Functional Histology

A Core Text

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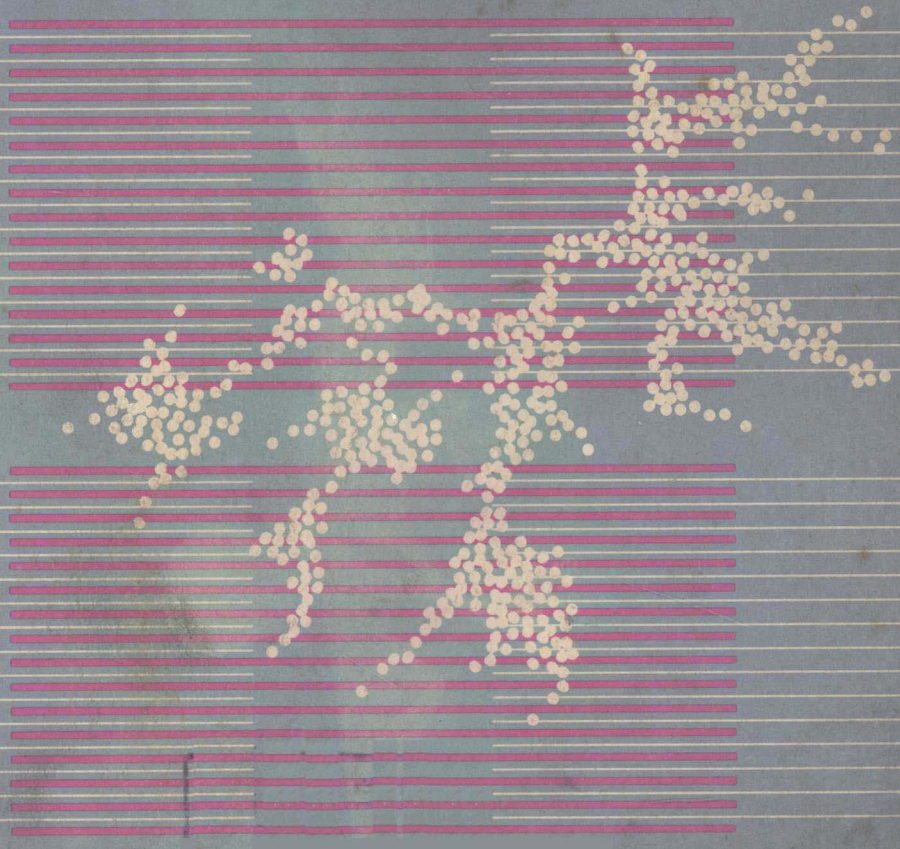

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PREFACE

This book was developed from a syllabus that has been distributed with favorable response to students of medicine and dentistry at Tufts University during the past five years. Its value stems from a concise presentation of material that integrates histology with corresponding biological functions. We tried to establish a conceptual understanding of the histological organization of cells, tissues, and organ systems by the use of representative diagrams. The use of a companion atlas to assist laboratory study is highly recommended. As a core text, this book provides a manageable reading load in today's stacked curriculum, allowing the student to concentrate on lectures rather than on the production of copious notes that often must be interpreted later.

Functional Histology reflects a team approach toward teaching, an approach in which the authors have borne an equal share from the time the original material was delivered in lecture form, through its incorporation into a course syllabus, and into its current form as a short text. Each author's contribution is reflected in the table of contents. As a group we are deeply grateful to Dr. W. Duane Belt, Professor of Anatomy at Tufts University School of Medicine, who has shared his extensive understanding of histology with each of us.

The book evolved with the support of a large base of very cooperative individuals. At every stage we have solicited and incorporated suggestions made by some of the more than 1500 medical, dental, and graduate students who have used the syllabus material out of which this book developed. The original versions of the syllabus were typed and produced, often on very short notice, by Bonnie Goldman, Kathy Boudrow, and Fran Patterson. The book chapters were typed and retyped, untiringly, by Tricia Waneck. We are indebted to those people for their continued interest and dedication to the educational process. Dr. Karen Hitchcock, chairman of the Tufts Department of Anatomy, has been constantly supportive of our efforts and has done much to expedite the book's production through use of departmental facilities.

Many of the prototype diagrams for the text, and some of the finished diagrams as well, are the skillful work of Daniel Casper, a graduate student in the Department of Anatomy at Tufts. The bulk of the diagrams were expertly prepared by the medical illustrator, Mary Brown Allen. We also acknowledge Tony Ross who helped with the illustrations.

Finally, we most gratefully acknowledge the people of Little, Brown and Company for their guidance and support and wish to thank Mary Ellen Arkin specifically for her excellent contribution as copyeditor. Her careful work has been a substantial aid in producing what we hope is a clear and coherent text.

M. B.
J. B.
T. B.
A. G.

FUNCTIONAL HISTOLOGY

CONTENTS

1

A BRIEF INTRODUCTION TO HISTOLOGICAL ORGANIZATION AND TECHNIQUES OF MICROSCOPY

OVERVIEW OF THE RELATIONSHIP BETWEEN CELLS, TISSUES, AND ORGANS

The Cell

Cytology is the study of the cell, which is the basic unit of living matter. In their most primitive state cells are autonomous, unicellular organisms like amoebae or paramecia, which are capable of carrying out all metabolic processes and reproduction alone. Even in this simple context, however, cells begin to cooperate to enhance their chances of survival, as shown by the phenomenon of conjugation between paramecia. Simple cells may aggregate into colonies, such as volvox, to which the beginnings of multicellular organisms may be traced. As cells begin to cooperate and to share labor among themselves, different cells become suited to carrying out particular functions. Even in a two-cell layered organism such as a hydroid, some cells are differentiated into digestive cells, while others function as a combined muscle and covering cell. Certain of these two basic cell types differentiate further to become either reproductive cells or a special kind of defense cell. In this way, each cell type develops its own specialty, giving the organism the capacity to interact with increasing specificity to its environment. In contributing to a more complex whole, however, individual cells lose their autonomy, or their ability to exist independently.

The Tissues

In more highly evolved species, three cell (germ) layers develop in the embryo; these are endoderm (inner), mesoderm (middle), and ectoderm (outer) cell layers. These basic layers give rise to four functional groupings of similar cells, called *tissues.* Study of the four tissue types and their particular specializations is called *histology.* The tissue types and their basic germ layer derivations are as follows:

1. Epithelium: Arises from all three primary cell layers
   - Endoderm: epithelium lining the digestive tract and its glands, epithelium of respiratory tract and its glands, epithelium of bladder and certain parts of the urinary and reproductive systems.
   - Mesoderm: epithelium (endothelium) lining the blood vessels, mesothelium lining serous membranes (pleural cavity, pericardium, peritoneum), epithelium of a large portion of the urogenital system.
   - Ectoderm: epithelium covering the body surface (skin), epithelium of the anus and oral cavity glands opening into the mouth, taste buds, enamel of teeth, and epithelium lining parts of the eye, ear, and nose.
2. Connective tissue: Primarily mesodermal, except some of the neuroglia
3. Muscle: Mesodermal, except for the smooth muscle of sweat glands and pupillary muscles of the eye
4. Nerve: Ectodermal

Each tissue can be further subdivided, resulting in variations on a basic theme. For instance, there are three types of muscle tissue that vary in the organization of the major contractile protein filaments, actin and myosin. The muscle proteins are best organized in the fast-contracting skeletal muscle fibers, whereas the slow, rhythmic contraction of smooth muscle is subserved by a more diffuse arrangement of component filaments. The intrinsic rhythmicity of cardiac muscle, however, relies both on the relationship between nerve and muscle cells, and also on the particular arrangement of protein filaments within the cells. In all cases, the important message to grasp is that *structure follows function.* Through the ages, organisms have evolved that are best able to

adapt to the environment; this is also true on the cellular level. The various parts present within a cell reflect the function that these parts have evolved to perform. Therefore, simple examination of a cell's component parts will reveal its particular function. When a functional approach is used in the study of histology, cell physiology will naturally unfold because the two together form a unified whole, and the need to memorize will be largely replaced by simple deductive logic.

The Organs

The four tissue types are further organized into *organs* and *organ systems.* For example, the digestive system is a series of hollow tubular organs with regional functional specializations of the component tissue types to form esophagus, stomach, and intestines. In addition, the large epithelially derived glands, liver and pancreas, as well as the gallbladder, contribute secretions to the digestive tract. The entire organ system functions in the intake, breakdown (digestion), and absorption of food. Each organism is composed of several organ systems, which can be explored from the gross to the cellular level. Control and integration of the various systems are functions of both the nervous and endocrine systems. A basic knowledge of histology, therefore, provides both *morphological* (structural) and *physiological* (functional) understanding of the delicate homeostatic mechanisms involved in the interaction of an organism with the environment.

TECHNIQUES OF MICROSCOPY

Measurement

Just as an organism is composed of organ systems, each performing a specified task, so each cell is composed of miniature organs, or *organelles.* The same basic organelles are present in every cell, but their arrangement and degree of development vary with the particular function of the cell. In order to describe the microscopic anatomy of the cell, a system of metric measurement is employed:

1 inch = 2.54 centimeters (cm)
1 cm = 10 millimeters (mm)
1 mm = 10^3 micrometer (μm) (formerly = μ, micron)
1 μm = 10^3 nanometer (nm) (formerly = mμ, millimicron)
1 nm = 10 angstroms (Å)
1 Å = 10^{-7} mm

Techniques of Specimen Preparation

Typically, the study of cytology and histology pivots on the use of preserved (fixed) specimens, which are viewed by either light or electron microscopy. It is important to understand the basic processes involved in tissue preparation, so that artifacts (structural alterations resulting from tissue preparation) can be easily recognized as such. Once a fresh specimen of tissue is obtained, it is subjected to various procedures, as described in the following sections.

Light Microscopy

Fixation. By the use of protein coagulants, precipitants, or cross-linking agents, much of the cellular protein is denatured and retained in situ. Intracellular enzymes that would normally putrefy tissue are therefore rendered inactive, and the basic framework of the cell is preserved as a result of fixation, in a state intended to resemble the in situ structural condition, ideally like a snapshot. However, it is important to remember that fixatives, especially those used for light microscopy, often result in shrinkage of proteins, so that spaces may occur as artifacts at natural boundaries (e.g., around the nucleus), between adjacent cells, or at the interface between different tissue types.

Dehydration. The specimen is run sequentially through a series of organic solvents of increasing concentration to extract water. The purpose of this procedure is to facilitate embedment of the tissue in a hard paraffin or plastic matrix that is miscible with organic solvents but immiscible with water. The hard embedding medium supports the tissue so that it can be sliced into sections. Dehydration extracts many lipids and other important constituents, which can result in another artifactual set of intracellular spaces in certain cell types.

Embedment and Sectioning. The tissue is infiltrated with paraffin or plastic, which is subsequently hardened or polymerized. The specimen is then trimmed and put in a microtome (tissue sectioner), where slices thin enough to transmit light are cut. Sections for light microscopy range from 1 to 40 μm in thickness. The act of sectioning itself can produce artifactual separation between tissues of different consistencies.

Staining. For the light microscope, there are many combinations of dyes used to stain particular parts of the cell. The most common combination is hematoxylin and eosin. Hematoxylin is itself a weak dye, but when oxidated to purple or blue color (hematein) and mordanted with a positively charged metal, it is strongly attracted to negatively charged tissue components such as nucleic acids, so that nuclei and cytoplasmic RNA stain blue. Ionic interactions are the attractive forces most often involved between tissue elements and the portion of the dye imparting color to the tissue. Tissue components staining with hematoxylin are inaccurately but consistently called *basophilic,* because they attract so-called basic dyes. These tissue components are more accurately called anionic. Eosin is a red dye which stains the cytoplasm rather diffusely but which appears bright red when attracted to a concentrated region of positive charge, for instance, certain protein granules manufactured for secretion by a cell. Regions staining with eosin are called *acidophilic,* but they are actually attracted to the negatively charged component of the dye. There are many other stains used for special purposes, some of which show the phenomenon of *metachromasia.* For instance, the basic dye toluidine blue stains areas of net negative charge *blue.* However, in the case of certain compounds such as mucopolysaccharides, many negative charges are closely aligned. The dye molecules line up similarly, and when light passes through, the stacked-up dye molecules appear red, the *metachromatic color* of toluidine blue. A whole range of chemical reactions, which are beyond the scope of this book, can be exploited to identify the molecular nature of intracellular regions. This branch of histological technique is called *histochemistry.*

Resolution. The resolution of the light microscope is 0.25 μm, with resolution being defined as the distance between two points when they can be resolved as two distinct entities, instead of one. In other words, a light microscope is capable of differentiating two points separated by a distance of 0.25 μm or more. This concept differs from magnification, which merely means making things larger without necessarily revealing more detail. A good example of this would be a blowup of a newspaper photograph, in which the image is made up of relatively few coarse spots. Magnification of such a picture would make it larger but would not yield increased detail. In fact, the blowup would be fuzzy. The process of magnifying an object without increasing resolution is known as *empty magnification.* Resolution is an optical phenomenon involving the wavelength of the light used to illuminate the specimen, as well as certain properties of the lens system. The resolution of the light microscope is limited principally by the long wavelength of the visible spectrum.

Special Techniques. In addition to the examination of specimens by conventional light microscopy, a host of special microscopes and methods of specimen preparation exist for special purposes. Two of the most commonly used techniques are listed below:

1. Phase contrast microscopy. Living specimens can be observed without the need for fixation and staining. Small differences in the refractive index of cellular organelles are amplified and converted into visible differences in intensity, so that various organelles have different contrasts and can thus often be identified without requiring staining.
2. Fluorescence microscopy. Monochromatic light (consisting of only one wavelength) is used to illuminate the specimen. If the specimen contains molecules that absorb this light, it will re-emit light of a longer wavelength. A filter system is used to exclude emissions of extraneous wavelength. Molecules that fluoresce, therefore, appear luminous. This is a widely used technique both clinically and experimentally; in both situations the fluorochromes may be either endogenous

(naturally occurring within a cell), or applied exogenously by an experimenter as a tag for a molecule that is known to bind to cells. Both fixed and living tissue can be examined in this way.

Transmission Electron Microscopy

A similar rationale for fixation, embedment, and sectioning applies to preparations for the electron microscope. Common fixatives for electron microscopy are gentler than those used for light microscopy, however. In general, *aldehydes* are used, which cross-link proteins and keep them as close to their original position and form as possible. Furthermore, the image visualized in the electron microscope is due to variations in density recorded on black and white film. The variations in density within the tissue section are due to (1) a second fixation step using *osmium tetroxide* (OsO_4) which is reduced so that osmium, a heavy metal, is deposited on certain tissue elements, imparting density to them; (2) staining with uranyl and lead ions which give contrast to the specimens. The tissue elements stained with heavy metals impede the flow of electrons and are called *electron dense.* Therefore, very few electrons penetrate these areas to excite the underlying fluorescent viewing screen on the microscope, which consequently appears dark. Areas of the section that are less dense, or *electron-lucent,* allow the electron beam to penetrate and excite the underlying screen which fluoresces, producing an image of the tissue section. In order for electrons to penetrate a tissue section, such a section must be cut very thin. A typical ultrathin section would be only 60 to 100 nm thick and has to be cut on a special ultramicrotome. Since illumination is provided by a beam of electrons, glass lenses cannot be used since they would absorb the incident electrons. Instead, the course of the beam is controlled by electromagnetic lenses. The resolution of the transmission electron microscope is about 0.1 to 0.5 nm.

At the electron microscopic level there are also a number of special microscopes and techniques. Two of the more common techniques are discussed below.

Scanning Electron Microscopy. Scanning electron microscopy differs from transmission electron microscopy in that only the *surface* architecture of cells is visualized. A gold replica is made of the cellular surface, and this replica is scanned by an electron beam that builds up an image on a cathode ray tube, similar to a television. In this way, striking three-dimensional images of the cellular surface are formed. The resolution of the scanning electron microscope is only about 10 nm, compared to the 0.1 nm resolution of the transmission electron microscope.

Freeze-Fracture Technique. In recent years the freeze-fracture (freeze-cleave) technique has come into wide usage. A tissue specimen is frozen in liquid nitrogen cooled isopentane and then fractured with a blade; the resulting fracture face is replicated under a high vacuum with platinum (at a 45-degree angle) and carbon. The platinum shadowing creates an effect similar to a snowfall propelled by a stiff wind. The platinum, like snow, accumulates on the near side of elevations, and is absent on the far side. Conversely, the near side of a depression would be empty, while platinum would pile up on the far side. When viewed in the transmission electron microscope, areas where platinum has accumulated will impede the flow of electrons and appear dark; areas devoid of platinum appear as white shadows. The carbon does not yield further electron density, but acts to stabilize the platinum shadowed replica of the fractured surface, so that the replica can be separated from the frozen tissue and mounted on a grid for electron microscopy. The advantage of this technique is that fracture occurs along the plane of least resistance, and in cells, one such area is between the hydrophobic acyl tails of membrane phospholipids. Thus the cell membrane is split down its middle – a technique that has opened up new realms to membrane biologists.

Part I
CELLS AND TISSUES

2
CYTOLOGY

The cell can be viewed as a microcosm of the body, with organelles that are analogous to the bodily organ systems. The central coordinator of the cell, and the archive of information, is the *nucleus.* The cell is capable of receiving and transducing a wide range of stimuli, which trigger nuclear control mechanisms. Different genes become activated or repressed, and the genetic message is converted into an appropriate response by messages relayed to the cytoplasm. The delicate balance within the cell and the organelles responsible for maintenance of this balance are the subject of this chapter.

The cell is a mass of *protoplasm.* This protoplasm is divided into two compartments: (1) *cytoplasm,* that which lies between the cell membrane and the nuclear membrane; and (2) *nucleoplasm* (karyoplasm), that which fills the nucleus. These "plasms" are colloidal, that is, they may express as either sols or gels and are combinations of various organic molecules, salts, and water. They are the background, or medium, in which the specific cellular machinery is suspended, and through which various metabolites diffuse. They constitute the primordial "electrolyte sea" that conducts electrical energy throughout the organism.

Suspended within the protoplasm are the various *organelles,* or miniature organs, which are always present within a given cell, and *inclusions* such as lipid droplets or pigments, which are not constant cellular features (Fig. 2-1). Inclusions may be present in some cell types but not in others, or they may appear only under particular circumstances.

CYTOPLASMIC ORGANELLES

The Concept of Membrane

The most ubiquitous structure in the cell is membrane. All cells are separated from the external environment by the *plasmalemma* or *cell membrane,* which acts as a selective barrier, recognizing and admitting some molecules while excluding others. In addition, the large size and diversity of functions of each cell require some sort of compartmentalization within the cytoplasm to spatially segregate different classes of reactions. This compartmentalization is achieved through a system of *intracellular membranous organelles.* Membranes can enclose specific regions of cytoplasm and actively modify the environment they enclose through a variety of transport systems suited to the particular need of the organelle in question. All membranes, both the plasmalemma and those forming the membranous organelles, are complex structures composed of a *lipid bilayer* that is closely associated with a variety of *proteins.* The general composition of the cell membrane is similar to that of internal membranes, but it does differ in the types of lipid and protein present and in the additional presence of *sugar groups,* which project from the cell surface and are important in the recognition of signals (such as hormones) impinging on the cell from its environment.

Recent investigations have revealed that cellular membranes "flow," and that some of the external cell membrane actually moves inward to contribute to cytoplasmic membranous organelles, and vice versa. The molecular details of this process have not yet been worked out completely but are exemplified by two familiar phenomena, *phagocytosis* and *exocytosis.* In phagocytosis (Fig. 2-1) a particle from the external milieu is engulfed by a pouch of plasma membrane, which subsequently pinches off from the surface and is liberated as a round vacuole into the cytoplasm. Exocytosis (Fig. 2-1) is the reverse phenomenon, whereby some cellular product is discharged to the external milieu when the membranous vacuole enclosing it fuses with the cell membrane. An obvious question that arises when considering exocytosis concerns cell types that are rapidly secreting. In this case, one would predict that a large bulge of membrane would accumulate at the apical (secretory) end of the cell as more and more vacuoles fused with the cell membrane. This does not happen, however, and recent evidence suggests that the membrane is rapidly cycled back into the cytoplasmic compartment through one of the membranous organelles, possibly the Golgi complex.

In considering organelles, it is helpful to group them into two basic categories, membranous and nonmembranous.

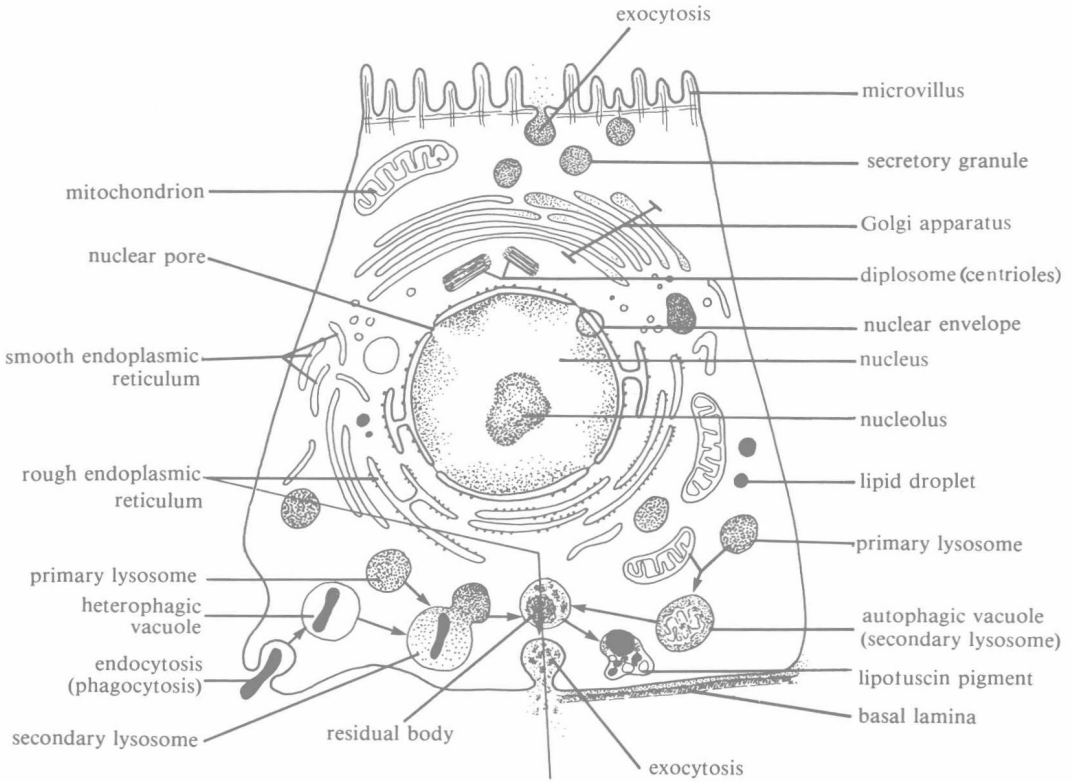

Figure 2-1. A cell. The major organelles and some inclusions are shown. The exocytosis of a secretory granule is depicted at the apex of the cell, while the cellular digestive system is portrayed at the base of the cell. At the bottom right, a lysosome combines with a worn-out organelle in the phenomenon of autophagy; the resultant body is a secondary lysosome. A fully loaded lysosome may condense into an inactive inclusion, lipofuscin pigment, or it may be released by exocytosis. In the case of heterophagy, or phagocytosis (bottom left), the cell takes in a particle enclosed in plasma membrane which combines with a lysosome to form a secondary lysosome. The secondary lysosome may then condense into a residual body and follow the same variety of pathways as indicated above. Because of these possibilities, it is important to understand that lysosomes may assume a number of appearances.

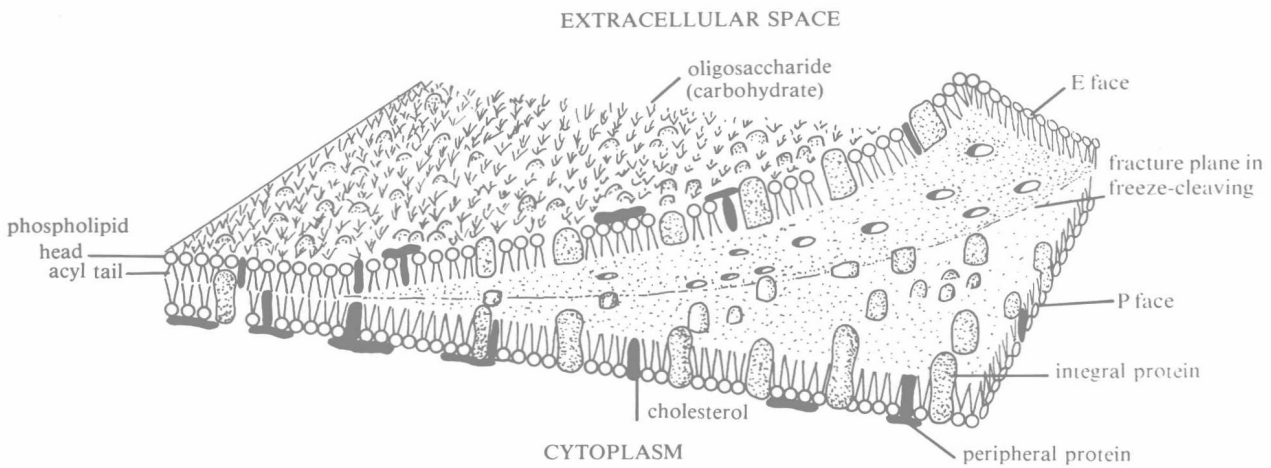

Figure 2-2. The cell membrane. In freeze-cleaving, the membrane is fractured between the nonpolar acyl tails. The half of the membrane facing the cytoplasm is referred to as the P *or protoplasmic* face *and retains most of the integral membrane proteins which give it a particle-rich appearance. The half-membrane facing the extracellular space is called the* E face *and is particle poor.*

Membranous Organelles

Cell Membrane (Plasmalemma)

The cell membrane (Fig. 2-2) is not visible with the light microscope but can be visualized with the electron microscope as a set of electron-dense lines, separated by a clear or lucent space. This "unit membrane" has been compared to railroad tracks; the width of this three-layered structure is usually 7 to 8 nm. The field of membrane science has rapidly advanced in the past 10 years with the advent of several specialized physical techniques to explore the arrangement of lipids and protein within the membrane. These physical methods, coupled with direct observation of the membrane interior through freeze-fracture electron microscopy, have yielded new concepts of membrane structure that are still evolving.

It should be stressed that many models exist for possible arrangements of membrane molecules, and that currently there is no one model that is satisfactory on all fronts. At a very basic level, however, the cell membrane can be described as follows:

1. *A bimolecular leaflet of phospholipids* is the backbone of the membrane (see Fig. 2-2). Hydrophilic phosphate head groups are attached to long hydrophobic hydrocarbon tails (fatty acids). The hydrophilic (polar) heads are directed outward to abut on the aqueous cell cytoplasm or external milieu. The nonpolar hydrocarbon tails point inward, creating a hydrophobic environment sequestered in the membrane interior. These fatty acid tails can exist either as a rigid crystalline lattice or as a fluid phase in which the hydrocarbon tails are more freely mobile. This property, which can affect the location of membrane proteins that are inserted into the bilayer, forms the basis of some of the newer membrane models.
2. *Protein molecules,* which account for 60 to 70 percent of the membrane mass, are associated with the lipid bilayer in two ways:
   a. *Peripheral proteins* are external to the bilayer. They are associated with the polar lipid head groups by a variety of weak bonds, which can be broken by changing the pH or ionic strength.
   b. *Integral proteins,* on the other hand, can only be liberated from the membrane by the use of drastic measures such as detergents, which break up the lipids into micelles. Even this harsh treatment may not fully separate proteins from lipids. Integral proteins are actually inserted directly into the bilayer and extend either partly or completely through the lipid layer. These proteins are *amphipathic,* con-

taining both hydrophilic and hydrophobic regions which allow them to penetrate the bilayer. Freeze-fracture electron micrographs reveal the inner region of the membrane because fracture occurs along the path of least resistance – between the nonpolar lipid tails (see Fig. 2-2). Such micrographs reveal numerous particles within the membrane that have diameters of 7 nm; these particles have been identified as membrane proteins. Membrane proteins have a variety of functions, many of which are enzymatic. They participate in a range of reactions, from monitoring the ionic equilibrium within the cell to transducing messages impinging on the cell surface into molecules that can trigger a specific nuclear and/or cytoplasmic response.

3. *Cholesterol* is a lipid that is present in nearly the same molar concentration as phospholipids in most cell membranes. Intracellular membranes, however, generally contain far less cholesterol. The cholesterol molecule readily associates with the fatty acid tails of membrane phospholipids and changes their molecular motions. In other words, cholesterol can determine whether the fatty acids are in crystalline or "loose" packing, and it is critical in determining how fluid the membrane is.
4. *Carbohydrates* are linked to either proteins or lipids, exclusively on the *outer* surface of the membrane abutting the external environment. These sugars serve as specific receptor sites for a whole host of incoming stimuli and also bear a net negative charge that is carried mainly by the amino sugar, sialic acid. The binding of particular molecules, such as hormones, to their specific receptor sites brings about local changes within adjacent membrane proteins (enzymes), which release messenger molecules such as the cyclic nucleotides (cyclic AMP and cyclic GMP) into the cytoplasm. These molecules can then trigger intracellular responses appropriate to the given stimulus.

The membrane is now viewed as potentially "fluid" in nature; that is, the lipids and also the proteins are capable of some degree of lateral motion. Certain proteins and species of lipid may occupy different sites, or *domains*, in the membrane which can provide a functional mosaic displaying various receptor sites and enzymes. This dynamic concept of two-dimensional fluidity was developed by Singer and Nicolson and is known as the *fluid-mosaic* model of membrane structure. Although the membrane can behave as a fluid mosaic, the control mechanisms governing the disposition of the membrane proteins and the mechanics of membrane flow from the cell membrane to cytoplasmic membranous organelles are just beginning to yield to investigation.

Mitochondria

Mitochondria are the energy plants of the cell, utilizing an orderly sequence of membrane-bound enzymes to generate adenosine triphosphate (ATP) by the process of *oxidative phosphorylation.* In addition to providing energy, these organelles also actively sequester calcium ions, which are stored within their matrix as granules of $CaPO_4$ and are released in response to the cell's changing needs. When living cells are observed with a phase contrast microscope, mitochondria appear as threads that are about 0.2 μm in diameter and up to several micrometers in length. They can be observed to move around within the cytoplasm in quite an autonomous manner and also to divide by binary fission. The *matrix* of the mitochondria contains closed loops of DNA similar to those of bacteria, as well as particles resembling ribosomes. These molecules allow mitochondria to function as partially independent organelles, since they can self-replicate and synthesize some of the proteins they require to function.

When viewed with the electron microscope (Fig. 2-3), mitochondria show a highly plicated (folded) membranous structure, which mirrors their function as generators of ATP. These organelles are composed of two membranes, a smooth outer limiting membrane and an inner membrane convoluted into a series of folds or *cristae.* The numerous cristae dramatically increase the surface area available for the disposition of the respiratory chain enzymes involved in the generation of ATP. The surface of the cristae facing the interior of the mitochondrion is further studded with a series of particles similar in

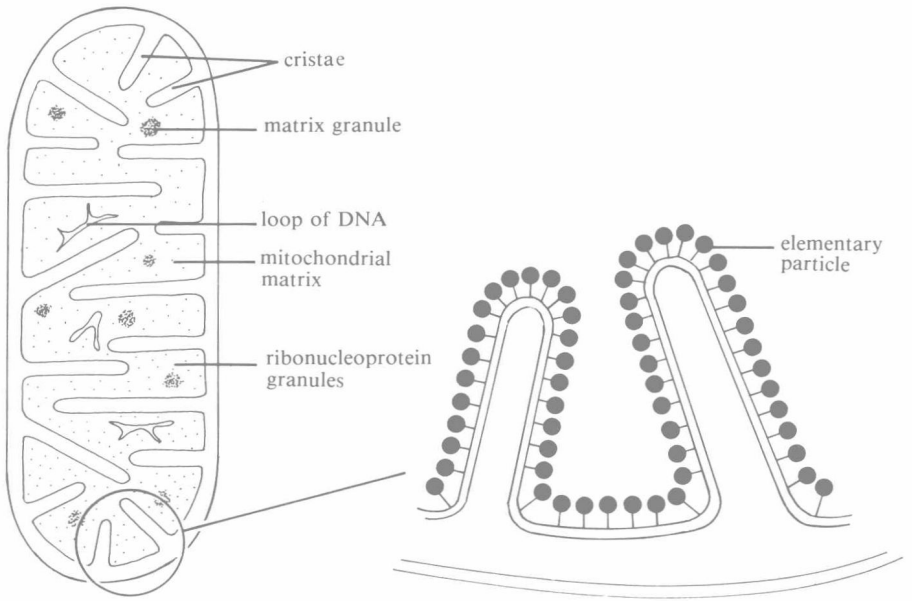

Figure 2-3. A mitochondrion.

shape to lollipops, the *elementary particles.* The round 10-nm head of the elementary particle is attached via a slender stalk to the inner membrane. The region of the mitochondrion enclosed by the folded inner membrane houses the *matrix.*

Mitochondria receive *pyruvate,* which has been generated from the metabolism of glucose, and they generate ATP by the process known as *oxidative phosphorylation.* The final generation of ATP occurs in a series of steps that are localized to certain compartments of the mitochondrion, as follows:

1. Krebs cycle enzymes are located in the mitochondrial matrix. Pyruvate is converted to carbon dioxide and reduced coenzyme (NADH).
2. Respiratory chain enzymes (dehydrogenase, flavoproteins, cytochromes) occupy the inner mitochondrial membrane (cristae). For each pair of hydrogen ions entering the chain as reduced coenzyme, three ATP molecules will ultimately be generated.
3. The elementary particles house the actual enzymes of oxidative phosphorylation and ATPase. Energy released from the respiratory chain oxidation-reduction reactions is stored as a high-energy phosphate bond in ATP. The energy required for the actual phosphorylation of ADP to ATP is now thought to result from a *chemiosmotic gradient* created by the respiratory chain enzymes, which pump their H^+ ions unidirectionally across the inner and outer membranes to the cytoplasm (Mitchell's chemiosmotic theory). The resultant gradient appears to drive the synthesis of ATP, which occurs in the elementary particles.

Lysosomes

Lysosomes are a rather heterogeneous class of organelles with a multitude of functions. While inconspicuous in the light microscope, they appear in the electron microscope as round, dense bodies 0.25 to 0.50 μm in diameter. Lysosomes are membrane-bound vacuoles containing hydrolytic enzymes with acid pH optima (acid hydrolases). These bags of enzymes are, in effect, mobile digestive systems that sequester lytic enzymes away from the cytoplasm at large. Cells digest two basic categories of substances. Digestion of a cell's own worn-out organelles or substances is called *autophagy,* while digestion of particles phagocytized from the external environment is called *heterophagy.*