Introduction to DYNAMIC MORPHOLOGY

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Preface

Morphology, the study of visible structures of organisms, occupies places of variable importance in different fields of biology. The role of morphology is obvious in surgery, embryology, and taxonomy. It is less obvious in chemotherapy, genetics, and enzyme biochemistry. *Dynamic morphology* includes static structures as well as development and functional changes of structures.

Morphological procedures range from Roentgen-ray analysis to histological staining, from mechanical dissection to electron microscopy, from perfusion of an isolated heart to centrifugation of living amebas. In the face of ever-increasing specialization, this book offers (1) a survey of areas or problems in which morphological procedures are needed, and (2) a review of morphological procedures in their relation to various types of projects.

The material is organized in such a fashion that continuity is maintained between macroscopic, light microscopic, and electron microscopic levels of analysis. Interaction between morphological and biochemical approaches is stressed throughout. Pathological phenomena are classified from both the biological and the medicopathological points of view.

The book is intended primarily for pharmacologists, microbiologists, biochemists, chemists, physicists, and statisticians who need an understanding of morphology but who have had no training in this discipline. Therefore, detailed discussions are devoted to matters that most morphologists take for granted. The principles governing morphological studies are identical with those of natural science in general, but certain rules of procedures in morphology are stated explicitly. The term "introduction" in the title indicates that this book does not aim at completeness; it is not supposed to replace textbooks or laboratory manuals.

Readers trained in normal and pathological morphology may use this book as a guide for traveling unusual routes through familiar scenes. They also may enjoy occasional excursions into unfamiliar areas at the borders of dynamic morphology.

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Members of the Photographic Department of Lederle Laboratories cooperated effectively. All India-ink drawings were made by Mr. W. Hearn, either from my pencil sketches or from published originals. I appreciated the constant assistance of our librarians, in particular Mrs. J. M. Fantini and Miss G. A. Irby.

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Finally, I wish to thank the publishers and scientists who permitted reproduction of illustrations. Personal communications are acknowledged in the text.

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INTRODUCTION

Purpose of the Book

The systematic study of visible structures of organisms, briefly biological morphology, is part of the curricula for students of medicine, veterinary medicine, zoology, and botany. A number of useful textbooks and reference books are available for students of these sciences. The present book is not intended to compete with these books. It is meant for scientists who need an understanding of morphology, but who have had no satisfactory training in this discipline.

I have had the opportunity to collaborate over a period of forty years with physicists, chemists, biochemists, pharmacologists, and bacteriologists. This collaborative work was done in universities, in institutes for fundamental research, in hospitals, and, during the last eighteen years, in industrial research laboratories. In all these places peculiar difficulty was encountered when morphological information was needed by scientists experienced in other areas: they could rarely understand morphological publications or textbooks.

These difficulties proved to be more formidable on the microscopic than on the naked-eye level and appeared to be almost insurmountable when pathological conditions were involved. Before discussing the probable causes of this problem, I will give a few examples to illustrate how physicists and other nonmorphologists get involved in morphological matters. Physicists who are occupied with ionizing radiation find themselves faced with biological effects, such as leukemia and mutations, which are characterized by important morphological aspects. Bacteriologists recognize the necessity of analyzing morphological changes in infected experimental animals. Biochemists interested in the enzymes of the kidney have to determine the localization of enzymatic activity in the intricate structures there. Pharmacologists who study the distribution of drugs in the animal body are forced to consider the morphological as well as the chemical composition of the different organs. Why is it that these highly trained scientists are not able to use the existing textbooks in order to obtain morphological information?

The first complaint concerns terminology. Conventional morphological terms which seem to be satisfactory for communication among insiders, present puzzles to the outsider. Who can guess that the term cuboidal epithelium refers not to cubes but to hexagonal prisms in which the height does not exceed the width? Who can guess that the term fibroblasts refers in ordinary histology to fiber-forming cells, but in tissue culture language to a cytoplasmic network pattern with or without fibers? Who can guess that a malignant tumor characterized by an aggregation of oval nuclei and the absence of any recognizable pattern of cell bodies is called carcinoma when it occurs in the lung and sarcoma when it occurs in the leg?

The second complaint is that photographs and drawings of microscopic structures seem to show what they are supposed to show only to the eyes of the initiated. Besides uncertainty in identifying the structures, three-dimensional interpretation of two-dimensional sections seems to be particularly difficult for persons not trained in morphology.

The third complaint is that there is a lack of continuity between the presentations of normal and pathological morphology. Illustrations of pathological changes of microscopic structures are rarely accompanied by a picture of the normal condition. The M.D. is supposed to remember the normal condition, or to refresh his memory with a textbook of normal histology. This raises too many barriers, however, for a physicist who wants to make a reasonable effort to understand bone marrow injury by radiation.

One may advise students of any branch of natural science to acquire a sound training in mathematics, but I do not feel that every scientist should study normal and pathological morphology even though he may need this knowledge on special occasions.

In my association with other scientists, I have made numerous attempts to explain morphological points by using a minimum of technical language, by selecting exceptionally clear drawings and photographs on the microscopic level, and by making extensive use of diagrams including three-dimensional ones. This proved helpful in many cases. Yet there remained a mysterious veil which seemed to cover morphology. Finally it became clear to me what prevented my colleagues from understanding morphology. In modern science it seems to be obvious that *results should be presented as functions of procedures.* Physical, chemical, physiological, genetical, and bacteriological results are not given without stating the particular procedures by which particular results were obtained. This is true not only of research publications, but also of textbooks. Students and technicians are taught in this way. Morphology textbooks are the only exception: as in textbooks of history the emphasis is on results, and procedures or documentation may or may not be mentioned. What is missing in both the textbooks and the technical manuals of morphology are the *principles of morphological procedures,* or, in other words, *explicit statements of the rules of the morphological game.* This

generalization needs some supplementary comments. Morphological papers contain descriptions of material and methods. However, these publications take for granted a certain amount of morphological background which is presumably to be found in textbooks. Unfortunately, too much is taken for granted. The situation varies in different areas of morphology. Most laboratory manuals of macroscopic dissection serve their practical purpose in schools of human and veterinary medicine. Yet there is rarely a discussion of the differences between the appearance of organs in a fresh and a preserved cadaver, of the principles of dissection, of the different ways of dissecting a brain, of the role of injection techniques, or of the use of a magnifying glass or dissecting microscope. By and large, the modern branches of morphology such as histochemistry and electron microscopy have cultivated the habit of detailed presentation of procedures and careful derivation of results. In traditional morphology, the special textbooks of anatomy and histology of the nervous system show a laudable tendency to coordinate presentations of procedures and results. Many general textbooks of normal or pathological morphology contain paragraphs, or even whole chapters, in which results are properly backed by procedures. As an example I mention W. E. Le Gros Clark's book (1958), in which anatomical methods are given for the study of nervous tissues, lymphatic vessels, and postembryonic growth of bone (increase in size), but not for blood, bone marrow, skin, or other organs. I do not know of any textbook on morphology in which the relation between procedures and results is established as a principle and followed throughout. Similarly, most books on microscopic techniques give satisfactory descriptions of procedures, but do not include the principles of interpretation of morphological phenomena. The *third dimension* of biological structures presents special problems of technique and interpretation that are treated in various ways in existing textbooks. In the present book substantial space will be given to topographic and three-dimensional analysis on macro- and microscopic levels.

One of the most important tasks of the modern morphologist is the *dynamic interpretation of static pictures.* How does one know whether certain structural variations seen in fixed and stained sections represent a chronological sequence? It seems that the rules for handling such problems have never been published. They will be discussed in detail since time-associated changes of structures are of particular interest to pharmacologists, biochemists, and bacteriologists.

Special efforts will be made to *connect results with procedures* in the same way in which it is done in physics, chemistry, bacteriology, physiology, or pharmacology, and to present pathological conditions in close connection with normal ones. The fact has to be faced that the purpose and efficiency of morphological procedures are obscure to scientists other than morphologists. This uncertainty produces a peculiar paradox. In the mind of some scientists, morphology still enjoys the authority it had during the 19th century, whereas in the opinion of others the study of visible structures is superseded by modern biochemistry and

biophysics. Physiologists, biochemists, and pharmacologists may, at unpredictable occasions, place great confidence in the morphological verdicts of the pathologist, or they may adorn their publications with histological data and pictures because it is done traditionally. The present book will point out what to expect and what not to expect from morphological analysis.

Organization and Scope of the Book

Macroscopic and microscopic structures, normal and pathological, are the subject of this book. The relations between structural and functional aspects will be discussed in detail. However, the organization of the book is based on *procedures* rather than on structural or functional classifications. The term procedure is meant here to include technical manipulations as well as intellectual planning and interpretations. Seemingly unrelated biological items will frequently be tied together by a common procedure. Macroscopic and microscopic examples can illustrate the same principle, if no scaling problems are involved. The banding of birds and the vital marking of embryonic cells will be treated together as tagging procedures. Potential spaces will be discussed first on a macroscopic level, with the pleura cavity as an example, and then on the microscopic level of tissues and cells. The subject of axial polarity of biological structures will be introduced by the planes of orientation in the mammalian body and concluded by comments on polarity of cells. Under the common heading of "Natural and Artificial Units" the reader will find the isolated heart-lung-kidney preparation of a dog, and also the question of applicability of the cell concept to protozoa.

In contrast to tradition, the present book does not start with the cell, for a number of reasons. What is simpler, a cell or a dog? Claude Bernard (1866) stated that a highly organized animal offered simpler conditions for experimentation than so-called lower organisms did, since the latter seemed to perform the same basic functions—metabolism, motility, reproduction—without visible specialized structures. At the present time the tissue cell has become complicated for the opposite reason: electron microscopy and cytochemistry have revealed a wealth of functional structures within each cell, and new ones are being discovered at a high rate. To emphasize this change, Novikoff (1960) published an impressive set of illustrations: liver cells as presented by Rudolf Virchow in 1858; the generalized cell as conceived by Edmund B. Wilson in 1896; and a diagram of a rat liver cell based on recent cytochemical and electron microscopic information. The last picture not only is very involved, but some of its features are better established than others. Sjöstrand (1956) pointed out difficulties in judging the *average picture* of a cell because of the limited number of cells that can be examined under the electron microscope and because of the variability of structure in different functional stages which are not synchronized from one cell

to another. Moreover, cells of different tissues and species now show so many differences that one should be reluctant to start a textbook of histology with the description of "the generalized cell." In organizing the present book along procedural lines I was guided, to some extent, by Eugen Albrecht's (1907, p. 247) comment that Virchow's cellular pathology produced the side effect of "cellular myopia." Now there is also the danger of subcellular myopia. Probably the best prophylaxis is a balanced consideration of macro-, micro-, and submicroscopic levels. The importance of maintaining procedural continuity between different orders of magnitude will be stressed at many occasions. It is well to remember P. W. Bridgman's (1927, p. 51) statement that "the large may not always be analyzed into the smaller."

The present book is not a systematic treatise on morphology. No attempt will be made to give complete descriptions of all morphological structures of each system and organ. Neither is it within the scope of this book to discuss, or even mention, all the harmless and pathological variations which may occur in the different organs. The normal and pathological morphology of systems and organs is described with great completeness in the existing textbooks. Guided by the present book, scientists without morphological training should be able to utilize the material in these textbooks.

It is obvious that the choice of procedures should depend on the purpose and nature of each study. The selection of the best technique is not always simple, and an investigator may not know what techniques are available to tackle his problem. In the present book a large variety of procedures will be mentioned and their application will be illustrated by examples. Some procedures will be described briefly, others more elaborately. The reader will be introduced to the principles of histological staining by a detailed discussion of one of the simplest stains, hematoxylin. However, this description will hardly enable anyone to stain a section in a satisfactory way. The present book cannot replace laboratory experience and the use of technical manuals, but it should give an insight into the workshop of the morphologist.

The examples which illustrate procedures are taken mostly from the morphology of man and laboratory animals, but references to comparative morphology and physiology will be frequent. This means that not only vertebrates, but also invertebrates, protozoa, and plants may be discussed. Examples from bacteriology will be used extensively.

Embryological material is found in all parts of the book. Modern experimental embryology is particularly suitable for demonstrating the relation between morphological and physicochemical procedures. An understanding of visible structures and their variability is hardly possible without a study of their production during embryonic and postembryonic life. Most of the pharmacologists, bacteriologists, chemists, and physicists with whom I have been associated showed no desire to be informed on problems of experimental embryology. If static mor-

phology was hardly accessible to them for the reasons stated before, the mere idea of unstable, changing structures was certainly forbidding. Moreover, these scientists may have been so fascinated by genetics and the evolution of species that no enthusiasm was left for the problems encountered in the study of embryos. It is hoped that a greater interest in experimental embryology will be kindled by the present book, in which embryology is presented within the framework of physiology.

This book is devoted primarily to the study of visible structures of organisms, or biological morphology. Certain activities of the organism produce visible structures, and, in turn, visible structures are necessary for particular activities. The activities of organisms are called functions. As a rule, functions and visible structures are related in some way, but there is a great variety of relations, many of which will be discussed in the book. These relations will be compared to similar relations in man-made machines. The study of biological functions is known as physiology, and the study of visible structures in their relation to functions is known as *functional or dynamic morphology*. The first parts of this book deal with fundamental aspects of physiology in order to supply the background for the subsequent parts, which are devoted to functional morphology. Therefore the first parts will contain physiological items which seem remote from the study of visible structures. There will be analyses of muscular contraction and of the different phases of sunburn; there will be discussions of genetics and of immunology; and there will be dose-response curves. Attempts will be made to consider the various areas of physiology from the common view of stimulus, response, and reactivity. All this is necessary in order to place morphological procedures in their proper relation to the problems of physiology.

The same item may be discussed in different contexts. Thus, the contractility of the dog's spleen will be mentioned in several places of the book: illustrating procedures for observing internal organs during life; requiring special postmortem techniques; playing an important role in the distribution of blood in the body; and finally resembling other erectile organs. This type of repetition had to be faced as inherent to the plan of the present book. I was encouraged by a statement in Albert Einstein's 1918 semipopular presentation of the theory of Relativity: "For the sake of clarity, frequent repetitions were deemed necessary while no attention was given to elegance of presentation: I followed conscientiously the advice of the great theoretician L. Boltzmann, that elegance should be left to tailors and shoemakers" (translated by E. M.).

The last part of the book will be devoted to the classification and identification of biological structures under normal and pathological conditions. In classifying organs, tissues, and cells, not only structural and functional characteristics are used but also origin, potentialities, and immunological relationships. Pathological phenomena may be classified according to abnormal stimuli, such as bacterial infections, or according to abnormal responses, such as neoplasms (cancer).

Therefore, many items, which were treated in earlier chapters from the procedural point of view, will appear again in the discussion of alternative classifications.

Terminology

A term expresses a concept or a definition. It is hardly worth while to argue over a term if the concept is sound and the definition is clear. The rules applied in the present book can be characterized as follows.

(1) Clarity is given preference over traditional use, and plain English over unnecessary technical terms. The open space inside a tube will be called the bore, instead of the lumen, which is the customary term in biological morphology. In my experience, nonmorphologists expect the word lumen to mean unit of illumination. Certain cells which have the shape of hexagonal prisms will be called prismatic rather than cuboidal, although the latter term is generally used. At many occasions the self-explanatory descriptive terms will be supplemented with the conventional terminology.

(2) Controversial terms will be avoided, especially if they are not needed for the objectives of this book. The word *protoplasm* will not be used. Some authors apply the term protoplasm both to cell bodies and to nuclei; others exclude the nuclei and, therefore, consider cell body, cytoplasm, and protoplasm as synonymous. Moreover, the traditional definition of protoplasm as living substance cannot be easily applied to intercellular substances.

As a rule, the term *degeneration* will not be applied to microscopic structures. Microscopic structures should be interpreted either as living or dead, and, if living, it should be stated whether the structures are in good or poor condition; criteria of this appraisal should be given. The term degeneration is always properly specified with respect to the nervous tissue. For instance, "degeneration of a tract" in the spinal cord means the loss of myelin sheaths of the nerve fibers in that particular tract.

(3) Ill-defined general terms will be replaced by specific terms or by qualifications of the general term. Instead of using the term *growth,* reference will be made to increase in length, surface, volume, cell number, intercellular substances, wet weight, dry weight, total protein, nucleic acids, or other components. If the term growth is intended to summarize the effect of several factors, the factors will be listed, and the result will be called the balance sheet of growth. The term *norm* will be qualified by distinguishing the statistical from the teleological norm. The statistical norm refers to an arbitrary area on both sides of the mode of a frequency distribution curve. The teleological norm, synonymous with the evaluating, desirable, or idealistic norm, refers to the optimum of health. Besides these two useful concepts of norm there are also bizarre norms such as the textbook norm, which offers nonexisting or exceptional conditions as standards; and the

hospital norm, which interprets observations in hospital patients as being representative of the population.

(4) Terms that imply different criteria of classification will be explained. The term *epithelium* may refer to the visible pattern of a cell aggregate (mosaic pattern), or to the origin of mature cells from specific precursors (cytogenesis). According to a widespread but unsubstantiated opinion, the two criteria of epithelium are *always* correlated. To avoid this problem, the term epithelium will be replaced as follows: liver cord cells for liver epithelium; lining of thyroid follicles instead of thyroid epithelium; cells of renal tubules instead of kidney epithelium. *Problems of nomenclature are inseparable from problems of classification.* Organic chemists as well as botanists and zoologists will agree to this statement. In a handbook used for the registry of diseases, entitled "Standard Nomenclature of Diseases," the editors are constantly faced with problems of classification.

(5) Some conventional terms are not used in the same sense by all investigators. To be on the safe side, the terms reproducibility, precision, and accuracy will be defined operationally in the present book, in agreement with those authors who follow the same principle. Preference will be given to the term reactivity over its synonyms irritability, excitability, responsiveness, and susceptibility. Although the latter four terms are found more frequently in the literature, reactivity has the advantage of not being associated with special branches of biology (see Table 2).

In the present book organisms and man-made machines are compared on various occasions. Neither machines nor biological objects can be described without evaluating teleological concepts such as order and randomness, or fuel materials and waste products. The philosophical implications of teleology have been the subject of a number of treatises. I mention Immanuel Kant's "Kritik der Urteilskraft," which appeared in 1790, and Morton Beckner's book "The Biological Way of Thought," which appeared in 1959. These books represent *philosophies of biology.* I do not pretend to offer more than a *philosophy of morphological procedures* or, perhaps, a philosophy of biological procedures with special emphasis on morphological techniques.

Comments on the Literature Selected

It is hoped that the scientists for whom this book is written will benefit from it in two ways: they should be able to understand morphological matters, and also be able to judge the merits of morphological data and interpretations. References to textbooks of physiology, histology, and pathology are given at many occasions. Morphological and histochemical laboratory manuals are mentioned for those who wish to acquire familiarity with special procedures.