GENERAL CYTOLOGY

De ROBERTIS

NOWINSKI

SAEZ

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SECOND EDITION

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Preface to the Second Edition

THE PROGRESS in some of the fields of Cytology represented in the first edition of this book (1948) has been so rapid and revolutionary that it has demanded a complete revision of its content.

Profound changes have been made in Chapters II, VI, and X on the physicochemistry of protoplasm, the plasma membrane, and cell enzymes, and a new section on cell metabolism has been included. Chapters VIII and IX have been completely revised with inclusion of two new sections on the action of radiations and chemical agents in cytogenetics.

About half of Chapter III now deals with the cytochemical organization of the cell. Chapters IV and V have been completely changed and a new section on plastids has been incorporated. Chapter VII, on the cytochemistry of the nucleus, has been entirely rewritten. New material, particularly in the realm of ultrastructure and cytochemistry, has been included in Chapters XI and XII. In order to correct to some extent the greater emphasis on the animal cell of the first edition, more material on plant cells has been added in several chapters.

In spite of all these changes, the original plan and the goals pursued in the former English and Spanish editions have been maintained. In our task we have been encouraged by the warm reception of this book both in the Spanish and English languages and by the fact that it is ready to appear in Italian and to be translated into Japanese and German.

We have been even more encouraged by the kind criticisms and suggestions received from colleagues all over the world. Particularly helpful for this new edition have been the suggestions of Professors Giuseppe Levi, Robert Chambers, Hans Holter, J. Holtfreter and A. W. Pollister to whom we are profoundly thankful. We want also to express our gratitude to Professors Keith Porter, A. W. Pollister, S. H. Bennett and O. Bessey, and to Drs. M. Alfert, L. Ornstein and S. Hughes-Schrader for the critical reading of different parts of the manuscript. To Professor Daniel Mazia, who read the entire manuscript and made many valuable criticisms and excellent suggestions, we extend our deep gratitude.

Although the first English edition was a translation from the Spanish by Professor Warren Andrew, this edition has been pre-

pared directly in English. In the different phases of the preparation of the manuscript and the compilation of the index we have been generously assisted by Dr. Magdalena Reissig, Mr. C. L. Solari, M. E. Drets, R. Ruibal, C. Lewontin, and Miss Leedia M. Greve.

Last but not least our thanks go to all the members of the W. B. Saunders Company for their painstaking attention to all details in the publication of this book.

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Preface to the First Edition

THE PRESENT English edition of our book is not a mere translation of the original work, published in Argentina in 1946, but a complete revision. We took this opportunity to bring the text up-to-date and to add thirty-four new illustrations. Many changes were made in Chapters II, IV and VI, and new material was included in Chapters V, VII, VIII XI and XII. However, the scope, plan and main material of the Spanish edition are kept intact here.

The book originally arose from the necessity for a synthesis in the Spanish language of the most important aspects of modern cytology.

In recent years this branch of biology has shown rapid progress and has been converted into the fundamental basis for study of the structure and function of living organisms in normal and pathological conditions. The cell can be regarded as the vital unit of organisms and the anatomical and physiological substrate of the biological phenomena. In its morphological aspect modern cytology has gone beyond simple description of structures visible to the light microscope, and by the application of new methods has begun analysis of the submicroscopic organization which deals with the architectural arrangement of the molecules and micelles composing living matter. In its functional aspect, modern cytology has transcended the stage of pure description of physiological changes, and seeks an explanation of these changes in the intimate physicochemical and metabolic processes of protoplasm. Finally, as a corollary, modern cytology, on the basis of the physicochemical changes of the nuclear structures, has tried to interpret and explain the phenomena of heredity, sex, variation, mutation and evolution of living organisms.

In the present book an attempt has been made to stress the morphological, physiological and genetic aspects of modern cytology. Chapter I presents an introduction to the problem of the organization of living matter and a short historical summary leading to the Cell Theory and the Protoplasm Theory. Chapter II gives the chemical and physicochemical foundations of the structure and function of the cell. Chapter III describes the general microscopic organization of the cell. Chapter IV considers the submicroscopic organization or ultrastructure of protoplasm. Chapter V deals with the structure, composition and functional significance of the cytoplasmic organoids. In Chapter VI the molecular structure of the plasma membrane and the phenomena of permeability are considered. Chapter VII describes the structure and chemical composition of the nucleus. Chapter VIII discusses the morphology and internal organization of the chromosomes and their behavior in the process of cell division; and Chapter IX correlates the chromosomes and genetic phenomena (cytogenetics).

In Chapter X the enzyme systems which take part in metabolism and respiration in the cell are described. In Chapter XI the visible manifestations of cellular activity, and in Chapter XII the phenomena of differentiation, senescence and death of the cell, are considered.

One of the most important factors stimulating the progress of cytology has been the development of new methods, in many cases derived from related sciences such as physics and biochemistry. Hence we have included brief descriptions of some of these modern techniques. The descriptions are not given as separate, isolated sections, but closely correlated with the results obtained and with considerations of the progress in the field of cytology which these techniques have made possible.

Since many of the theories seeking to interpret cytological phenomena are still in a state of discussion, we have sought to avoid them as far as possible, and to present the reader only with established facts. Nevertheless, we have tried to point out that cytology is in a state of constant change, and that many concepts remain yet to be developed and facts to be cleared before we can establish an integrated picture of the cell from morphological and dynamic points of view.

This book is intended primarily for students of medicine, agronomy, veterinary medicine, and the biological and other natural sciences, and for those persons who, for the purposes of teaching or investigation, wish to obtain a general view of some modern aspects of cytology. With this in mind, we have included a bibliography at the end of each chapter which contains works of reference and recent papers mentioned on it.

In connection with the present English edition we should like to express our gratitude to Drs. J. Biesele, H. Bunting, A. R. T. Denues, D. E. Green, M. A. Jakus, C. D. Leake, A. Ormsby, C. M. Pomerat, F. O. Schmitt, J. Scott and I. Sizer for reading various parts of the manuscript and for many suggestions. We want particularly to thank Prof. H. Stanley Bennett for his valuable suggestions and generous assistance in the preparation of all parts of the American edition. The critical comments and useful suggestions of these and other

colleagues have been of great benefit to the book. Last, but not least, our thanks are due to Dr. Warren Andrew for having accomplished the difficult task of translation, to Mr. Donald Macdonald for his help in compiling the Index, and to the publishers, W. B. Saunders and Co., for their cooperation and generous acceptance of all our wishes.

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I Introduction

THE CELL is a fundamental morphologic and physiologic unit in the structure of living beings, just as is the atom in chemical structure. In addition to the organization visible with the aid of the light microscope, the cell has a further organization which, passing through micelles and organic and inorganic molecules, leads to the protons, neutrons and electrons of the atom and constitutes the "ultrastructure" of the cell.

If by mechanical or other means the cellular organization is destroyed, the function is likewise altered and, although some of the vital properties may persist (for example, the activity of many cellular enzymes), the cell loses its significance as an organized unit and dies. This means that under these circumstances, autoregulation (Roux), the most important property of the living cells, has ceased to exist both in its morphologic or static sense and in its physiologic or dynamic sense.

This fact gives rise to a problem which during many centuries has been fundamental in biology: Are the vital processes of a purely mechanical nature, that is, may they be interpreted on a mechanical basis (*mechanicism*), using the word "mechanical" in the Kantian sense, or may they be considered as regulated by a special force existing in the organism? In the latter case, the structure would only be a frame by means of which the vital force is manifested (vitalism). Fortunately for modern biological investigations, the problem is more of a metaphysical nature. The only possibility to perceive and investigate vital phenomena is by means of physical and chemical methods. All the experimental attempts made to demonstrate the presence of a special agent, for example, the entelechy* (Driesch), have failed.

This failure as well as many characteristics of the living organism which are considered inexplicable in terms of physics and chemistry become understandable in terms of the theory of open systems as advanced by von Bertalanffy (1952). The inorganic sciences deal primarily with closed systems, in which no materials enter or leave and whose aim it is to reach a thermodynamic equilibrium. The organism, on the other hand, represents an open system, i.e., there is

* Entelechy, from the Greek *entelecheia*, means to have an end, a final cause. Driesch set forward the hypothesis of a principle which would regulate the unity of development and which, following Aristotle, he called "entelechy."

a continual input and output of matter and energy, but the system as a whole is in a steady state, i.e., it does not change.

The steady state explains many organic phenomena, which up to now seemed to be very difficult to understand in terms of mechanicism and vitalism. A closed system, for instance, does not need energy after an equilibrium is reached, but the open system needs a permanent supply of energy. Such a system, therefore, tends to reach an equilibrium but never achieves it. In physical closed systems, the final state depends upon the initial conditions. However, in open systems, the steady state which is achieved is independent of the initial conditions and determined only by the parameters of the system (for example, rate of reaction, rate of inflow and outflow, etc.). This corresponds to the equifinality found in biological phenomena. The fact, for instance, that a whole embryo develops from one blastomere is not, as Driesch thought, the action of entelechy, but a result of the tendency of the developing organism to reach a steady state; hence the regulations and compensations of the early damage finally produce a normal embryo. One of the very important characteristics of the open systems is the fact that they can be expressed in terms of physical and mathematical equations of which von Bertalanffy (1951) gives a series of examples.

The mechanicist theory, as well as the vitalist, resulted from the ancient tendency in biology to separate form from function. This, in part, may have been due to the outstanding influence of Aristotle, in whose Logic form is a quality but function is not. We believe today that form and function constitute an inseparable unity: the living organism. As a consequence of this belief, a new concept was introduced into biology, that of organicism or holism.

The doctrine of organicism is based on the premise that the whole is more than the sum of its parts. In other words, the separation of a complex structure into its constituent elements does not give us an exact idea of the functioning of the structure as a whole. If, for example, a watch is separated into its different parts, from the observation of these isolated parts one cannot deduce the function of the whole, nor understand that its movement is in relationship with the rotation of the earth about its axis. In spite of its manifestations, organicism, as the above quoted example demonstrates, does not imply in itself anything supernatural, and the psychologists who first developed these ideas (Gestalt theory, Köhler, Koffka and others) did so in accord with demonstrable facts.

The principle of holism is applicable to the inorganic world as well as to the biological. For example, glycogen has properties very different from those of the molecules of glucose composing it; sodium chloride is a salt having characteristics different from the two ions combined in it.

The past century was a period of great synthesis; in contrast we are now living in an analytical period of science. We analyze the biological processes, that is, we separate them into their elements and, in order to be able to create an image of the whole, we must carry out integration and synthesis in the mental realm.

This holistic principle which is applicable to the entire organism can be applied in a very special way to the interpretation of cellular function. The cell can be considered as an organism in itself, often very specialized and composed of many elements, the sum of which not only constitutes the cellular unit but has a particular significance in the organism as a whole.

The Greek philosophers and naturalists, particularly Aristotle and Theophrastus, arrived at the conclusion that "All animals and plants, however complicated, are constituted by few elements which are repeated in each one of them." They were referring to the macroscopic structure of the organism, such as roots, leaves and flowers common to different plants, or to the segments or organs which are repeated in the animal kingdom.

After many centuries, thanks to the invention of magnifying lenses, it was discovered that a whole world of microscopic dimensions exists beyond the macroscopic structure. The cell came then to be regarded as "the unit of living matter" (H. Spencer) or "the primary representative of life" (Claude Bernard). The single cell can constitute in itself the entire organism, as in the case of the Protozoa, or it can be grouped and differentiated into tissues and organs, as in the multicellular organisms.

The development of microscopic techniques made it possible to obtain a better knowledge of cellular structure, not only as it appears in the dead cell after fixation but also as seen in the living cell. Biochemical studies, on the other hand, demonstrated that the products of living matter, and even living matter itself, are composed of the same elements which make up the inorganic world. Biochemists were able to isolate from the complex mixture which composes the cell, not only fundamental substances, such as proteins, fats, glycogen, nucleic acid, and so forth, but also other specialized substances such as hormones, vitamins and enzymes. Many of these substances can be synthesized with the methods of organic chemistry.

The advance of knowledge concerning the composition of the cell, and in particular those advances which result from the application of modern physical methods, such as polarization optics, x-ray diffraction, the ultramicroscope and the electron microscope, will produce a fundamental change in the interpretation of cellular structures.

To these discoveries one must add the viruses, known until recent times only by the pathologic alterations they caused in cells and by their ability to pass through filters, but which in the last few years have been observed with the electron microscope. Viruses,