Cytology and Cell Physiology

Edited by

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THIRD EDITION

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Preface to Third Edition

It is now 12 years since the second (postwar) edition of "Cytology and Physiology" was published. In this time there have been major developments in our knowledge of cell physiology and in the morphology of cells. The third edition has seen many changes in authorship, and the Editor welcomes the new band of distinguished contributors to the volume.

Many workers in this field, upon hearing that a new edition of the book was in preparation, made a special plea that much of the historical material be retained since "Cytology and Cell Physiology" was one of the few places where students could find this material. The detailed and patient work that went into the study of cytology for 100 years before the development of the electron microscope and differential centrifugation of tissue homogenates is often overlooked by investigators now concerned with new techniques; yet, without the assiduous work of hundreds of light-microscopy cytologists, much of the modern work would still be meaningless. Dr. Ross McArdle has written an historical chapter to serve as an introduction to the new volume, and the Editor's own chapter has retained most of the historical material found in the first and second editions. This was made possible by the coverage given to modern studies on mitochondria and the membranes of cells by Dr. Schneider and Dr. Kuff in Chapter 2 and Dr. Sjöstrand in Chapter 7.

The graduate students who found the first and second editions of "Cytology and Cell Physiology" helpful in their studies of the cell will, we hope, still find much to interest them in the third edition even though many are now distinguished faculty members of many universities. Also, we feel that present graduate students in biology, biochemistry, physiology, and anatomy will discover that the third edition is as helpful to them as the second was to their predecessors. Investigators in the field of pathology who used the second edition will find of special interest in the third edition the chapters on the pathology of the cell and the cytology of the cancer cell both written by Sir Roy Cameron and the chapter on viruses in cells by Dr. Kingsley Sanders.

The book also covers the recent advances in the new techniques of studying cells and includes descriptions of cell membranes, cytoplasmic constituents, nucleus and nucleocytoplasmic reactions.

It is perhaps invidious to refer to any specific chapters and it is not possible to refer to all of them in the preface, but the Editor has enjoyed reading them all and hopes that other readers of this volume benefit as much as he has.

GEOFFREY H. BOURNE

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January 12th, 1964

Preface to First Edition

The phase of purely morphological investigation of cells is now changing into a period in which the interpretation of structure in terms of chemical composition and function is the aim of many cytologists. This does not mean that we have learnt all that the morphologist has to tell us, for there are many problems of cell structure which he has yet to solve. But it means that the morphologist will need to work, not as before in a watertight compartment, nor even in a compartment which is covered with a semi-permeable membrane, but in one which will permit an intimate mixing of his knowledge with that of the physicist, the biochemist, and the physical chemist: for so complex are cellular organization and function that the brain of no one man can hope to envisage their manifold complications.

In this book an attempt has been made to bring together chemical, physicochemical, and morphological aspects of the study of cells. It has not been the aim to cover the whole field of cytology or of cell chemistry, indeed it would take a series of volumes to do so. The best that one can do is to choose a number of subjects which are representative of different fields of the study of cells and which relate as far as possible, one to the other, and to bring them together within a single cover.

The problems of producing such a book as this in war-time are not inconsiderable, and the editor wishes to thank the contributors, who are all scientists working in war-time Britain, for the way they overcame their many difficulties and the speed with which they produced their various chapters.

The whole book was written during the course of the 1940–1 air blitzkrieg on Britain, and there are probably no chapters of which part was not written within the sound of bursting bombs. One contributor, in fact, wrote almost his entire chapter by candle-light in an air-raid shelter during the worst bombing attacks on London. Another author wrote his while on sick leave from one of the fighting services, and a third produced his contribution chiefly in railway carriages while travelling from one urgent war duty to another. Most authors have written their chapters in what little time they had left over from war research. Others who have not been occupied directly in this way have had their time severely curtailed by other war duties such as acting as air-raid wardens, &c.

Incidental difficulties have harassed the authors in various ways. Those who were working in areas subject to frequent air attack had to contend with the closing of libraries while air raids were in progress, or with the evacuation of libraries to other parts of the country. The latter difficulty often resulted in long and tedious journeys to obtain essential periodicals.

G.H.B.

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OXFORD
December 1941

Preface to Second Edition

In the new edition of this book which is published some eight years after the first edition and during a period of peace, the same general form and all the existing chapters have been retained. All the chapters have been revised and rewritten with new material added, and in some cases substantial changes have been made. The importance of the pathological aspect of cytology has made it necessary for this particular chapter to be greatly enlarged. There are two new complete chapters, one on "Histogenesis in Tissue Culture" by Honor B. Fell, and one on aspects of evolutionary cytology by E. N. Willmer. Two portions of chapters are contributed by F. K. Sanders and R. Barer, who wrote on "Special Methods" of cytological investigation and microscopy. Dr. Sanders has also assisted in the revision of Chapter VIII.

I am indebted to the Ministry of Supply by whose kind permission the electron microscope photographs in Plates 3 and 4 illustrating Dr. Barer's article on microscopy are reproduced.

G. H. B.

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WHITECHAPEL, E. 1
December, 1951

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Introduction

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I. Historical Aspects of Cytology

Cytology in its widest sense extends into every branch of biology that is concerned with cell phenomena, including those of pathological states. It comprises not only structure and structural transformations of the cell, but also molecular biology and cell physiology. It is ever more apparent that the accuracy of modern cytology, whether by the intrepidity of electron microscopy or by the light of Leeuwenhoek's lantern, depends upon the prerequisite of an extensive knowledge of the cell by light microscopy.

The history of the problems of the individual cell as an organic unit of structure and function is of paramount importance to the student of this highly specialized field of cytology. Scientific knowledge does not come to us as a direct Promethean gift of the divinity. Too few scientists know the available literature of the past. Editors unfortunately discourage and usually prohibit historical accounts of a problem. Cytology is a science that demands extensive training in classical and modern cytology, physiological histology, cellular biology, biochemistry, and general physiology. The research student should possess a wide laboratory experience in the structural and functional characteristics of different cells, including the complex protozoa, in different physiological and pathological states, in order to secure a sound basic knowledge of the cell as it is revealed by the light microscope. Cellular pathology is rarely studied by the cytologist, yet the pathologist has contributed much fundamental knowledge to this field. Modern cytologists study chiefly the old problems with which biologists have dealt in classical cytology for nearly a century by light microscopy and physiological experiment. A richly rewarding experience awaits the cytologist who reads the remarkable history of the problem of the gene theory of heredity and the cytological basis of protein synthesis, a study begun in the 1860's by Miescher (pupil of the histologist His), who discovered *nuclein* (Miescher, 1871). This led Altmann (1889) to isolate nucleic acid, and Kunitz in 1940 to crystallize ribonuclease. Brachet (1941), and later Caspersson (1941), proposed that ribonucleic acid (RNA) serves as a template upon which proteins are formed. Since then, Palade (1955) made the very significant discovery by electron microscopy of the cytoplasmic ribosomes which are now known to serve an essential role in protein synthesis. Leblond and his associates (1957; Leblond and Amano, 1962) have shown clearly that the nucleolus is a site of formation of RNA which then migrates to the cytoplasm. It is interesting to note that early in the 1840's, Barry described the nucleolus as a center of synthesis. Nirenberg and Matthaei in 1961 and Nirenberg *et al.* (1962), in a brilliant study of *Escherichia coli* that produces a specific RNA, were the first to break the genetic code.

Nearly every historian divides the development of cytology into three great periods: viz., the nucleus and mitosis from 1875 to 1900; chromosomes and genetics from 1900 to 1925; and the cytoplasm from 1925 until 1940. However, another period of the development of electron microscopy and differential centrifugation from 1940 to the present may now be added. It is generally stated that the science of cytology first began in 1875 as a study of the nucleus that continued until 1900, when, upon the rediscovery of Mendel's description of his experiments on peas, cytology turned to genetics to prove the chromosome theory of heredity, and then in 1924 to a study of the cytoplasm, only to return to the nucleoproteins in the last twenty years. Such categorization of this basic field of research, although partly true, indeed, may be somewhat misleading. The fact is that cytologists have studied the anatomy and physiology of both cytoplasmic and nuclear components of the cell continuously since 1865 when Valette St. George (1865) discovered mitochondria which he called *cytomicrosomes,* and Oscar Hertwig (1875) demonstrated the fate of the spermatozoon within the egg. The entrance of the spermatozoon into the egg was first described by Newport (1854), who applied spermatic fluid on the point of a pin to the ripe egg of the frog. Fol (1879) gave the first explanation of the process of its penetration which he observed in the egg of the sea urchin. Cytology as a subject was first recognized perhaps a hundred years ago when these remarkable researches on fertilization and cleavage began. Achromatic lenses were discovered in 1820 to 1830, and precise methods of staining and fixation were developed from 1880 to 1900. Jacobson (1833) described chromic acid and its salts as preservatives. Sections were cut free-hand by a sharp blade. The first studies of the central nervous system began in 1850 when Clarke (1851) studied the spinal cord of mammals by free-hand sections. In the 1870's, cytologists began to use paraffin wax as support for a specimen during cutting of sections. Mayer (1880) melted the paraffin so that it would infiltrate the specimen. Two years later (1882), Threlfall (cf. Threlfall, 1930) discovered that consecutive sections could be fused at the edges to form a continuous ribbon. This led to the development of the first automatic microtome driven by a water motor. The early 1880's found cytologists in command of good technical methods for cutting and staining sections. At this same time, Ernst Abbé, with Schott, a glass manufacturer, had developed the first Jena glasses, by adding boron and phosphorus to the silicon base of the glass. Abbé (1886) produced the new *apochromatic* objectives with a limit of resolution that remained until the present time, nearly eighty years later. The electron microscope, which has been known for more than twenty-five years, has only recently begun to reveal information about the fine structure of the cell. The development of the ultramicrotome, which is capable of cutting extremely thin sections, was responsible for the advancement of electron microscopy in cytology.

The problems of modern cytology are pursued with the ingenious modifications of old methods and with remarkable new instruments, especially differential centrifugation, radioautography, fluorescence microscopy, interference microscopy, and electron microscopy. Cytologists have only recently analyzed the significance of the old Pappenheim (1901) pyronin-methyl green method of 1899 which colors deoxyribonucleic acid green, and ribonucleic acid red in the same cell. It is said that modern cytology, in contrast to the cellular morphology of the past, deals with the dynamic and quantitative aspects of the cell. Perhaps the fine structure of electron microscopic anatomy, with its physiological interpretations derived from enlargements of photographs of limited areas of cells fixed in osmic acid, may not qualify in the dynamic category of the brilliant physiological experiments of Driesch (1892), Hertwig, Boveri, Wilson, Bensley, and others of the past. However, the elegant modern cytological studies of such problems as the synthesis of proteins, the formation of RNA, isolated respiring cytoplasmic components such as mitochondria and Golgi apparatus, the rate of turnover of cells as demonstrated by the use of tritiated thymidine (Leblond *et al.*, 1957), and the studies of the continuity of protoplasm at the molecular level, certainly can be classified as dynamic and quantitative cytology. Modern cytologists like those of the nineteenth century consider the cell as a unit of structure and function, albeit an ever-changing cell under the influence of the medium that bathes it.

Electron microscopy at the macromolecular level has revived the cytomorphology of the nineteenth century, especially with respect to the cytoplasm, so that the bridge between biochemistry and cytology is at least passable. It has shown that the cytomorphologists of the past were correct in believing the organization of the cytoplasm to be structural. A major problem in electron microscopy today is the fact that few cytologists trained in light microscopy have entered the fields of electron microscopic anatomy and differential centrifugation, and it is imperative that more classical cytologists embark on these important areas of research. It is a bit surprising to find that some modern cytologists dealing with electron microscopic anatomy can be born overnight, prepared without benefit of basic classical cytology or physiological histology to publish observations on the fine structure and even the functional properties of a single cell as determined by still electron micrographs. It is as though the electron microscopist believed cytology had not existed before 1950, and as though every fine structure were a brand new copyrighted discovery. On the other hand, electron microscopy has given much to our knowledge of the fine structure of intracellular elements, already observed by light microscopy. The discoveries of the membrane systems (endoplasmic reticulum) of cells described so well by Fawcett (1961), of the ribosomes, of the internal structure of mitochondria, of the fine structure of the Golgi complex (Dalton, 1961), the fine structure of cylindrical centrioles that had been detected earlier by light microscopy in *Polychoerus* by Costello (1961), and of the structure of cilia represent perhaps the great contributions of classical cytologists in electron microscopy. It remains to be determined whether the typical cell of E. B. Wilson (1924), or the typical cell of electron microscopy, by different fixatives and different methods of observation, is the correct one. The existence and continuity of the centrioles from one generation to another as described by E. B. Wilson and A. P. Mathews (1895) and others are now firmly established. Costello showed that the curved rod-shaped centrioles are oriented at right angles to each other which determines the path of separation of the daughter centrioles.

The nature of the spiral mitochondrial filament of the spermatozoon and its role in fertilization and cleavage of the egg was never clearly understood. Valette St. George (1886) and Meves (1900) studied the confusing development of the helical spiral mitochondrial filament of the middle-piece of the spermatozoon. Benda (1903) believed that the male mitochondria might play a definite role in fertilization. Meves (1914) showed in the sea urchin that the middle-piece of the spermatozoon with its mitochondrial filament was clearly distinguishable in one blastomere of the 32-cell stage. In the fertilized ovum, Held (1916) found that the paternal mitochondria stained red, while the maternal ones were black. There is still no satisfactory evidence that mitochondria play any part in embryonic development except perhaps to facilitate the penetration of the head of the spermatozoon at fertilization. In some forms, the middle-piece does not even enter the ovum. The fine structure of the mitochondrial filament of the spermatozoon was not known until recently when the cytologists revealed it in the stimulating studies by electron microscopy.

Thus, cytology began with the early embryologists of the late nineteenth century who discovered that the apparatus of cell division in the tissue cells of the adult is the same as that of the ovum and blastomeres. Flemming proposed the term mitosis in 1882. Chromosomes had not been discovered and defined as hereditary bodies until they were observed independently by Oscar Hertwig in 1876, and by Strasburger in 1877. They stated that the nucleus carries the physical basis of heredity. Van Beneden's study in 1883 of *Ascaris megalocephala* demonstrated that the chromosomes of the offspring are derived by meiosis in equal numbers from the nuclei of the two conjugating germ cells, and hence equally from the two parents. The reader should read the excellent accounts by Rhoades (1961) and Mazia (1961). Cellular embryology was begun in 1844 by Kölliker when he described the cellular development of cephalopods from the blastomeres and from the corresponding chemically differentiated regions of the unsegmented egg. The pioneer studies of C. O. Whitman (1878) on the cell lineage of leaches was the sequel to Kölliker's brilliant analysis. Experimental embryology quickly followed in the classical investigations of Roux (1883), of Driesch in 1883 (1892), and the remarkable experiments of the Hertwigs in 1886 on the influence of chemical agents on the development of the sea urchin egg, which led Mead (1898) to the discovery that the blocked metaphase of the unfertilized *Chaetopterus* egg, normally completed only after fertilization, could be stimulated chemically to complete division by adding KCl to the sea water without fertilization. Jacques Loeb made the brilliant discovery in 1899 that the unfertilized egg of the sea urchin can be induced artificially to cleave by placing it in hypertonic sea water where it produces normal larvae by parthenogenesis. In this scholarly environment dominated by studies on the nucleus and chromosomes that pre-