Functional Histology

Second Edition

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Preface

This book was originally developed from a syllabus that has been distributed with favorable response to students of medicine and dentistry at Tufts University. Its value stems from a concise presentation of material that integrates histology with corresponding biologic functions. We have tried to establish a conceptual understanding of the histologic organization of cells, tissues, and organ systems through the use of representative diagrams. 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.

During the years following the first edition we received many comments, suggestions, and criticisms from both students and colleagues that have contributed significantly to the second edition. We believe that we have succeeded in producing a *practical* textbook, one that is both concise and comprehensive, functionally oriented, and well illustrated. The book has been thoroughly revised and updated to reflect current concepts and new developments. The incorporation of photomicrographs and additional diagrams is intended to complement the text and emphasize the link between structure and function. *Functional Histology* provides the student with a core of relevant information that can serve as a foundation for many other biomedical courses.

We are indebted to many people for their continued interest and dedication to the educational process. Karen Hitchcock, Ph.D., Chairperson of the Tufts Department of Anatomy and Cellular Biology, has been constantly supportive of our efforts and has done much to expedite the book's production through the use of departmental facilities. The encouragement of David Moffatt, M.D., Chairman of the Anatomy Department at the University of Missouri–Kansas City School of Medicine, is also acknowledged with appreciation. We thank Bruce Crary, Ph.D., for his many hours in the darkroom, printing and reprinting many of the micrographs and diagrams; and Philistia Bronston for the tedious job of typing much of the manuscript. We gratefully acknowledge the original contributions of Joan Borysenko, Ph.D., and Alvar Gustafson, Ph.D., who were among the authors of the first edition but have now moved on to other worthwhile endeavors. Finally, we thank all those, too numerous to name, who have kindly provided us with diagrams and micrographs of their original work and who have given us help and encouragement in the evolution of this book.

M. B.
T. B.

Contents

1

A Brief Introduction to Histologic Organization and Techniques of Microscopy

Overview of the Relationship Among 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 amebae 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 *morphologic* (structural) and *physiologic* (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, the osmotic properties of most 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 that 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 cellular components. The most common combination is hematoxylin and eosin (H and E). 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 a basic (cationic) dye. These tissue components are more accurately called *anionic*. Eosin is a red dye that stains the cytoplasm rather diffusely but that 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;* they are attracted to the negatively charged (anionic) component of the dye. There are many other stains used for special purposes, some of which show the phenomenon of *metachromasia*. The basic dye toluidine blue stains anionic regions blue, its orthochromatic color. However, in the case of certain compounds such as sulfated glycosaminoglycans, 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 tissue elements. This branch of histologic 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 as follows:

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 technique is widely used 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 because 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 in the following paragraphs.

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 5 nm, compared with 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.

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Cells and Tissues

I

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 genetic information is the *nucleus*. The cell is capable of receiving and reacting to a wide range of stimuli that trigger nuclear control mechanisms. Different genes become activated or repressed, and the genetic message is relayed to the cytoplasm, where it is converted into an appropriate response. 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* surrounded by a cell membrane called the *plasmalemma* (or *plasma membrane*). The cell's 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 (Fig. 2-1). These "plasms" are colloidal in that they may occur in the form of sols or gels and are combinations of various organic molecules, salts, and water. Protoplasm is the medium in which the specific cellular machinery, the *organelles*, is suspended and constitutes the primordial "electrolyte sea" or intracellular environment. The organelles that are suspended in this protoplasm conduct specific cellular activities.

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, which acts as a selective barrier, recognizing and admitting some molecules while excluding others. In addition, the diversity of functions performed by each cell require compartmentalization

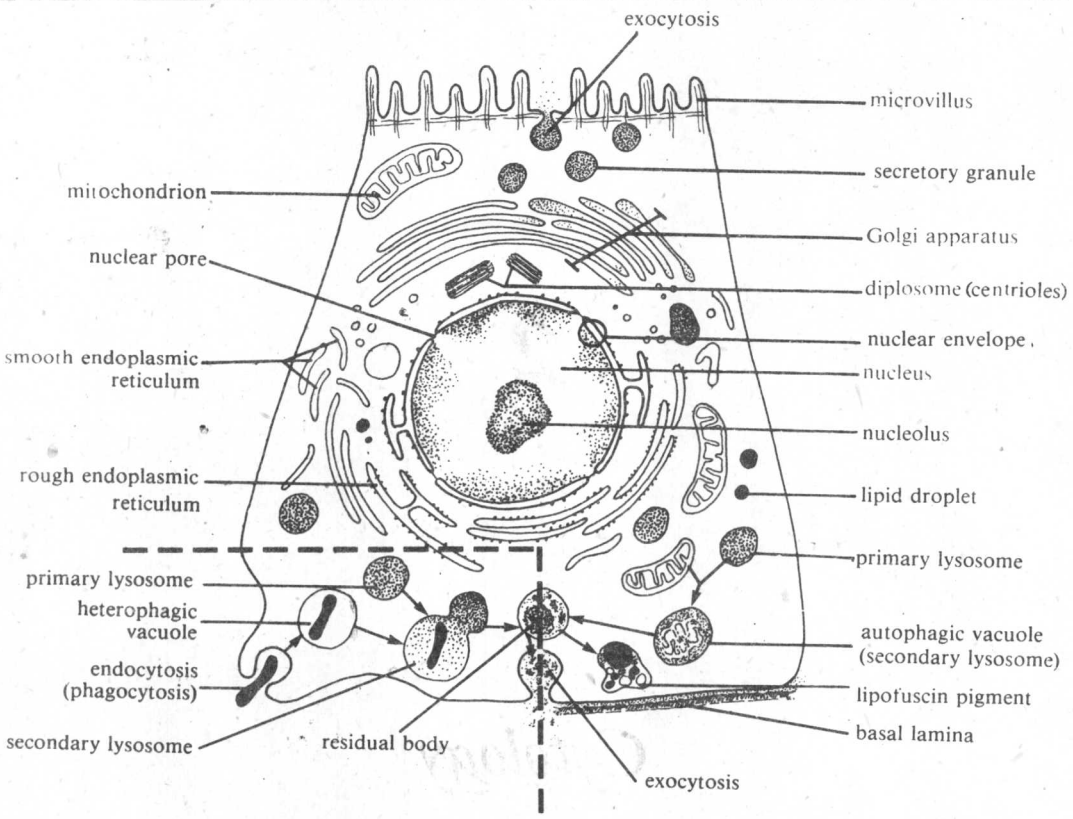

Fig. 2-1. A cell. The major organelles and some inclusions are shown. The exocytosis of a secretory granule is portrayed at the apex of the cell, while the cellular digestive system is depicted at the base of the cell.

within the cytoplasm to spatially segregate different classes of cellular activities. 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. The plasmalemma and the membranous organelles are complex structures composed of a *lipid bilayer* that is integrated with a variety of structural and functional proteins. The general composition of the plasmalemma is similar to that of internal membranes, but it differs in the types of lipid and protein present and in the additional presence of carbohydrate groups that project from the cell surface; these carbohydrate groups serve as receptors important in the recognition of molecular signals, such as hormones, impinging on the cell from its external environment.

Specific membrane proteins, which either are integral parts of the membrane or merely associated with its surface through relatively weak molecular bonds, are responsible for most of the physiologic activities conducted by that membrane. Hence, the membranes of different organelles that perform different functions within the cell are biochemically distinct with regard to at least some types of membrane proteins. Furthermore, regions of a membrane may exhibit molecular domains preferentially occupied by aggregates of one type of membrane protein that may be sparse or absent elsewhere in the same membrane. This has the effect

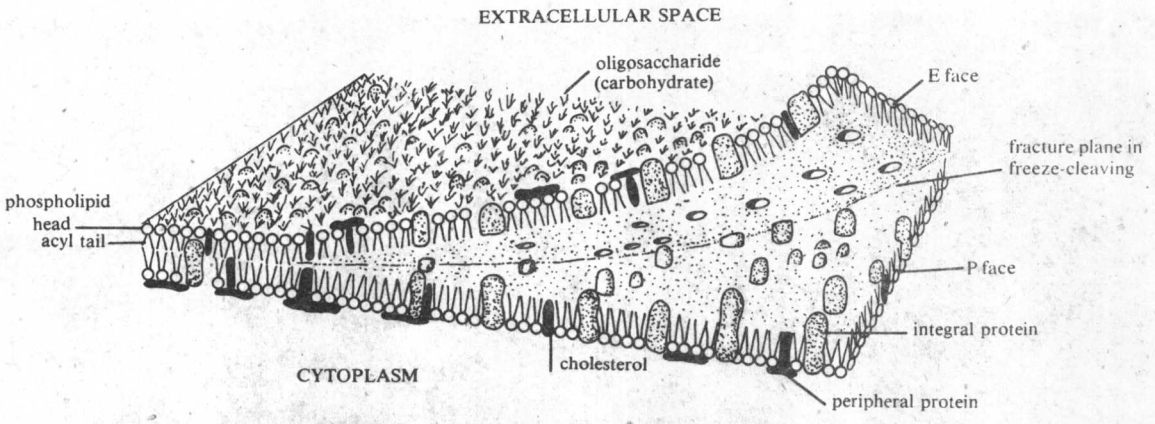

Fig. 2-2. The cell membrane or plasmalemma. In freeze-cleaving, the membrane is fractured along the lipid bilayer between the nonpolar acyl tails. The half of the membrane facing the cytoplasm is referred to as the P or protoplasmic face and retains most of the integral membrane proteins which give it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

of focusing membrane activities in specific sites and is only one example of the complex level of organization displayed by the cell (Figs. 2-2, 2-3).

Another level of functional and structural organization is found within the cell's *cytoskeleton*. The cytoskeleton is composed of a variety of protein filaments that confer structural support and help direct the functions of the organelles to specific cellular localities.

This section will describe the various organelles beginning with the plasmalemma. The cytoskeleton, sometimes referred to as a nonmembranous organelle, will be described in a following section.

Membranous Organelles

Cell Membrane (Plasmalemma)

The cell membrane (Fig. 2-4) 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 with 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 (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

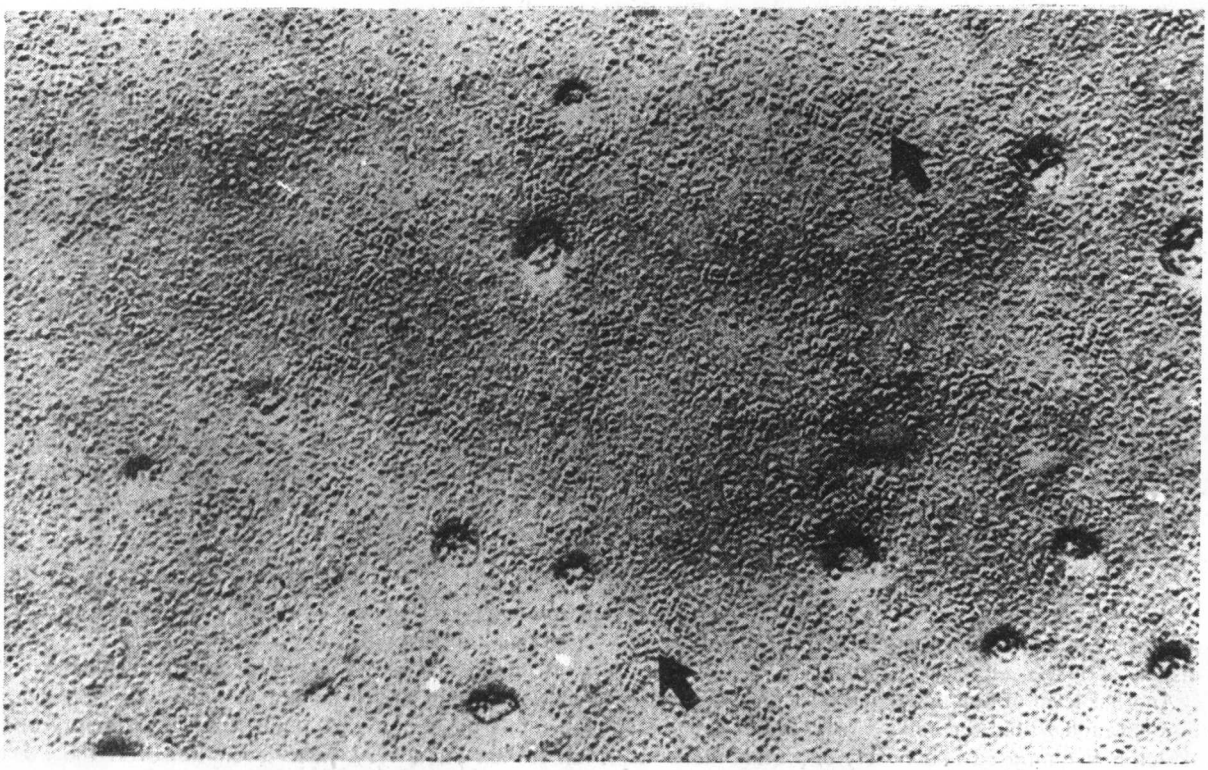

Fig. 2-3. Freeze-fractured replica of muscle cell plasmalemma. The outer or E-face leaflet of the plasmalemma has been fractured away to reveal intramembranous particles embedded in the protoplasmic leaflet of the plasmalemma. These particles are believed to represent macromolecules within the membrane. Some of the particles are aggregated into clusters (arrows), *but most have a less organized distribution.*

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 be liberated from the membrane only 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*, containing both hydrophilic and hydrophobic regions that allow them to penetrate the bilayer. Freeze-fracture electron micrographs reveal the inner region of the membrane because the fracture occurs along the pathway of least resistance—between the nonpolar lipid tails (see Figs. 2-2, 2-3). 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 wide range of activities from maintaining different ion concentrations on opposite sides of the membrane to transducing messages impinging on the cell surface into molecules that can trigger a specific nuclear 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