Second Edition *Asian Edition*

MEDICAL LABORATORY TECHNOLOGY

AND CLINICAL PATHOLOGY

B. SAUNDERS COMPANY · PHILADELPHIA · LONDON · TORONTO · 1969

W. B. Saunders Company: West Washington Square
Philadelphia, Pa. 19105

12 Dyott Street
London W. C. 1

1835 Yonge Street
Toronto, Ontario 295

Medical Laboratory Technology and Clinical Pathology

PREFACE TO THE SECOND EDITION

The success which met the first edition of *Medical Laboratory Technology* has answered the authors' question as to the value of their effort, at that time, to present a single volume which incorporated not only the "how" but also the "why" of laboratory technology.

Since 1963, laboratory medicine and medical technology have continued to grow in logarithmic progression. Also, there is now less time between discovery and application. Not only is communication rapid but competing laboratory suppliers are swift to make apparatus and convenient reagents available.

We have tried to keep the length of the text to a single volume, and in this effort some material may have been excluded. Nevertheless, in places the frontiers of medical knowledge are visited, especially where these further vistas help in conveying a better appreciation of older and often previously empiric areas. If acts of omission have been made, acts of commission, we hope, have been avoided.

That the text has grown appreciably in size is due to the expansion of knowledge and to the inclusion of new topics, as well as a wider coverage of clinicopathologic aspects of laboratory medicine. The result, we hope, will prove useful to technologists and pathologists alike, both in training and in practice, and to teachers of laboratory medicine at all levels.

Naturally, in a work of this scope much is owed by a few to many. Among the many are the following: Dr. J. A. Lowden, The Hospital For Sick Children, Toronto, for the chemistry of neurolipids and their analysis by thin-layer chromatography; Dr. P. E. Conen and Mrs. N. Czegledy-Nagy, The Hospital For Sick Children, Toronto, and Dr. D. H. Carr and Mrs. M. Corin, University of Western Ontario, London, Canada, for the many karyotypes reproduced in Chapter 49; Dr. D. W. Thompson, Toronto General Hospital, for the use of many of his excellent exfoliative cytology smears; Dr. C. Ezrin, the Banting Institute, Toronto, for making freely available the results of his researches on staining of the adenohypophysis; Dr. J. Bain and Mr. G. Penz of Dr. Ezrin's laboratory for their precision immunofluorescence technique; Dr. H. Z. Movat and Dr. J. W. Steiner, University of Toronto, for electron micrographs; Dr. M. Moscarello, The Hospital For Sick Children, Toronto, for help and advice on methods for immunoglobulin assay; Dr. Jan Schwarz, Cincinnati, Ohio, and Miss

M. Finlayson, Toronto, for constructive criticism of the section on microbiology; Mrs. Carolyn B. Telford, The Hospital For Sick Children, Toronto, for the many sections prepared for new illustrations and for her helpful suggestions and critical reading of the section on histopathologic technique; Mr. William Wilson, The Hospital For Sick Children, Toronto, for his modification of the Bowie stain; Mr. William H. Bryson, Department of Visual Education, The Hospital For Sick Children, Toronto, for the countless photographic prints from which most of the new illustrations have been selected; and Miss E. Forster, Department of Ophthalmic Pathology, University of Toronto. Finally, our thanks are due to Mr. Robert B. Rowan, Medical Editor, and to Mr. Carl M. Katila, Jr., Production Manager, and to other members of the staff of the W. B. Saunders Company for their patient cooperation.

M. J. L.

S. S. R.

L. D. M.

P. D. S.

M. J. I.

CONTENTS

SECTION THREE MICROBIOLOGY

SECTION FOUR HISTOLOGIC TECHNIQUES

SECTION ONE

GENERAL KNOWLEDGE AND CHEMICAL PATHOLOGY

CHAPTER 1 Cell Organization and Function*

An average mammalian cell is some 15 to 20 μ in diameter and has a volume of 3000 to 6000 μ^3. Within the confines of this tiny space is a completely automated, computerized factory of an elegance and complexity that surpasses the imagination. In the short space of the past 15 years, utilizing and expanding the store of knowledge that man has laboriously amassed to develop refined techniques and apparatus, we have come to learn a great deal about this basic unit of life. By combining cell fractionation studies of the biochemists with electron microscopy, which gives a resolution of 10 to 5 Å (Å = one ten-millionth mm.), i.e., some 200 to 400 times the resolving power of the light microscope, many of the secrets of the cell have been laid bare. We shall begin here with a brief description of the cell and its component parts and follow with a more detailed account, in which function will be related to structure.

ANATOMY OF THE CELL

Each cell is bounded by a limiting membrane, within which are three main compartments, the cytoplasm, spaces and channels separated from the cytoplasm by membranes, and the nucleus. The cytoplasm itself may be regarded as a mixed aqueous and colloid solution which manifests many of the properties of a gel. In some cells the rim of cytoplasm just beneath the membrane is more viscous and gel-like and is called the ectoplasm, as distinct from the more fluid endoplasm. Clear, fluid areas of cytoplasm are sometimes designated hyaloplasm.

Some 10 to 20% of the cell mass is composed of protein, but most of this is in the framework of the various membrane-bounded structures. An elastic structural protein, stromatin, is present in the cytoplasm, but its amount varies greatly with cell type. Dissolved in the cytoplasm are: (1) basic raw materials such as electrolytes, amino acids, lactic acid, pyruvate, glucose, many other precursor substances, and intermediary metabolites; (2) many of the cell's small, mobile machine tools, e.g., transfer ribonucleic acids (t-RNA), enzymes subserving intermediary metabolism of the cell, such as those involved in the initial steps of glucose breakdown, anaerobic glycolysis, and the phosphogluconate shunt, and probably also those that break down lipids to acetoacetate and those that synthesize and break down glycogen, and many more; (3) waste products, e.g., creatinine, uric acid, and urea. Small to large stores of "fuel" are often seen in the cytoplasm, e.g., glycogen granules and lipid droplets.

Cytoplasmic Ribosomes

Also free in the cytoplasm are some of the tiny "jigs" that manufacture protein, i.e., the ribosomes. These are frequently arranged in groups of five, sometimes more, sometimes less. They make the proteins required for "home use" and are numerous in actively dividing cells and also in normoblasts and reticulocytes, where they are busy making hemoglobin. Abundance of cytoplasmic ribosomes accounts for the

*The beginner will find it more convenient to read this chapter only as far as "The Cell Membrane," page 6, at first. Later, when a wider appreciation of the whole field of laboratory medicine and techniques has been gained, the entire chapter may be read with greater ease and profit.

diffuse basophilia of such cells with conventional stains.

Membranous Structures in Cytoplasm

Many membrane-bounded structures may be seen in the cytoplasm. Most prominent, especially in cells such as those of pancreatic acini or plasma cells which manufacture proteins "for export," is the *endoplasmic reticulum* (ER). In digestive enzyme-secreting cells, like those of the acini of the pancreas, tubules of ER are most abundant in the basal portions of the cytoplasm. Here, the outer aspects of the ER membranes are studded with tiny granules. These are fixed ribosomes (as distinct from those free in the cytoplasm), and they tend to be attached in spirals, curved rows, and double rows to the cytoplasmic aspects of the ER membranes. This system of tubules studded with ribosomes is descriptively called the rough-surfaced endoplasmic reticulum (r-s ER), or ergastoplasm. As such it is distinct from the smooth-surfaced endoplasmic reticulum (s-s ER), which is a similar system of membrane-bounded tubules but without ribosome granules. The s-s ER almost certainly communicates with the r-s ER and may actually be a continuous part of the latter. Indeed, there is strong evidence for an all-pervading, intercommunicating network of channels within the cell: thus the ER communicates with the membranous channel or space around the nucleus, the perinuclear cisterna, and with the membranous complex next to be described.

Golgi Complex

Near the nucleus, and generally on its luminal aspect in the case of secretory cells, is the Golgi apparatus or complex, a system of flattened cisternae stacked on top of each other with vesicles and outpouchings at its periphery, all bounded by smooth membranes. Bordering the Golgi complex one frequently sees elements of both s-s and r-s ER; unequivocal evidence of a functional communication between all three elements exists.

Secretion Granules

Between the Golgi complex and the luminal border of an exocrine cell large, spherical, dense bodies bounded by smooth membrane may be seen. These are secretion or zymogen granules waiting to be discharged into the lumen of the

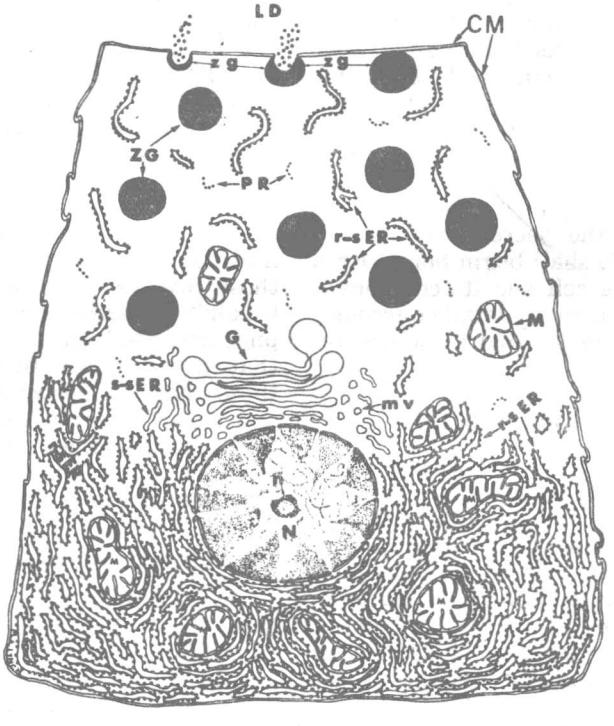

Figure 1-1. Pancreatic acinar cell. *LD*, Lumen of ductule; *ZG*, zymogen granules; *zg*, same in process of secretion; *PR*, polyribosomes; *r-s ER*, rough-surfaced endoplasmic reticulum, very abundant in basal (lower) part of cell, where it has long been referred to as ergastoplasm; *G*, Golgi complex; *mv*, microvesicles; *M*, mitochondrion; *s-s ER*, smooth-surfaced endoplasmic reticulum; *N*, nucleus; *n*, nucleolus; *CM*, cell membrane.

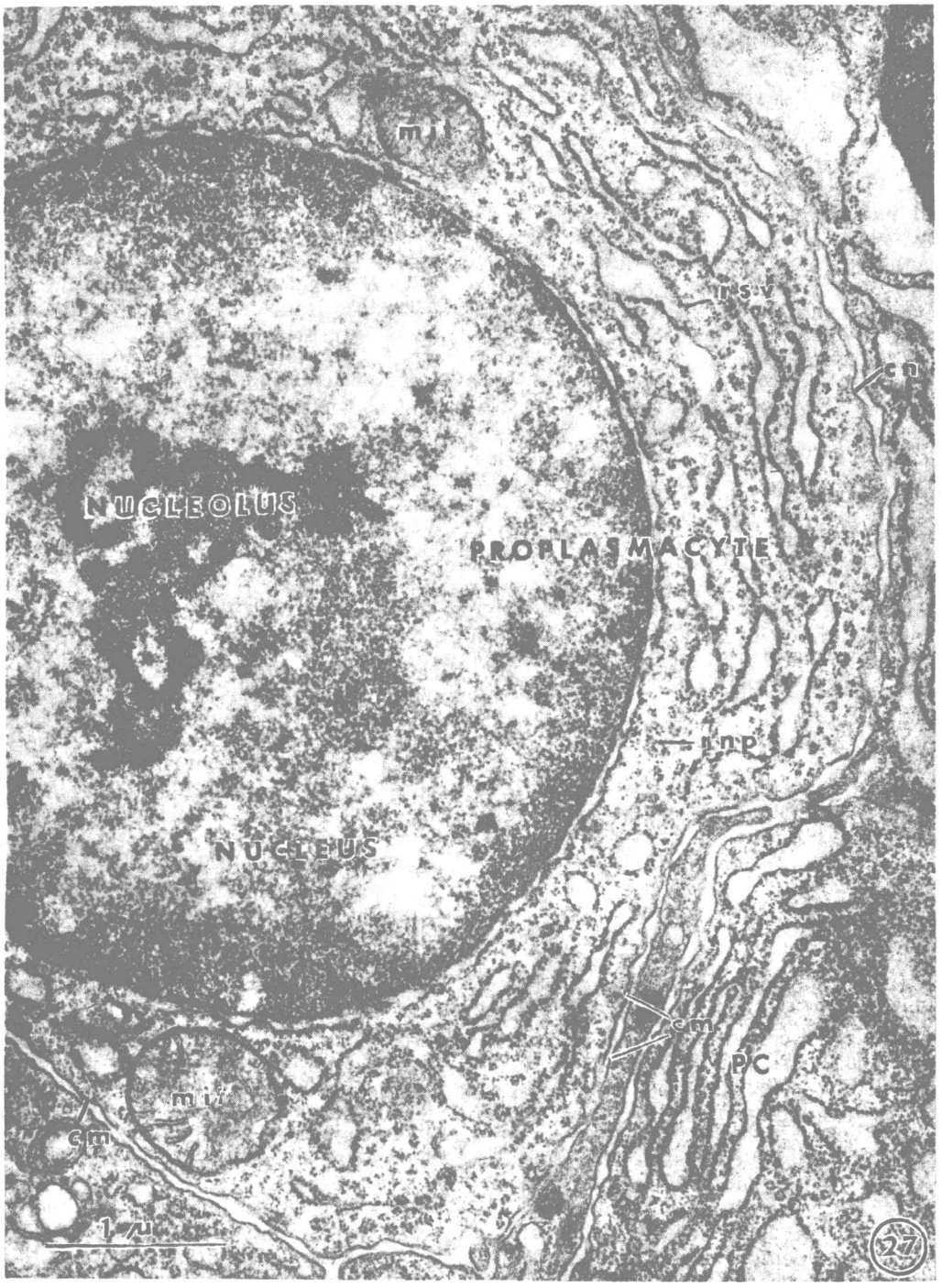

Figure 1-2. Portion of proplasmacyte. Note the large nucleus and nucleolus; also, numerous, moderately dilated rough-surfaced vesicles of endoplasmic reticulum (*rsv*). This cell still resembles the plasmablast, but is unlike the mature plasma cell in possessing fairly numerous free ribonucleoprotein granules (*rnp*) in its cytoplasm between the rough-surfaced vesicles. *mit*, Mitochondrion; *cm*, cell membrane. Lead hydroxide × 24,000. (From Movat, H.Z., and Fernando, N.V.P.: The fine structure of the lymphoid tissue during antibody formation. Exp. Molec. Path., 4:155, 1965. By kind permission of Dr. Henry Movat of the Banting Institute, Toronto, and the publishers, Academic Press Inc., New York.)

acinar ductule. In this region, scattered between the secretion granules, some tubules of r-s ER may be seen, but they are very much less numerous than in the basal portion of the cell.

Mitochondria

These are sausage-shaped structures having a double-layered membranous wall. Their inner membrane forms many projections or partitions that jut into the cavities of these bodies, much like the plates of a storage battery. Indeed, these are the "powerhouses" of the cell, supplying 70 to 90% of its energy requirements. An average cell contains about 200 mitochondria, but an active liver cell may contain well over 1000. A typical mitochondrion is 2 to 2.5 μ in length and 0.5 to 0.75 μ in width, but mitochondria may be as large as $7 \times 1\ \mu$. Their shape varies greatly.

Lysosomes

These are round, membrane-bounded bodies, approximately 0.25 to 0.75 μ in diameter. In the inactive state they contain finely granular material of low electron density; in the active state they contain variably dense or even vacuolated material, in which the remains of organized structures may be seen.

Microbodies (Peroxisomes)

These have often been confused with lysosomes. So far, they have been found only in liver and kidney cells. They are round bodies about 0.5 μ in diameter and often display a dense core.

Nucleus

In most cells the nucleus is situated near the middle of the cell body, but there are many exceptions; e.g., in epithelial cells it is commonly in the basal half of the cell. In an average cell it is about 5 μ in diameter and has a volume of 16 μ^3. It is bounded by a double nuclear membrane and contains a finely granular material of variable density. This granular material represents the chromosomes, and the light material between the granules is the nuclear sap. Within the nucleus but not separated from it by any membrane or structure other than a slight concentration of chromatin (nucleolus-associated chromatin) are one or two nucleoli. These vary in size and number with cell type, but for each cell type the total nucleolar mass is constant. Theoretically, the maximum number of nucleoli in human cells is 10, but this number is never seen since they tend to fuse. Nucleolar size is large in cells actively synthesizing proteins.

RESUME OF CELL FUNCTION

Before entering into more detailed considerations it may be helpful at this juncture to give a brief outline of how the cell operates. In the opening paragraph it was stated that the cell is automated and computerized: if anything, that is an understatement. To do its work the average cell requires hundreds of enzymes: the figure may prove to be higher in some cases. These enzymes are protein and most are specific. Their specificity derives from certain sequences of amino acids often coupled with a specifically shaped molecule. The master record or "tape" controlling the manufacture of these enzymes resides in the deoxyribonucleic acid (DNA) strands of the chromosomes in the nucleus. When called upon, the DNA strands make a complementary copy of whatever section (gene) of its code the immediate needs of the cell, local tissue, or body as a whole demand. Toward the end of this chapter we shall consider how the cell responds to local and general body needs; here we shall confine ourselves to its own requirements.

Feedback Control in Cell

By means of a sensitive feedback system the nucleus senses the needs of its own cell body. If a certain substance, essential to the cell, is running low, the nucleus is alerted—either by the low concentration of the substance in the nuclear sap or by accumulation of other substances whose metabolism is contingent upon adequate supplies of the substance in question, or by both. The nucleus then "searches its memory" and within hundredths of a second it has produced several templates for the manufacture of the enzyme required to produce the substance. (The reverse of this control also operates; i.e., excess of the substance tells the nucleus to stop producing templates and, as if this were not enough safeguard, the excess clogs the enzyme itself and the ribosomal jigs producing it, so that the entire assembly line is quickly brought to a halt.) The templates that the nucleus produces are strands of messenger ribonucleic acid (m-RNA). These are carried out into the cytoplasm through pores in the nuclear membrane. In the cytoplasm an unemployed ribosome attaches to the end of the m-RNA strand bearing the start of the code for the enzyme to be manufactured; then, by a ratchet system, as it were, the ribosome advances along the strand of m-RNA, adding the specific amino acid called for by each cog of the ratchet. Somewhat later another free ribosome does likewise, and in due course some five ribosomes are spaced out along the m-RNA strand, each busy making a complete molecule of the enzyme protein in question. When a ribosome comes to the end of the m-RNA strand, it falls off, as it

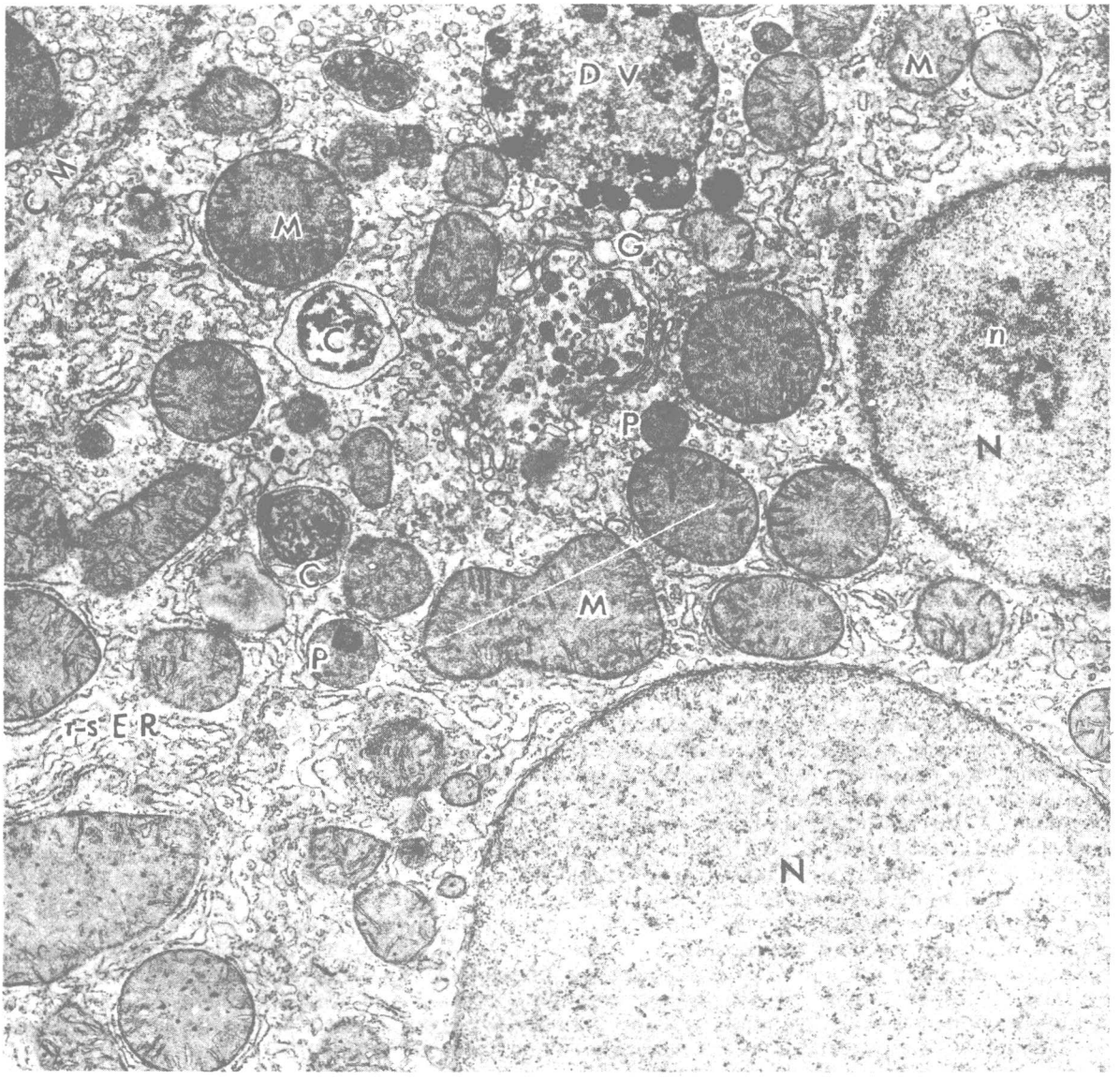

Figure 1-3. Rat liver cell 24 hours after ligation of common bile duct. Note two nuclei (*N*), with nucleolus (*n*), in one; Golgi apparatus (*G*) just above center; numerous mitochondria (*M*); two microbodies or peroxisomes (*P*); two cytosegresomes (*C*) containing recognizable remains of mitochondria; large digestion vacuole (*DV*), representing a later phase of lysosomal activity and containing electron-dense debris; moderately abundant rough-surfaced endoplasmic reticulum (*r-s ER*); cell membrane (*CM*). Osmic acid fixation; Epon-embedded; lead hydroxide. Print × 16,200. (Courtesy of Dr. Jan W. Steiner and Dr. A.M. Jezequel of the Banting Institute, Toronto.)

were, and the molecule of newly made enzyme is simultaneously set free.

If the enzyme proteins are for export they are made not by the free cytoplasmic ribosomes but by those attached to the outer aspects of the rough-surfaced endoplasmic reticulum (r-s ER). Once made, the enzyme molecules destined for secretion somehow are moved through the membranous wall of the r-s ER. Within a short time — less than 30 minutes — they are present within the cisternae of the Golgi complex. Here they may be modified in some way or concentrated. They are packaged in the periphery of the flat Golgi cisternae by formation of rounded protuberances from the latter. These excrescences, containing the zymogen granules, detach from the Golgi apparatus and make their way toward the luminal aspect of the cell, from which they will be discharged on receipt of the appropriate stimulus by the cell.

Obviously, other cell types, having different functions, display modifications of the modus

operandi just outlined. Thus plasma cells, concerned with the manufacture of antibody globulins, possess r-s ER in great abundance. In them the counterparts of the secretion granules are droplets of crystalline protein known in larger dimensions as Russell bodies. Liver cells, in addition to countless other chores, are busy making plasma albumin, fibrinogen, and other coagulation factors. Other cells, e.g., fibroblasts, make the structural protein, collagen; osteoblasts lay down osteoid and bone; muscle cells transform energy into actual physical work; the reticuloendothelial cells and neutrophilic polymorphonuclear leukocytes have as one of their prime functions garbage collection, in which their duty is to collect, engulf, and catabolize foreign, particulate, or noxious substances.

It will be obvious that every cell in the body, and indeed every part of every cell, is geared to work either continuously or to go to work the instant it is called upon. Hundreds — perhaps thousands — of functions are carried out in and by every cell, both to keep itself alive and to fulfill its duty to the community of cells that go to make up the entire organism. The orderly, safe and expeditious execution of these numerous and complex tasks is assured by the systems of membrane-bounded organelles already briefly described; these serve to segregate activities that otherwise might interfere with each other. As we shall see presently, the membranes have peculiar qualities fitting them for the selective transport of raw materials across them and of products and waste disposal within them. In addition to its general or basic duties, each cell type has one or more special functions. These sometimes demand special structural features, e.g., muscle and nerve cells, special organelles, or more of one type of organelle and less of other types. These varied and seemingly enormous work demands entail: (1) adequacy of supply lines and raw materials; (2) an efficient energy source or powerhouse, with emergency standby system; (3) safe disposal of products and waste and protection from dangerous reactants or products; (4) a fail-proof control system; (5) ability to expand facilities to meet growing demands, i.e., cell division.

THE CELL MEMBRANE

Over 30 years ago, with remarkable prescience Danielli and Harvey suggested that the cell membrane consists of a central bimolecular layer of polarized lipid molecules between two protein layers. Modern techniques have substantiated this "bread-and-butter sandwich" structure. The electron microscope shows that the membrane has three layers, each approximately 25 Å in thickness. In the center is the lipid layer, actually composed of two rows or sheets (micelles) of phospholipid molecules arranged back to back (Fig. 1-4.). In physical structure these molecules resemble tuning forks or clothes pins and may be exemplified by lecithin.

Other phospholipids present in the cell membrane are phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidic acid, the latter having no organic base; i.e., its molecule is somewhat like that of lecithin stripped of choline. These phospholipid molecules possess rather special physical properties. Their heads are made up of water-miscible substances, i.e., glycerol, phosphoric acid, and, attached to the latter, choline ethanolamine, serine, or the cyclic sugar alcohol, inositol. Their "legs," composed of long-chain fatty acids, repel water. Hydration of such molecules in vitro tends to produce curlicue shapes — "myelin figures."

On either side of the lipid layer is a sheet of protein: the entire membrane consists of 60% protein and 40% lipid. The existence of a further layer of globular protein on the external and internal aspects, making five layers in all, as shown in Figure 1-4, has not been fully substantiated.

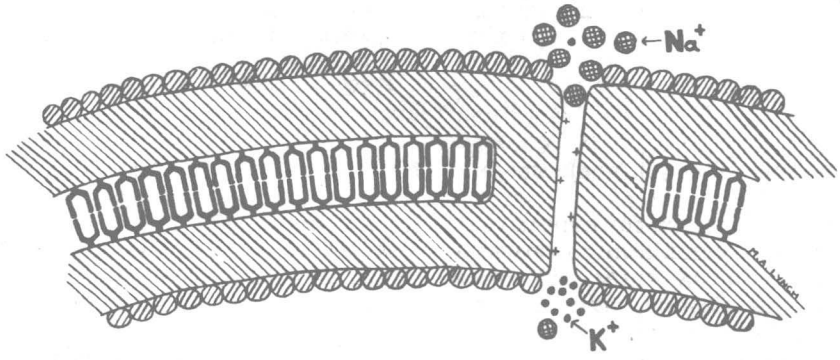

Figure 1-4. The cell membrane, showing the central lipid layer covered on either aspect by a layer of protein; also, a pore. An additional layer of globular protein is shown here, but its existence is still uncertain. Sodium and potassium ions are depicted in approximate relative sizes.

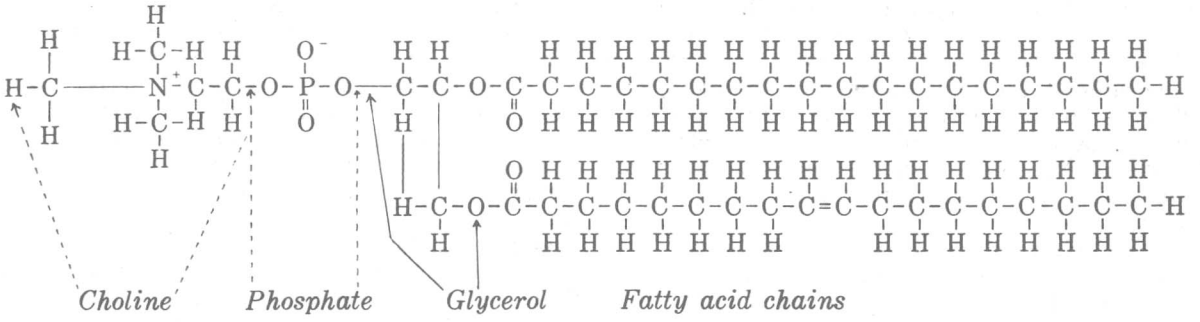

Figure 1-5. Structure of lecithin.

Passage of Substances Through the Cell Membrane

This is accomplished in several ways: (1) phagocytosis; (2) pinocytosis; (3) via pores; (4) simple diffusion; (5) facilitated diffusion; and (6) active transport.

Phagocytosis. Phagocytosis (phago = I eat) is the process by which a cell engulfs an entire particulate object such as a bacterium, portion of another cell, or granule of dust or pigment. When such a particle comes in contact with the cell membrane, the cytoplasm immediately underlying it seems to withdraw and the membrane sinks inward to form a pocket containing the particle. The narrow inlet to this pocket then closes and the apposed cell membrane fuses. At the same time the small pocket of what was cell membrane is nipped off and set free into the cytoplasm with the engulfed particle in it: this entire inclusion is then known as a phagosome.

Secretion. "Ejection Capsules." Reverse Phagocytosis. These terms all refer to the process by which zymogen granules and occasionally other membrane-enclosed objects are expelled from the cell. On contact with the internal aspect of the cell membrane (hormonal and neurosecretory substances apparently bring this about), the membrane enclosing the granule fuses with the cell membrane and both give way at the point of fusion. The contents of the secretion (zymogen) or other vesicle are then literally dumped to the exterior — into the lumen of a tiny ductule in the case of secretory cells such as those of the pancreas. The process is truly the reverse of phagocytosis.

Pinocytosis. Pinocytosis (pinein = to drink), which is effected in a manner precisely the same as phagocytosis, is applied to a cell's engulfing droplets of fluid and the substances dissolved therein. Its importance lies in the fact that it is the only method by which a cell can take in most large molecules, especially proteins. Ropheocytosis is a term sometimes used to describe pinocytosis on a minute scale. Endocytosis includes all processes by which the cell takes in materials from its environment.

Relation of Structure to Function in Cell Membrane. It might be said that the lipid layer of the cell membrane is more functional than structural. Indeed, much of the selective permeability, auto-sealing, and other peculiar qualities of the cell wall seems to be attributable more to the double lipid micelles than to the protein skins. Furthermore, the lipid core is the opposite to inert: it is metabolically and physiologically highly active. Hokin and Hokin have shown that when the exocrine pancreatic cells are stimulated to secrete by the natural stimulant, acetylcholine, there is a rapid and pronounced alteration in the proportions of phospholipids within their membranes, with notable increases in phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidic acid. However, the same workers have demonstrated that sufficient ionized calcium must be present in the extracellular fluid for the actual discharge of the secretion granules to be accomplished.

From the account of phagocytosis and pinocytosis it will be apparent that the cell membrane has an inherent ability to seal any breaches in it. The same is seen when the cell membrane is ruptured or punctured. Provided the tear is not too great, the adjoining membrane seals it before any great loss of cytoplasm can occur. Calcium ions are also essential for this: if the Ca^{++} in the extracellular fluid is below a certain concentration, repair is not effected.

Pores in the Cell Membrane. Although these are just too small to be seen even with the high resolution of the electron microscope, their existence is beyond doubt. They measure about 7.5 Å in maximum width. Eccles has postulated pores of two different sizes to account for the observed ion movements in nerve cells: more likely, pore size can vary. Although the pores are exceedingly numerous, their entire area accounts for only 1/1600 of the cell surface. Nevertheless, they are sufficient to