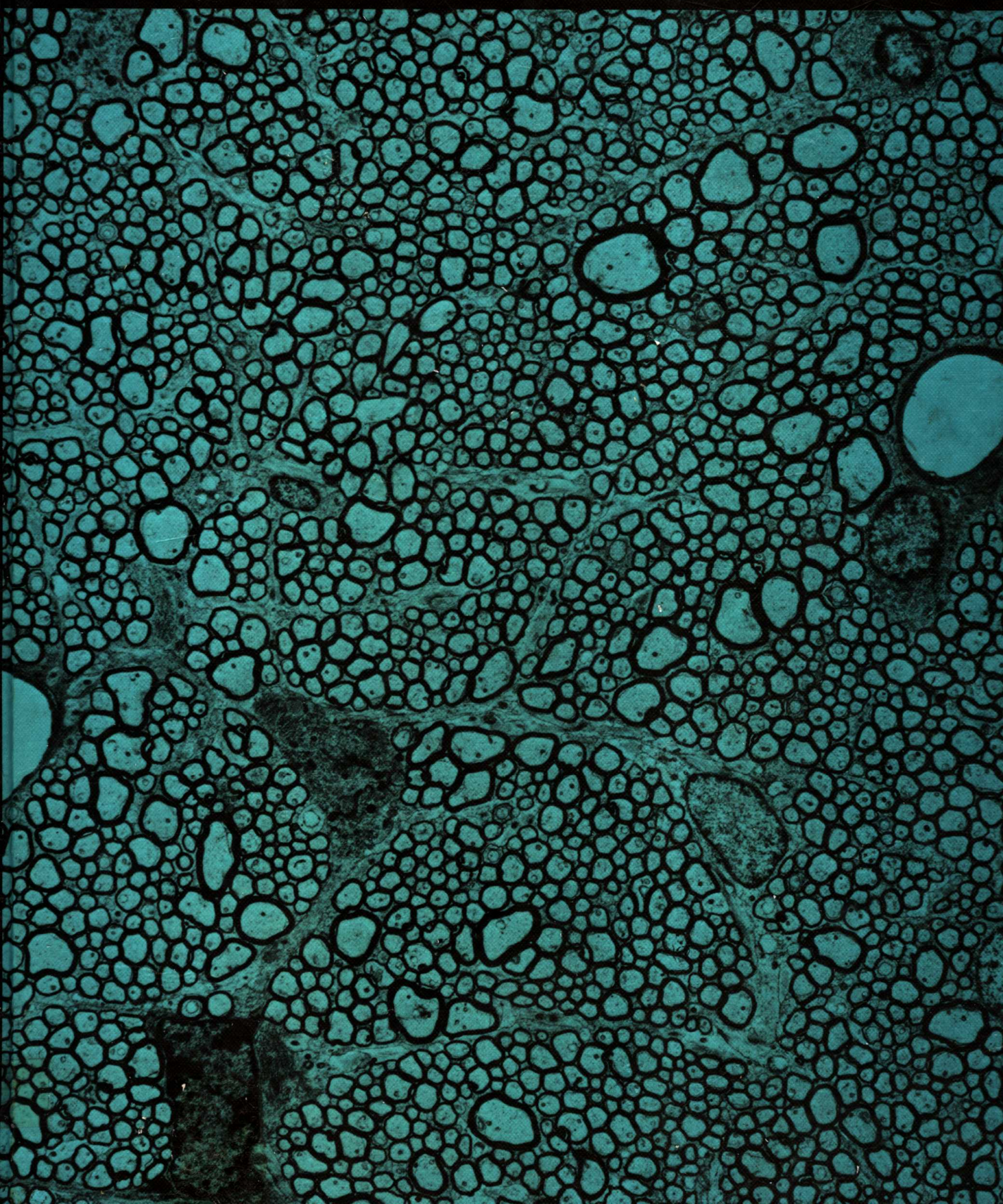
HISTOLOGY

A Text and Atlas

JOHANNES A. G. RHODIN



Histology

A TEXT AND ATLAS

Johannes A. G. Rhodin, M.D., Ph.D.

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COVER
Electron micrograph of numerous myelinated
nerve processes
in cross-sectioned optic
nerve of the rat. X 2650.

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Preface

This book has been created for college and medical students placed in an informal learning environment of a contemporary curriculum. The author does intend this volume to replace elaborate standard texts and atlases in the hope of assisting overburdened science students. The text is an up-to-date brief account of the structure, ultrastructure, and function of mammalian tissues, cells, and organelles. The photographs are carefully selected and organized to show sequential magnifications of the same field from light microscopy to low magnification electron microscopy as well as medium and high magnification electron microscopy. The numerous electron micrographs shown at magnifications ranging from 600× to 2000× are unique in their detailed and crisp outlining of organs, tissues, and cells. These illustrations will prove to be of particularly great value in helping the student bridge the difficult gap between light microscopy and high magnification electron microscopy.

The illustrations are found on the right hand page, the text and figure captions on the left. Thus, the book combines the concise and complete wording of a text with the descriptive legends and illustrations of an atlas, saving the student the time and effort involved in studying both a textbook and an atlas. Numerals are used to identify structures in the illustrations. The student will, therefore, easily find a specific item in the picture, and the micrographs can readily be used by the student and the teacher for testing purposes.

In the textual material, lengthy introductions were avoided on the assumption that at this level students have acquired some knowledge of the gross appearance and position of organs. Emphasis has been placed on present knowledge of structural architecture rather than on introductory historical background and accounts of the many special techniques utilized in arriving at a certain item of information. The author's experience in teaching has convinced him that histology should be presented in terms of the structural appearance of tissues as observed by both the light and the electron microscopes. Therefore, the text combines the light and electron microscope descriptions of organs, tissues, and cells.

The collection of illustrations is prepared by the author especially for this book. It includes analyses of many organs which have not previously been described adequately in the literature. The chapters on human blood cells and hemopoiesis represent the first comprehensive account of this

topic, and the thorough analyses of the ultrastructure of the spleen, lymphopoiesis, and blood vessels present many new functional aspects of these organs and their components. The chapter on the cell and cell organelles summarizes current knowledge in this field, and establishes a firm base for extended studies in cytochemistry, cell biology, and physiology. The light microscope preparations are almost exclusively taken from human or monkey tissues. The electron microscope preparations are taken for the most part from rats and cats. Human tissues were used in instances in which they differ considerably from those of lower mammals.

To achieve superior resolution numerous illustrations are taken from electron micrographs even though a similar magnification could have been achieved by the light microscope. The panoramic low magnification electron micrographs are intended to replace many of the drawings and composite diagrams of standard histology textbooks. The author does not believe that teaching histology should aim primarily at the "reading" of routine slides. Instead, it should enable the student to form some concrete visualization of structures and locations involved in the life processes. The cytologically informative and clearly delineated preparations reproduced in this book should provide the student with correspondingly clear concepts of biological structure.

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November 1973

Johannes A. G. Rhodin

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The cooperation and enthusiasm of Mr. William C. Halpin, Vice-President, and of his staff at Oxford University Press throughout the preparation of the text are deeply and sincerely appreciated.

Contents

1 Introduction

WHAT IS HISTOLOGY?

Histology deals with the normal microscopical appearance of the body. It is the basis for the understanding of physiological and pathological processes. The light microscope is used to observe an organ, either *in vivo* or after its removal from the body. *In vivo* observations are restricted to observing the general, coarse texture of the component parts, as well as the pattern of blood flow. After the removal of an organ, it can be examined with both the light microscope and the electron microscope following preservation by fixative solutions, dehydration, embedding, sectioning, and staining. This results in the light microscopical slides used routinely in the histological laboratory and surgical pathology. Entire organs or parts thereof can be sectioned. (Fig. 1-1) For electron microscopy, only small pieces can be preserved at a time, and the sections must be extremely thin (100 Å–1000 Å).

Light microscopy relies on the staining of the sections to bring out variations in color. This is desirable since the resolving power of the light microscope is in the order of 0.2 μ (2000 Å), and differences in optical density of various organ components are enchanced by the dye, aiding in the discrimination of one component from another. The electron microscope also relies on the staining of the sections, although to a lesser degree. The theoretical limit of the resolving power of the electron microscope is in the order of 2 Å. The practical limit of resolution in ultrathin sections is about 10 Å; in thin sections about 25 Å; and in thick sections about 50–100 Å. The resolving power (resolution) is dependent, therefore, on the thickness of the section and the wave length of the source of radiation. It is a linear measure of the smallest distance at which two structures can still be distinguished from each other. The following is a table for measuring structures in histology:

1 mm = 1000 microns (μ)
1 μ = 10,000 Angstrom units (Å)
In some texts, micron (μ) is often expressed as micrometer (μm) 1 μ = 1 μm.
Average diameter of a red blood cell = 7 μ
Average diameter of a ribosome = 200 Å

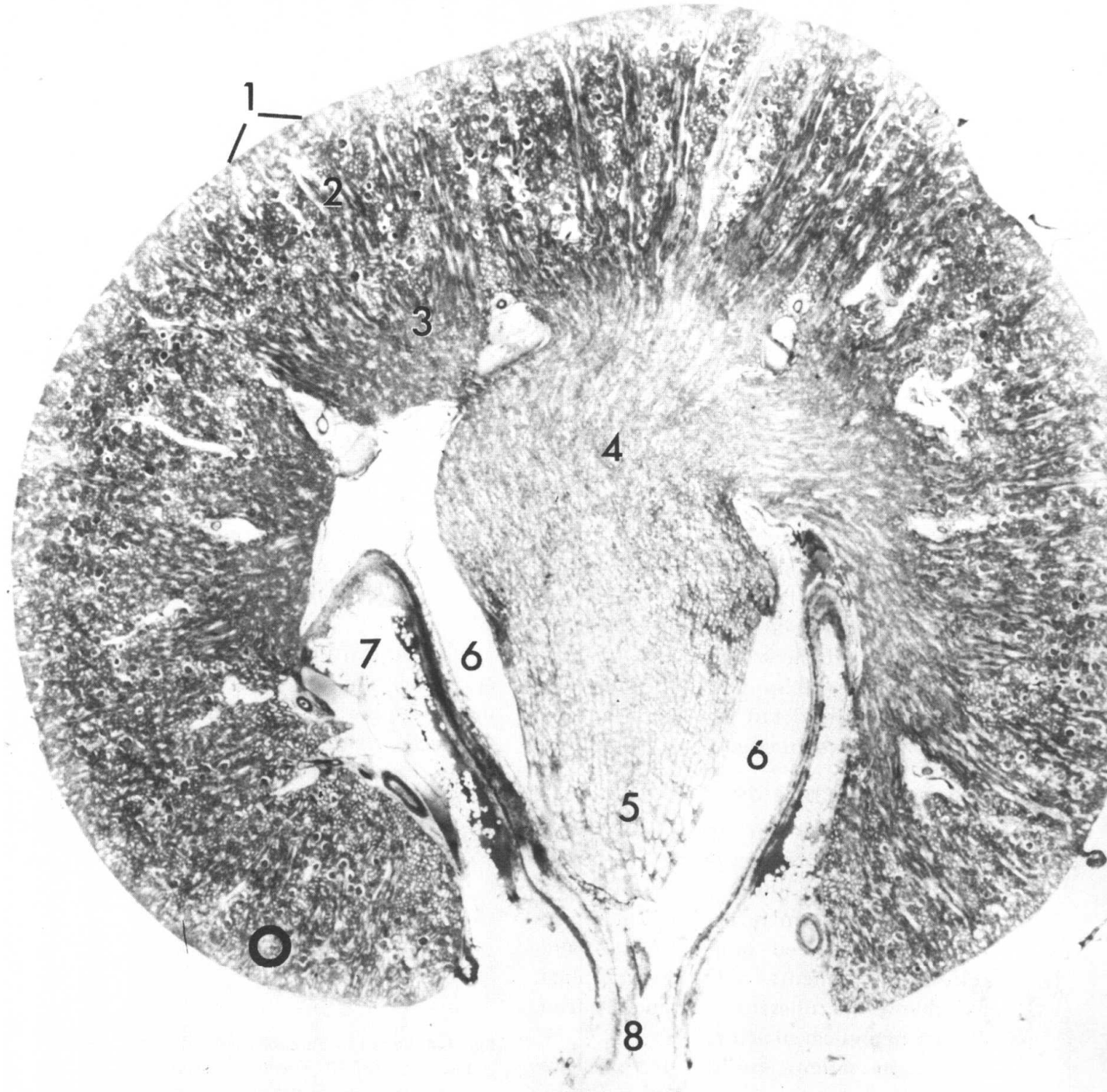
ORGAN COMPONENTS

Based on light and electron microscope observations, it has been established that organs consist of four primary tissues: epithelium, connective tissue, muscle tissue, and nervous tissue. Each tissue in turn consists of **cells** and **extracellular components.** Each cell consists of a cell mem-

Fig. 1-1. Section of entire organ. Kidney. Rabbit. L.M. X 15.5. This demonstrates the considerable amount of structural information which can be obtained at low magnification in the light microscope, if the tissue section is of proper thickness and the colored dyes carefully selected to show differences in optical density of different structures. **1.** Capsule. **2.** Cortex. **3.** Medulla. **4.** Pyramid. **5.** Papilla. **6.** Renal pelvis. **7.** Adipose tissue in renal sinus. **8.** Ureter. This section is enlarged further in Fig. 32-2 (p. 649).

Fig. 1-2. Proximal convoluted tubule of the nephron. Cross section. Enlargement of area similar to circle in Fig. 1-1. Kidney. Rabbit. L.M. × 1100. This demonstrates the practical limit of light microscopical resolution in a routinely prepared histological specimen. The preparation included formalin fixation, paraffin emebdding, and staining with hematoxylin and eosin. **1.** Lumen. **2.** Brush border zone. **3.** Nuclei.

Fig. 1-3. Proximal convoluted tubule of the nephron. Kidney. Rat. E.M. X 1100 (as in Fig. 1-2). This demonstrates the superiority of the preparation techniques and resolving power of the electron microscope. The tissue was fixed by intravascular perfusion of glutaraldehyde followed by intravascular perfusion of osmium tetroxide, embedding in epoxy resin, thin-sectioning, and staining with lead citrate. **1.** Lumen. **2.** Microvilli. **3.** Apical vacuoles (not present in Fig. 1-2). **4.** Nucleus. **5.** Cytoplasmic granules (mitochondria and secondary lysosomes). **6.** Peritubular capillary. Section of a similar area is enlarged further in Fig. 32-13 (p. 657).



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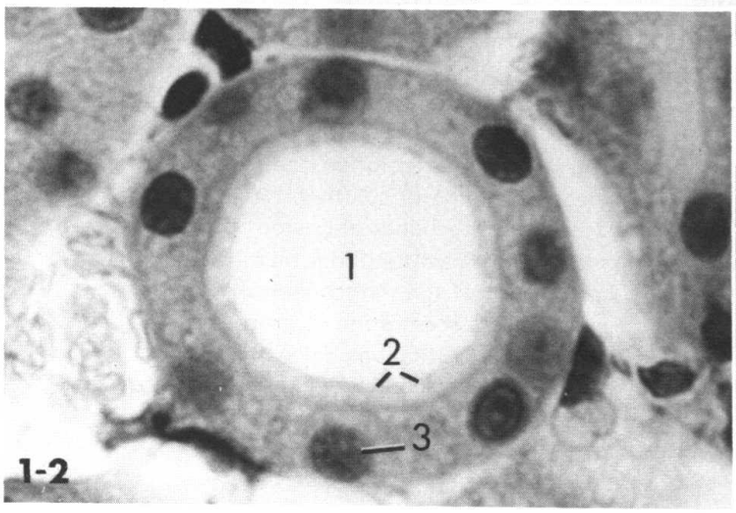

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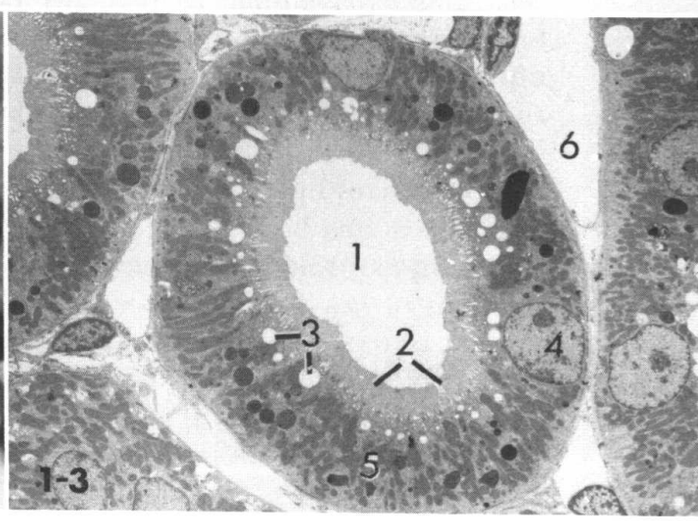

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brane, the nucleus, organelles, and inclusions. All organs, tissues, cells, and cell organelles of the mammalian body are described in subsequent chapters.

Knowledge of the structural architecture of living substance and its components is only one aspect of understanding the intricate mechanisms involved in the functions of organs, tissues, cells, and organelles. Many techniques are now available to identify biochemically the many varied components. During the last two decades, a combination of light microscopy, electron microscopy, histochemistry, radioautography, cell fractionation, differential centrifugation, and cytochemistry has contributed greatly to a better understanding of structural and functional interrelationships. It is considered beyond the scope of this book to describe these techniques which are accounted for in detail in standard textbooks of research methods in cell biology.

GUIDE TO ILLUSTRATIONS

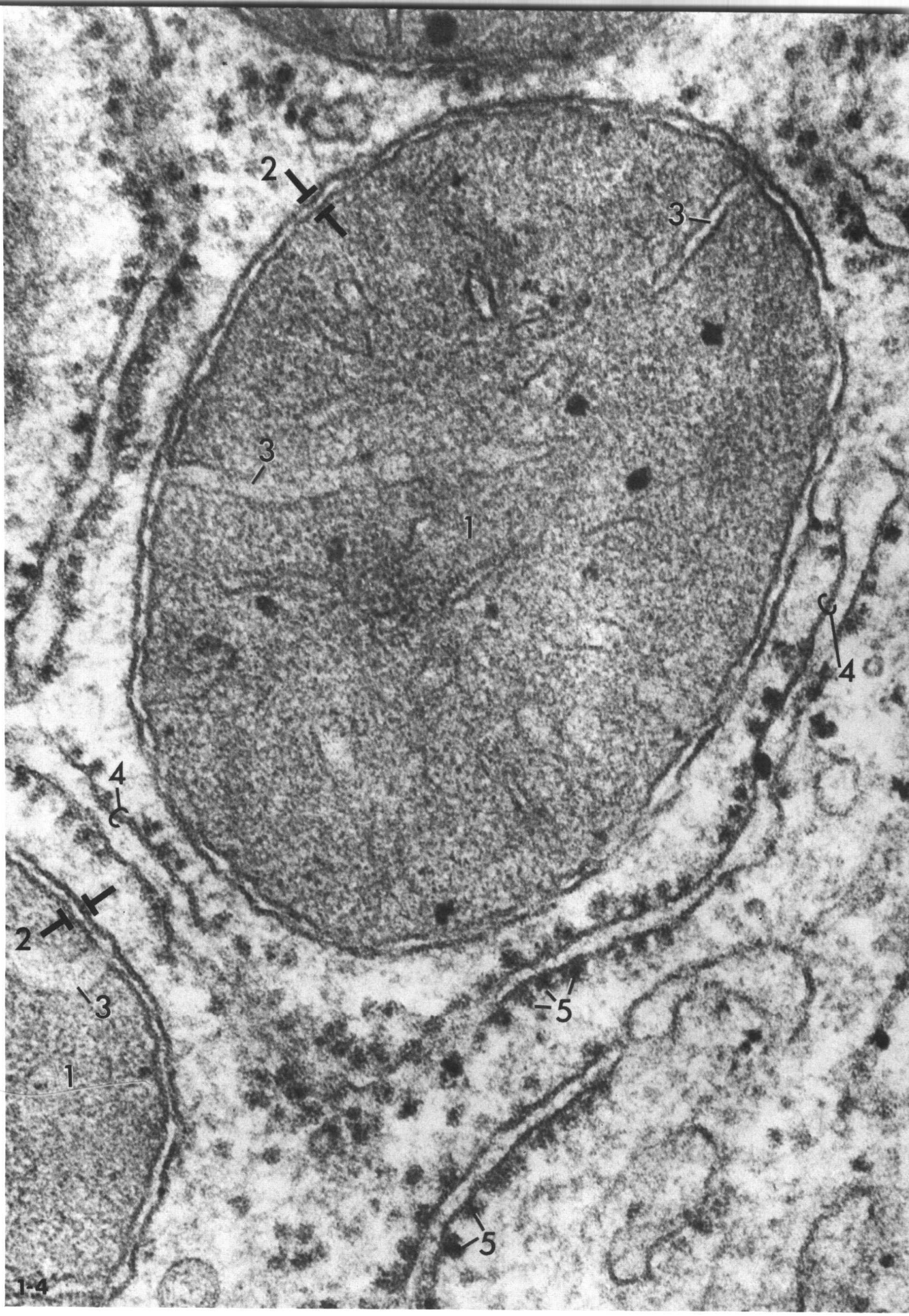
The illustrations in this book were obtained by photographing sectioned specimens under light and electron microscopes. In order to bring out differences in density between organs, tissues and cellular components, stains were applied to enhance the inherent light and electron density of a given structure.

In **light microscopy** the sections are stained with a variety of colored dyes, the most common being a combination of eosin and hematoxylin. The proteins of cells and extracellular components take on a red or pink color with eosin, whereas nucleic acids of the nuclei and certain components of the cytoplasm take on a deep blue color with hematoxylin. In **electron microscopy**, a solution of osmium tetroxide is used both as a general fixative and as an electron stain. In addition, salt solutions of heavy metals (lead, phosphotungstic acid) are used to enhance the electron density of tissue components. It should be kept in mind that these metals are not bound specifically to cellular or tissue components, although a **linear arrangement** of structures enhanced by the electron stain is generally accepted to indicate the location of membranes or filaments, whereas a **granular** configuration is believed to reflect a particulate arrangement of molecules. (Fig. 1-4)

The importance of staining a light microscope section by a variation of colored dyes is often overemphasized in the study of histological and pathological slides. Since the electron microscope cannot reproduce colors but depends entirely on differences in electron density of the tissue components, the light micrographs in this book are presented in black and white to facilitate the transition from light microscopy to electron microscopy. (Figs. 1-2, 1-3)

The adjectives **ultrastructural, submicroscopic,** and **fine structural** often used in contemporary textbooks of histology and cell biology express the details resolved by the electron microscope which lie beyond the resolving power of the light microscope.

Fig. 1-4. Detail of parenchymal cell. Liver. Rat. E.M. X 160,000. This demonstrates the resolving power of the electron microscope and the enhancement of the electron density of linear and granular components of the cell by osmium tetroxide fixation and staining of the section with lead citrate. **1.** Mitochondria. **2.** Mitochondrial external envelope, total thickness 200 Å. Each dense linear component of this envelope is 60 Å thick and consists of three subunits, each averaging 20 Å in thickness. The resolution achieved in this electron micrograph is therefore approximately 20 Å. **3.** Mitochondrial cristae, each originating from the inner membranous component of the mitochondrial external envelope. **4.** Membrane of granular endoplasmic reticulum, 60 Å thick. This membrane also consists of three subunits, each 20 Å thick. **5.** Ribosomes, particles averaging 200 Å in diameter.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2 Cells and organelles

GENERAL CONSIDERATIONS

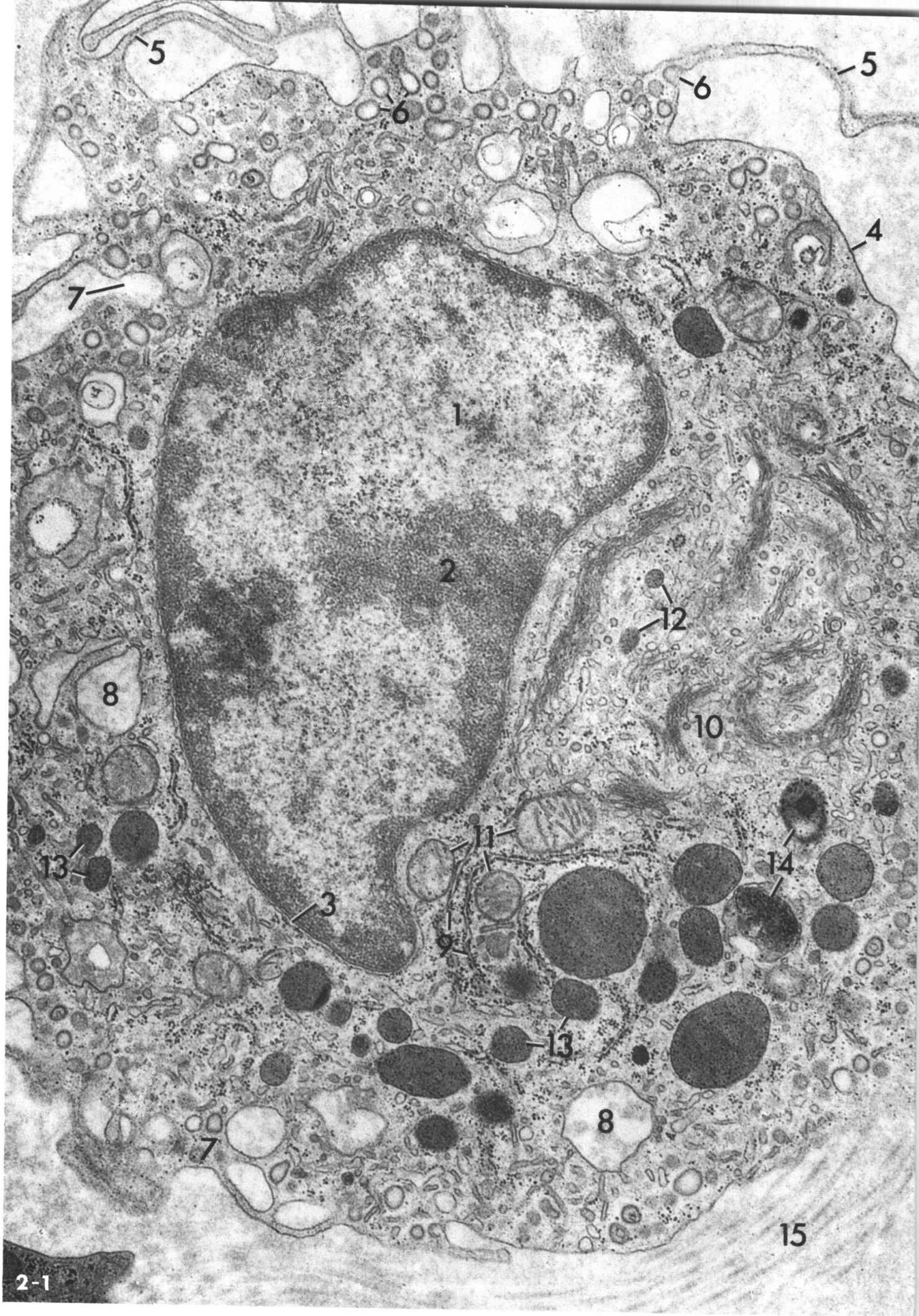
The cell is the functional unit of the mammalian body. It consists of protoplasm, bounded by a delicate cell membrane. The protoplasm consists of the nucleus (karyoplasm) and the cytoplasm. The cytoplasm contains a matrix (hyaloplasm), cell organelles, and inclusion bodies. The size, shape, and function of mammalian cells vary considerably and the study of cells and tissues deals with precisely these parameters. In general terms, the **protoplasm** is a heterogeneous aqueous phase which contains many biochemical components required for the varied metabolic processes that underlie and represent life. In addition, the protoplasm contains genetic material which determines the character of the cell in processes of cell multiplication and growth.

In mammalian cells the cytoplasm contains the biochemical components of the protoplasm, whereas the genetic material in the form of nucleic acids is concentrated largely in the karyoplasm (nucleus). The **cytoplasm** contains water (75%), salts (1%), proteins (20%), lipids (3%), carbohydrates (1%), and nucleic acids. The percentages indicated are approximate; they vary considerably from cell type to cell type. Some of the cellular **water** is present as bound water and held loosely by protein molecules, whereas most exists as free water, available for metabolic processes. Of the **salts,** cations are represented by sodium, potassium, calcium, magnesium, and minute concentrations of several other ions. Major concentrations of anions are phosphate, bicarbonate, and chloride. The concentrations of the various salts in the cell and in the extracellular milieu, and the transport back and forth across the cell membrane of these salts as a result of changes in membrane permeability, are vital for the maintenance and normal functioning of the cell.

Proteins and **lipids** are present as highly ordered arrays of molecules, forming **membranes,** both as part of the cell membrane and the membranes surrounding and constituting an essential part of the many varied subunits of the cell, referred to as cell organelles. Proteins also exist in the cytoplasmic matrix (hyaloplasm) in soluble form, and lipids can be stored as droplets. **Carbohydrates** may occur as glycogen, as small particles which are not membrane-bound, and represent a high polymer of the monosaccharide glucose. Carbohydrates are also conjugated with proteins to form mucopolysaccharides.

Cell **organelles** are structural subunits of the cytoplasm, each characterized by its specific enzymatic content, ultrastructure, and function. Considered as organelles (Fig. 2-1) are the ribosomes and polysomes, granular and agranular endoplasmic reticulum, Golgi apparatus, annulate lamellae, mitochondria, lysosomes and related particles, centrioles, microtubules, and filaments. Pinocytotic vesicles are intracellular derivatives of the cell membrane, and as such should be included among the organelles. The cell organelles are closely integrated with, and vital for, the metabolic processes of the cell. Structures not included among organelles were, in the past, referred to as **inclusion bodies.** Among these were such structures as pigment granules, stored lipids, and carbohydrates (glycogen), secretory granules, and crystals. Many of the inclusion bodies were considered non-living components of the cell and of little importance in maintaining cell life. Recent investigations seem to indicate that these structures,

Fig. 2-1. Macrophage. This connective tissue cell contains a fair representation of the many varied components of a mammalian cell. Interstitial tissue. Testis. Rat. E.M. X 22,000. **1.** Nuclear euchromatin. **2.** Nuclear heterochromatin. **3.** Nuclear envelope. **4.** Cell membrane. **5.** Microvilli or sheet-like cell processes. **6.** Pinocytic vesicles. **7.** Phagocytic vacuoles **8.** Phagosomes. **9.** Granular endoplasmic reticulum. **10.** Golgi apparatus. **11.** Mitochondria. **12.** Condensing vacuoles. **13.** Primary lysosomes. **14.** Secondary lysosomes. **15.** Extracellular collagenous fibrils.



2-1

with only few exceptions, are just as vital for the proper function of those cells where they occur as the ordinary cell organelles.

CELL MEMBRANE

The cell membrane, as seen in conventional, transmission electron microscope preparations, emerges as a 90 Å-thick, **trilaminar** structure, the outer surface of which is covered by a finely filamentous layer, the **glycocalyx** or cell coat. Its thickness varies from 75 Å to 2000 Å. The three leaflets of the trilaminar cell membrane vary in electron density. Two highly electron-dense leaflets, each about 25 Å thick, enclose a central electron-lucent layer, approximately 30 Å thick. (Figs. 2-2 and 2-3) This complex is often referred to as the **unit membrane.** Cell membranes preserved in a less conventional way seem to consist of particulate components with a range of dimensions from below 40 Å to 100 Å in diameter. Functional experiments indicate that the cell membrane may contain minute **pores** used by lipid-insoluble substances such as water and urea molecules. Their diameter would be 2–10 Å, but actual channels of this size have not been resolved with the electron microscope.

From correlated chemical and structural studies, it is known that the cell membrane, as well as other intracellular membranes, consists of lipids (phospholipids and cholesterol) and proteins. Small amounts of polysaccharides are also present in the cell membrane. On a weight basis, there are one to four times as much protein as lipid. However, since lipid molecules are smaller than protein molecules, there are many more lipid than protein molecules present.

It is presently not fully established how the lipid and protein molecules interrelate structurally. According to the concept presented by Danielli and Davson in the early 1930s, the backbone of the membrane is formed of a continuous bilayer of phospholipid molecules. The hydrophobic tails of the lipid molecules appose each other within the bilayer, the hydrophilic polar heads point outward, and globular protein molecules cover the lipid molecules on either side. In the conventional electron microscope image of the cell membrane, the central electron-lucent layer would be made up of the hydrophobic tails of the lipid molecules, and the two electron-dense layers would be formed by the hydrophilic polar heads and the globular proteins. In the unit membrane, as proposed by Robertson in the late 1950s, the electron-dense outer leaflets might correspond to flattened protein molecules, and the electron-lucent center to the bilayer of lipid molecules. In a more recent proposal, Sjöstrand suggested in 1968–69 that the mitochondrial membranes, and perhaps also the cell membrane, are composed of non-lamellar globular or polygonal lipo-protein complexes. The protein molecules are thought to project across the membrane in a repetitive fashion at a period of about 100 Å or less, separated by septa approximately 10 Å thick. In an attempt to reconcile the several proposals, it has been suggested that the cell membrane does indeed consist of phospholipids, arranged in a continuous bilayer formation with the polar lipid heads facing out toward either of the surfaces of the bilayer formation. Globular or irregularly shaped proteins may project at inter-

Fig. 2-2. Epithelial cell. Longitudinal section. Large intestine. Rat. E.M. X 211,000.
1. Lumen of gut. **2.** Finely filamentous glycoprotein coating (glycocalyx). **3.** Cell membrane. **4.** Microvillus. **5.** Tight junction (zonula occludens). **6.** Punctate contacts of fusion marked by **arrows.**

Fig. 2-3. Cross-sectioned microvilli. Epithelial cell. Large intestine. Rat. E.M. X 440,000. (1 mm=22 Å). **1.** Center of microvillus with core filaments, 45 Å in diameter.
2. Trilaminar cell membrane, 90 Å.
3. Electron-dense leaflet, 25 Å in diameter.
4. Electron-lucent middle layer, 35 Å in diameter. **5.** Dotted line indicates approximate height of glycoprotein surface layer (glycocalyx), 75 Å.