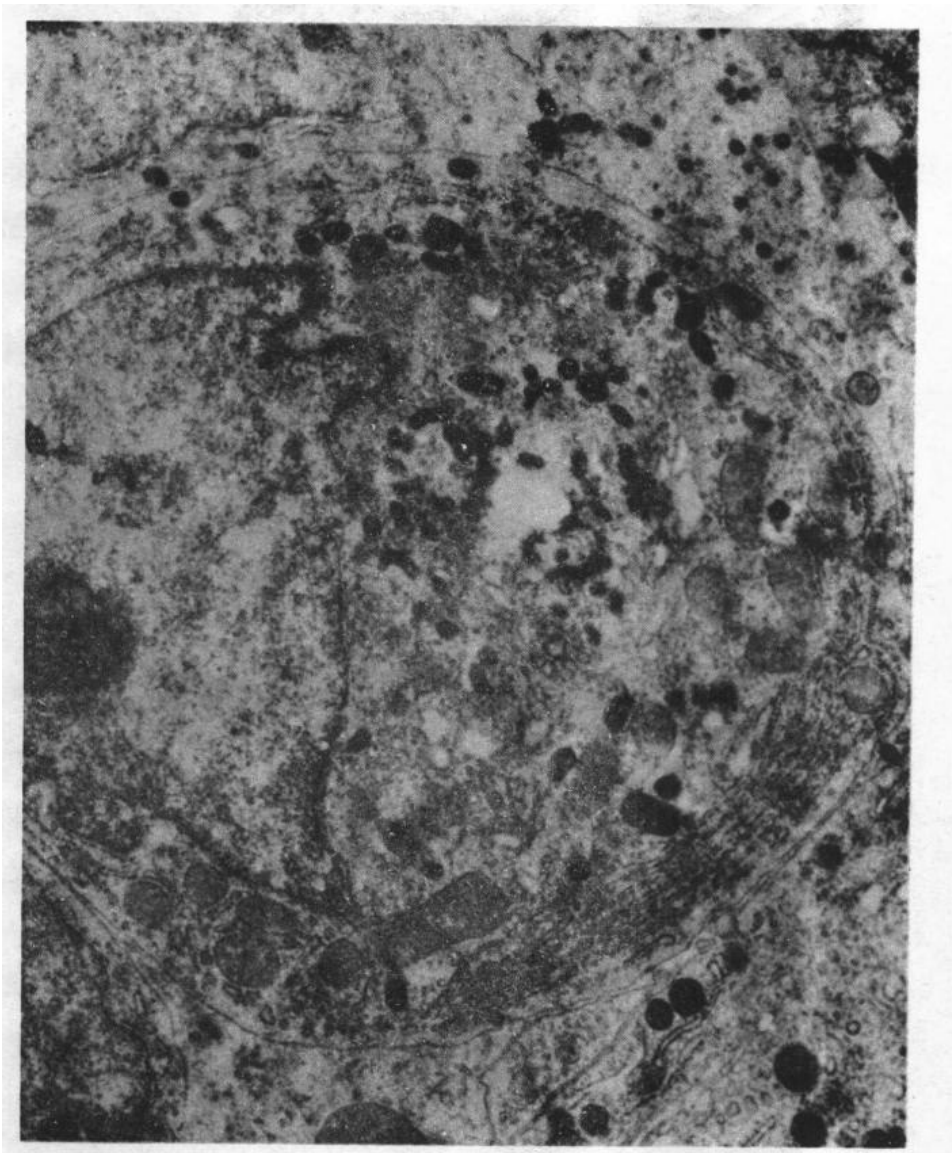

FIG. 1. Survey picture of cell of pituitary gland of the rat. For description see text. × 22,500. By courtesy of Mrs. B. Barnes, Cavendish Laboratory.

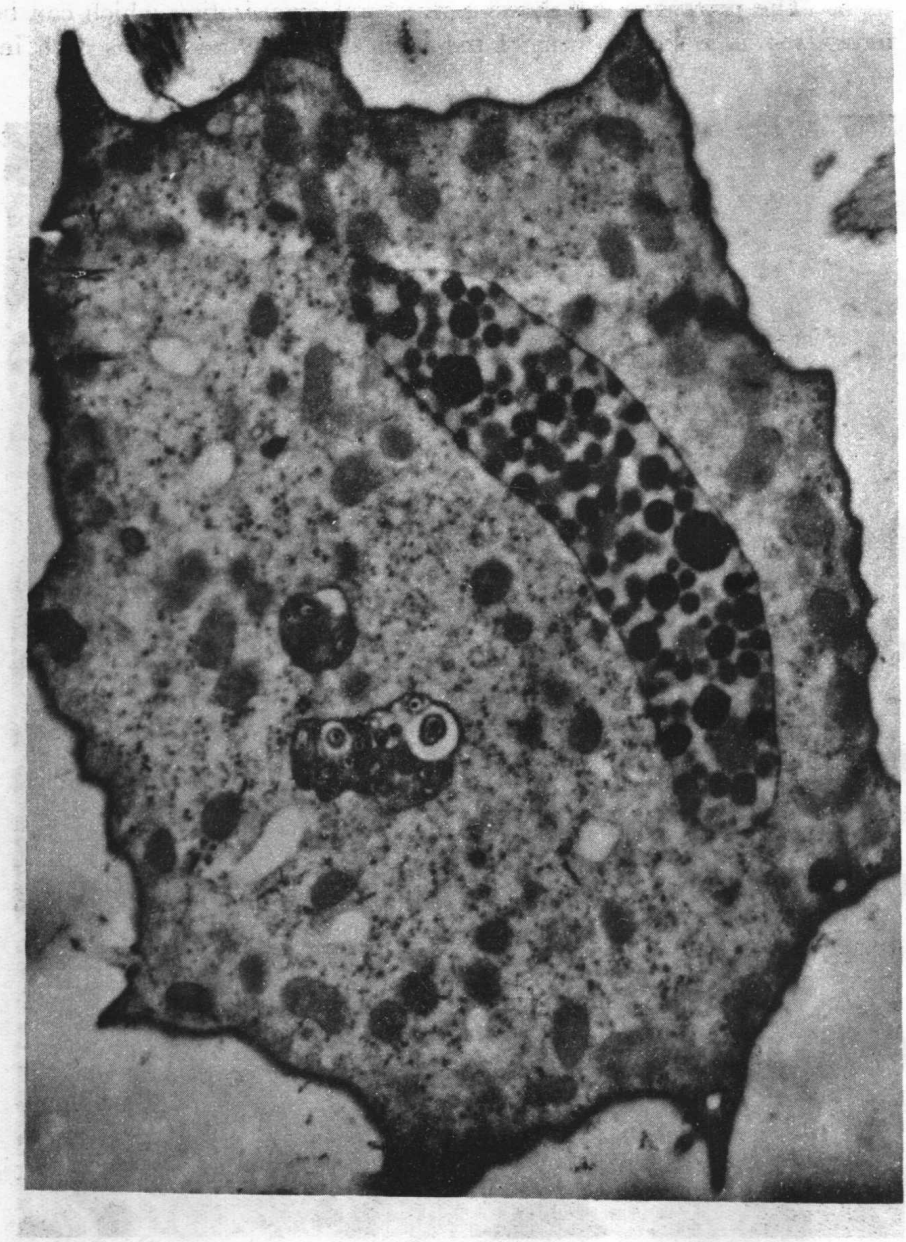

FIG. 2. Low power electron micrograph of the protozoon *Euplotes patella*. Note the large macronucleus with the nucleolar inclusions, and the granular structure of the cytoplasm. × 5,000. From Roth (1957).