THE CELL

FAWCETT

Second Edition

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DON W. FAWCETT, M.D.
Hersey Professor of Anatomy
Harvard Medical School

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CONTRIBUTORS OF ELECTRON MICROGRAPHS

Dr. John Albright
Dr. David Albertini
Dr. Nancy Alexander
Dr. Winston Anderson
Dr. Jacques Auber
Dr. Baccio Baccetti
Dr. Michael Barrett
Dr. Dorothy Bainton
Dr. David Begg
Dr. Olaf Behnke
Dr. Michael Berns
Dr. Lester Binder
Dr. K. Blinzinger
Dr. Günter Blöbel
Dr. Robert Bolender
Dr. Aiden Breathnach
Dr. Susan Brown
Dr. Ruth Bulger
Dr. Breck Byers
Dr. Hektor Chemes
Dr. Kent Christensen
Dr. Eugene Copeland
Dr. Romano Dallai
Dr. Jacob Davidowitz
Dr. Walter Davis
Dr. Igor Dawid
Dr. Martin Dym
Dr. Edward Eddy
Dr. Peter Elias
Dr. A. C. Faberge
Dr. Dariush Fahimi
Dr. Wolf Fahrenbach
Dr. Marilyn Farquhar
Dr. Don Fawcett
Dr. Richard Folliot
Dr. Michael Forbes
Dr. Werner Franke
Dr. Daniel Friend
Dr. Keigi Fujiwara
Dr. Penelope Gaddum-Rosse
Dr. Joseph Gall
Dr. Lawrence Gerace
Dr. Ian Gibbon
Dr. Norton Gilula
Dr. Jean Gouranton
Dr. Kiyoshi Hama
Dr. Joseph Harb
Dr. Etienne de Harven
Dr. Elizabeth Hay
Dr. Paul Heidger
Dr. Arthur Hertig
Dr. Marian Hicks
Dr. Dixon Hingson
Dr. Anita Hoffer
Dr. Bessie Huang
Dr. Barbara Hull
Dr. Richard Hynes
Dr. Atsuchi Ichikawa
Dr. Susumu Ito
Dr. Roy Jones
Dr. Arvi Kahri
Dr. Vitauts Kalnins
Dr. Marvin Kalt
Dr. Taku Kanaseki
Dr. Shuichi Karasaki
Dr. Morris Karnovsky
Dr. Richard Kessel
Dr. Toichiro Kuwabara
Dr. Ulrich Laemmli
Dr. Nancy Lane
Dr. Elias Lazarides
Dr. Gordon Leedale
Dr. Arthur Like
Dr. Richard Linck
Dr. John Long
Dr. Linda Malick
Dr. William Massover
Dr. A. Gideon Matoltsy
Dr. Scott McNutt
Dr. Oscar Miller
Dr. Mark Mooseker
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Dr. Greta Tyson
Dr. Wayne Vogl
Dr. Fred Warner
Dr. Melvyn Weinstock
Dr. Richard Wood
Dr. Raymond Wuerker
Dr. Eichi Yamada

PREFACE

The history of morphological science is in large measure a chronicle of the discovery of new preparative techniques and the development of more powerful optical instruments. In the middle of the 19th century, improvements in the correction of lenses for the light microscope and the introduction of aniline dyes for selective staining of tissue components ushered in a period of rapid discovery that laid the foundations of modern histology and histopathology. The decade around the turn of this century was a golden period in the history of microscopic anatomy, with the leading laboratories using a great variety of fixatives and combinations of dyes to produce histological preparations of exceptional quality. The literature of that period abounds in classical descriptions of tissue structure illustrated by exquisite lithographs. In the decades that followed, the tempo of discovery with the light microscope slackened; interest in innovation in microtechnique declined, and specimen preparation narrowed to a monotonous routine of paraffin sections stained with hematoxylin and eosin.

In the middle of the 20th century, the introduction of the electron microscope suddenly provided access to a vast area of biological structure that had previously been beyond the reach of the compound microscope. Entirely new methods of specimen preparation were required to exploit the resolving power of this new instrument. Once again improvement of fixation, staining, and microtomy commanded the attention of the leading laboratories. Study of the substructure of cells was eagerly pursued with the same excitement and anticipation that attend the geographical exploration of a new continent. Every organ examined yielded a rich reward of new structural information. Unfamiliar cell organelles and inclusions and new macromolecular components of protoplasm were rapidly described and their function almost as quickly established. This bountiful harvest of new structural information brought about an unprecedented convergence of the interests of morphologists, physiologists, and biochemists; this convergence has culminated in the unified new field of science called cell biology.

The first edition of this book (1966) appeared in a period of generous support of science, when scores of laboratories were acquiring electron microscopes and hundreds of investigators were eagerly turning to this instrument to extend their research to the subcellular level. At that time, an extensive text in this rapidly advancing field would have been premature, but there did seem to be a need for an atlas of the ultrastructure of cells to establish acceptable technical standards of electron microscopy and to define and illustrate the cell organelles in a manner that would help novices in the field to interpret their own micrographs. There is reason to believe that the first edition of *The Cell: An Atlas of Fine Structure* fulfilled this limited objective.

In the 14 years since its publication, dramatic progress has been made in both the morphological and functional aspects of cell biology. The scanning electron microscope and the freeze-fracturing technique have been added to the armamentarium of the miscroscopist, and it seems timely to update the book to incorporate examples of the application of these newer methods, and to correct earlier interpretations that have not withstood the test of time. The text has been completely rewritten and considerably expanded. Drawings and diagrams have been added as text figures. A few of the original transmission electron micrographs to which I have a sentimental attachment have been retained, but the great majority of the micrographs in this edition are new. These changes have inevitably added considerably to the length of the book and therefore to its price, but I hope these will be offset to some extent by its greater informational content.

Twenty years ago, the electron microscope was a solo instrument played by a few virtuosos. Now it is but one among many valuable research tools, and it is most profitably used in combination with biochemical, biophysical, and immunocytochemical techniques. Its use has become routine and one begins to detect a decline in the number and quality of published micrographs as other analytical methods increasingly capture the interest of investigators. Although purely descriptive electron microscopic studies now yield diminishing returns, a detailed knowledge of the structural organization of cells continues to be an indispensable foundation for research on cell biology. In undertaking this second edition I have been motivated by a desire to assemble and make easily accessible to students and teachers some of the best of the many informative and aesthetically pleasing transmission and scanning electron micrographs that form the basis of our present understanding of cell structure.

The historical approach employed in the text may not be welcomed by all. In the competitive arena of biological research today investigators tend to be interested only in the current state of knowledge and care little about the steps by which we have arrived at our present position. But to those of us who for the past 25 years have been privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of the major contributors to our present understanding of its structure and function. In venturing to do this I am cognizant of the hazards inherent in making judgments of priority and significance while many of the dramatis personae are still living. My apologies to any who may feel that their work has not received appropriate recognition.

It is my hope that for students and young investigators entering the field, this book will provide a useful introduction to the architecture of cells and for teachers of cell biology a guide to the literature and a convenient source of illustrative material. The sectional bibliographies include references to many reviews and research papers that are not cited in the text. It is believed that these will prove useful to those readers who wish to go into the subject more deeply.

The omission of magnifications for each of the micrographs will no doubt draw some criticism. Their inclusion was impractical since the original negatives often remained in the hands of the contributing microscopists and micrographs submitted were cropped or copies enlarged to achieve pleasing composition and to focus the reader's attention upon the particular organelle under discussion. Absence was considered preferable to inaccuracy in stated magnification. The majority of readers, I believe, will be interested in form rather than measurement and will not miss this datum.

Assembling these micrographs illustrating the remarkable order and functional design in the structure of cells has been a satisfying experience. I am indebted to more than a hundred cell biologists in this country and abroad who have generously responded to my requests for exceptional micrographs. It is a source of pride that nearly half of the contributors were students, fellows or colleagues in the Department of Anatomy at Harvard Medical School at some time in the past 20 years. I am grateful for their stimulation and for their generosity in sharing prints and negatives. It is a pleasure to express my appreciation for the forbearance of my wife who has had to communicate with me through the door of the darkroom for much of the year while I printed the several hundred micrographs; and for the patience of Helen Deacon who has typed and retyped the manuscript; for the skill of Peter Ley, who has made many copy negatives to gain contrast with minimal loss of detail; and for the artistry of Sylvia Collard Keene whose drawings embellish the text. Special thanks go to Elio and Giuseppina Raviola who read the manuscript and offered many constructive suggestions; and to Albert Meier and the editorial and production staff of the W. B. Saunders Company, the publishers.

And finally I express my gratitude to the Simon Guggenheim Foundation whose commendable policy of encouraging the creativity of the young was relaxed to support my efforts during the later stages of preparation of this work.

DON W. FAWCETT
Boston, Massachusetts

CONTENTS

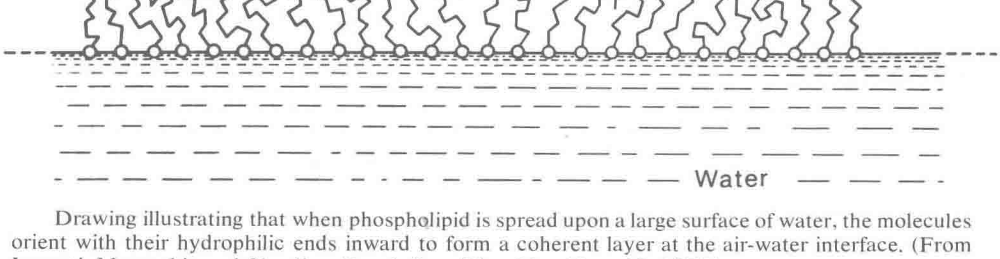
CELL SURFACE

CELL MEMBRANE

The cell surface regulates the traffic of ions and macromolecules in and out of the cell. Although deceptively simple in microscopic appearance, it is remarkably complex in its molecular organization, possessing devices for attachment to other cells, specializations for cell-to-cell communication, antigenic macromolecules that are the basis for cell recognition and tissue specificity, ion pumps for regulating the internal milieu of the cell, receptors for hormones and other environmental signals, and mechanisms for generation of second messenger molecules that activate the cell's physiological responses. Understanding the biogenesis, structural organization, and functions of the cell surface is now one of the major challenges in cell biology.

The presence of a limiting *cell membrane* was inferred from indirect evidence nearly a century before it could be visualized microscopically. Nägeli in 1855 described the formation of a protective film where outflowing cytoplasm of an injured cell came into contact with water. He called this the *plasma membrane,* showed that it was semipermeable, and speculated that it was responsible for the osmotic phenomena exhibited by living cells. Forty years later, the permeability of living cells was exhaustively studied by Overton (1899), who noted a correlation between the lipid solubility of substances and their rate of entry into cells and suggested that the cell surface was probably lipid in nature. However, the reality of the cell membrane was not widely accepted by morphologists until Chambers (1926) developed microsurgical techniques that made it possible to deform, tear, or penetrate it with fine glass dissecting needles.

The modern concept of the biochemical nature of the cell membrane dates from Langmuir's (1917) demonstration that when fatty acids or phospholipids are dissolved in benzene and a few drops are placed on a large surface of water, the molecules orient with their hydrophilic ends inward to form a coherent layer at the air-water interface.



Drawing illustrating that when phospholipid is spread upon a large surface of water, the molecules orient with their hydrophilic ends inward to form a coherent layer at the air-water interface. (From Luzzati, Mussachia and Skoulios, Farad. Soc. Disc. No. 43, p. 43, 1958.)

Taking advantage of this property, Gorter and Grendel (1925) extracted the lipids from erythrocytes, measured the surface area covered when spread as a monolayer, and found it to be twice the calculated surface area of the original erythrocytes. Therefore it was suggested that the cells were enclosed by a lipid layer two molecules thick. This interpretation of the cell membrane as a bimolecular layer of mixed phospholipids has withstood the test of time. Later investigations by polarization optics, x-ray diffraction, measurement of their thickness, and assessment of their electrical properties have all been consistent with orientation of the hydrocarbons of the bilayer perpendicular to the plane of the membrane.

The observation that the surface tension of artificial membranes composed entirely of neutral lipids is high while that of natural membranes is relatively low led Davson and Danielli (1935) to postulate a layer of protein on both sides of the lipid bilayer. This model dominated physiological thought concerning the organization of the cell membrane for the next 20 years.

With the advent of the electron microscope, the cell surface membrane was visualized in thin sections as two dense lines separated by a less dense middle layer. This trilaminar appearance was also characteristic of membranes in the interior of the cell and was termed by Robertson (1957) the "unit membrane." The three layers were thought to be the visual expression of a structural organization common to all biological membranes. The two dense lines were attributed to deposition of osmium from the fixative on the hydrophilic ends of the phospholipid molecules, and in outer layers of protein while the unstained middle layer was believed to represent the saturated hydrocarbon chains of the lipid bilayer.

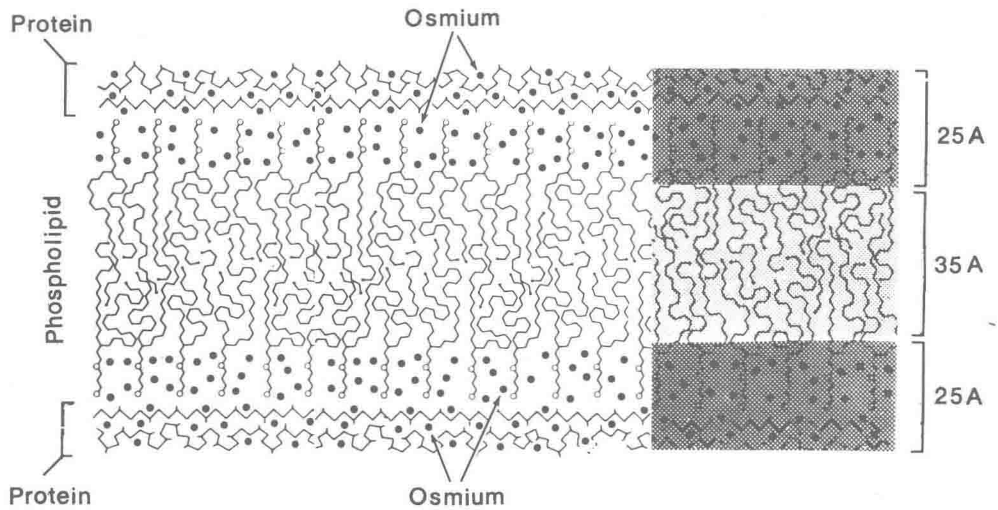


Diagram illustrating the initial interpretation of the trilaminar appearance of cell membranes in electron micrographs as a confirmation of the Davson-Danielli model. Deposition of osmium in layers of protein and in the hydrophilic ends of the phospholipids was thought to be responsible for the two dense lines. (Modified after W. Stoeckenius.)

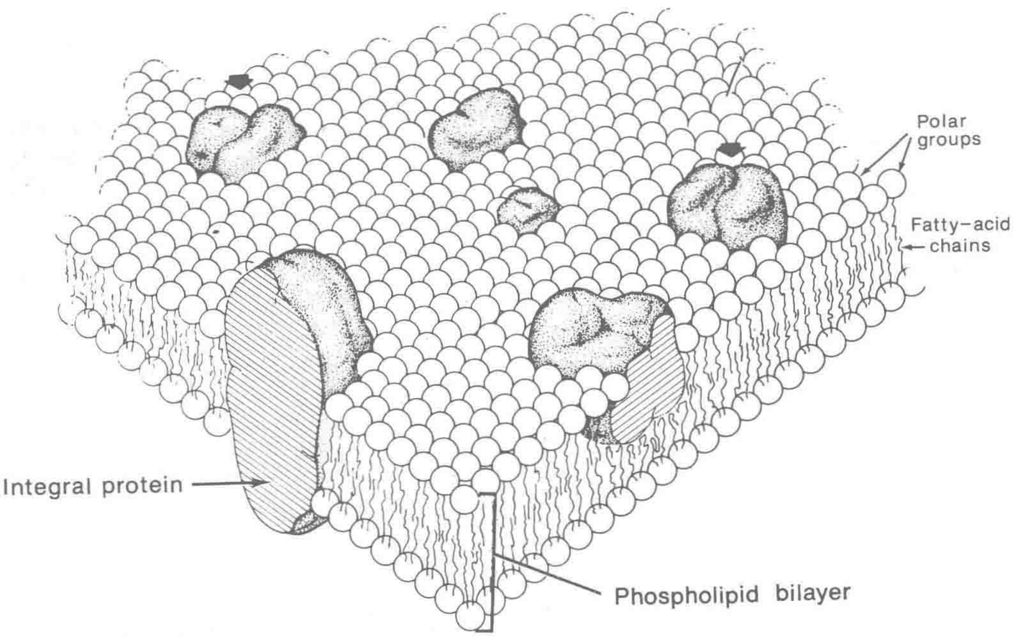
Thus the trilaminar appearance of the unit membrane was widely accepted as electron microscopic confirmation of the general features of the Davson-Danielli models, even though the observation that myelin forms of pure phospholipid had a similar trilaminar appearance in section (Revel, Ito, and Fawcett, 1953) should have cast some doubt upon this interpretation.

Throughout the 1950s, there was great emphasis upon the universality of the unit membrane and the continuity of the membrane systems of the cell. Diagrams depicting continuity of the plasmalemma with the endoplasmic reticulum were widely reproduced, and a hypothetical schema suggesting the origin of mitochondria from invaginations of the plasmalemma found its way into textbooks. These concepts gained considerable acceptance even though convincing electron microscopic evidence was lacking. On the other hand, some investigators felt that this emphasis on the universality of the unit membrane was diverting attention from important biochemical differences that must exist in the membranes of organelles having distinctive functions. Improved methods for isolation of membrane fractions for biochemical analysis were revealing significant differences in lipid composition and enzymatic activities of membranes from various organelles. It became apparent that the biochemical heterogeneity and physiological specificity of membranes were not expressed in morphological differences detectable in electron micrographs of thin sections. In the late 1950s, it seemed unlikely that electron microscopy would contribute further to an understanding of the internal organization of membranes.

This pessimistic prediction proved to be quite wrong. The timely development of the method of freeze-fracturing by Steere (1957) and Moor and coworkers (1961) made the interior of the membrane accessible to morphological observation and ultimately led to an entirely new interpretation of membrane structure. The method is based upon the principle that by evaporation of platinum and carbon in a vacuum, a high fidelity replica can be made of the surface of frozen hydrated biological material. Small blocks of tissue fixed in glutaraldehyde are immersed in a solution of a cryoprotectant, such as glycerin, to avoid severe distortion from ice crystal formation during freezing. Freezing rate is maximized by freezing in fluids that have a high heat capacity and a low freezing point. In the commonest procedure, the tissue fragments are immersed in the fluid phase of partially solidified dichlorodifluoromethane (Freon) cooled at $-150°$ C with liquid nitrogen. The rapidly frozen specimen is then fractured in a vacuum chamber by impact of the edge of a knife cooled at $-196°$ C. A replica of the fracture surface is next prepared by evaporation of a heavy metal such as platinum from a source at an acute angle to the fracture surface. Metal is thus deposited on all surface elevations on the side toward the source. This results in an enhanced three-dimensional appearance in electron micrographs, comparable to the exaggeration of surface contours that results from oblique lighting or the shading used in graphic arts. To provide greater coherence and stability, carbon is then deposited uniformly on the entire surface from a separate electrode directly above the specimen. The vacuum is broken and the coated specimen is immersed in a solution of acid or sodium hypochlorite to dissolve the tissue. The replica remaining behind is gently washed and picked up on a specimen grid for examination with the transmission electron microscope. Regions of dense metal deposit on convex surfaces toward the source deflect or absorb electrons while the metal-free areas transmit electrons. After the resulting negatives are printed, a three-dimensional image of surface relief is seen in which the elevations are dark and their shadows are light. When this method was first developed, it was thought that frozen cells fractured along membranes revealing their true surface. It was later shown by Branton (1966) that the fracture preferentially follows a path of least resistance through the hydrophobic region of lipid bilayers, thus cleaving membranes in half and exposing extensive areas of their interior.

The Davson-Danielli model of the membrane envisioned no heterogeneity within the plane of the lipid bilayer and would have led to the prediction of two smooth, featureless fracture faces. Instead, freeze-fractured membranes presented two distinct appearances. The outwardly directed inner half-membrane, called the *P-face*, contained numerous randomly distributed globular particles 6 to 9 nm in diameter. The inwardly directed outer half-membrane, called the *E-face*, was relatively smooth, containing only about a fifth the number of particles found on the other fracture face.

The finding of globules of protein within the plane of the membrane provided compelling morphological evidence for the *fluid-mosaic model* of the cell membrane proposed by Singer (1971) on the basis of thermodynamic considerations and the known properties of proteins.



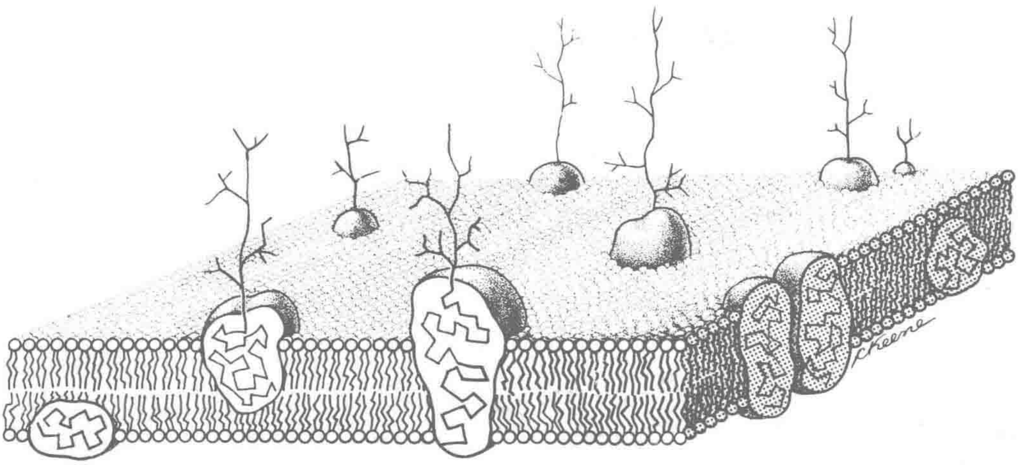
The current fluid-mosiac model of the cell membrane which envisions a two-dimensional solution of oriented lipids and globular proteins. The lipid bilayer is assumed to be fluid and the integral proteins are free to diffuse laterally within the plane of the membrane if not restrained by interaction with peripheral proteins in the underlying cytoplasm. (From S. J. Singer and G. L. Nicolson, Science 175, 720, 1972.)

According to this model, the membrane is a two-dimensional solution of oriented lipids and globular proteins. Like the phospholipid molecules of the bilayer, the protein molecules are assumed to be amphipathic. That is, they possess asymmetrical hydrophilic and hydrophobic regions. Thermodynamic considerations dictate that ionic amino acid and saccharide residues of integral glycoproteins would assume a position in the hydrophilic portion, and the non-ionic portions would preferentially localize in the hydrophobic interior of the membrane. Depending upon their secondary structure and the distribution of their hydrophilic and hydrophobic regions, the integral proteins would take up different positions in membrane. Some would have their oligosaccharide regions and polar amino acids exposed on the outer surface and their nonpolar region in the hydrophobic interior. Others with polar regions on either end and a nonpolar segment in the middle would extend through the entire thickness of the membrane. Freeze-fracture observations provided unambiguous evidence that protein particles are indeed embedded in membranes and not limited to layers on either side of the bilayer.

Using an electron microscopic technique for localizing specific antigenic proteins, Singer and Nicolson (1972) demonstrated that the integral proteins in unspecialized regions of membrane are essentially random in their distribution. Erythrocytes were treated with saturating amounts of antibody to Rh antigens and the membranes were then exposed to ferritin-labeled anti–human gamma globulin. The distribution of electron-opaque ferritin molecules visualized in the electron micrographs thus corresponded to the distribution of single antigen sites on the membrane. These were dispersed in a random two-dimensional array.

The fluid nature of the lipid bilayer and mobility of integral protein particles within the membrane were demonstrated in ingenious experiments by Frey and Edidin (1970). Human and mouse cells in coculture were induced to fuse, using Senai virus as the fusing agent. The distribution of human and mouse antigenic components was largely confined to their respective halves of the fused cell, but after 40 minutes they were intermixed and uniformly distributed over the entire surface, indicating that the proteins had diffused within a fluid membrane. Numerous additional demonstrations of lateral mobility of membrane proteins have since been reported.

In the 1960s, morphologists and biochemists seemed to be on diverging paths, with the structural uniformity implicit in the unit membrane concept at odds with the accumulating evidence of biochemical diversity among membranes. Freeze-fracturing and other technical advances have now brought about a gratifying convergence of the morphological and biochemical concepts of membrane structure. The number of particles per unit area in freeze-fracture replicas of membranes from different organelles correlates well with analyses of the protein content and degree of physiological activity of the same membranes. The fluid mosaic model is consistent with the great majority of morphological, biochemical, and immunological observations and is now generally accepted as the basis for further investigation of membrane-mediated phenomena.



Schematic representation of amphipathic proteins taking up positions relative to the lipid bilayer that are determined by the distribution of their hydrophilic and hydrophobic residues. Saccharide residues of integral glycoproteins projecting from the outer surface contribute to the glycocalyx.

When it became possible to examine thin sections of cells with the electron microscope, the plasmalemma always appeared as a linear profile consisting of two dark lines, about 3.5 nm thick, separated by an intermediate light zone of similar width. This came to be called the *unit membrane* and was widely interpreted as a morphological confirmation of the Davson-Danielli model. The dense lines were thought to result from deposition of osmium in the protein and hydrophilic ends of the phospholipids, while the light line was believed to correspond to the hydrophobic region of the lipid bilayer. In light of the more recent demonstration by freeze-fracturing that the protein constituents do not form layers on either side of the lipid bilayer but occur in particulate form within the plane of the membrane, this interpretation has been modified. The protein of the membrane does not appear to contribute significantly to its density in electron micrographs. The assumption that osmium is deposited in the polar ends of the phospholipids but not in the hydrocarbon chains may still be valid.

Shown here are the surface membranes of two adjoining cells separated by a 15 nm intercellular cleft occupied by material of low electron density assumed to consist mainly of carbohydrate.

Figure 1. Boundary between two glial cells in the central nervous system of the annelid *Aphrodite*.

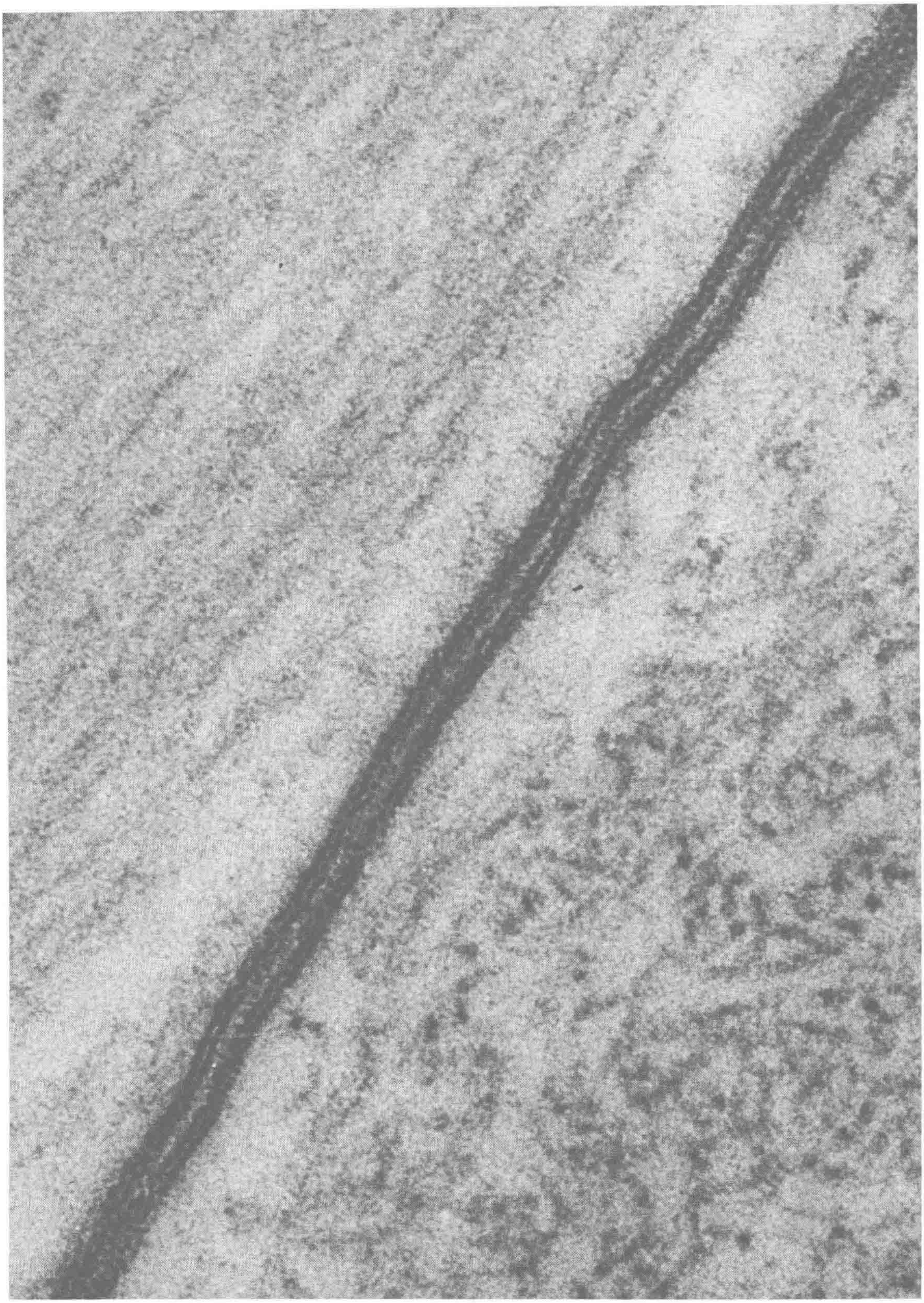


Figure 1

Where the plasmalemma has an uneven contour, its orientation with respect to the plane of the thin section changes and the trilaminar appearance of the membrane is not seen if the plane of section is slightly oblique. In the accompanying micrograph of the brush border of intestinal epithelium, the consistent orientation of the closely packed microvilli facilitated obtaining true transverse sections of their limiting membrane throughout the field. The cross section of each microvillus is bounded by two dense lines of similar thickness separated by a lighter intermediate zone. Not all cell membranes exhibit this degree of symmetry. In some the outer dense line is thinner than the inner.

Figure 2. Intestinal microvilli of a cat. (Micrograph courtesy of Susumu Ito.)