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# introduction to biological chemistry

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J. AWAPARA, *Introduction to Biological Chemistry*


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# preface :::

THE STUDY OF MODERN BIOLOGY IS BECOMING MORE DIFFICULT FOR students without a background in biochemistry. For this reason, at Rice University biochemistry is taught in the first semester of the junior year. In this way our students are prepared to grasp the foundations of modern biology or the foundations of other disciplines in which the language is largely biochemical in nature.

The choice of material for this book was based on my experience gained by teaching an introductory one-semester course in general biochemistry. Our junior students come into biochemistry with a sound background in general and organic chemistry. Nevertheless one-third of the book treats substances of biological importance.

One reason for emphasizing chemistry is that in most organic chemistry courses reaction mechanisms are stressed, leaving little time for substances of interest to the biologist.

Another reason is that quite often students who have learned a chemical concept in the context of a general chemistry course fail to see its immediate application to biochemical problems. Perhaps the reason is that junior students forget temporarily what they have learned in their freshman and sophomore years. Whatever

the reason, the student of biochemistry needs either to review or to learn principles in acid-base equilibria, optical isomerism, chemical equilibria and free energy, oxidation-reduction, and other chemical concepts. Some of these principles have been incorporated in the main body of the text in places where I thought they would be most useful. Others, because of their more general nature, have been presented parenthetically (that is, in smaller type and preceded and followed by a small square) to avoid discontinuity in the main theme of the book—*metabolism*.

Metabolism covers a rather large territory, of which only a minor portion can be covered in a one-semester course. Selection was difficult, but in my experience the students profit greatly from the discussion of a few basic principles in some detail.

The few basic topics discussed include enzymes and enzyme reactions, energy production, and biosynthesis. The two chapters on enzymes familiarize the student with the nature of enzyme catalysis and with common cellular chemical reactions.

In dealing with energy production an attempt was made to treat fatty acid oxidation and the citrate cycle as the terminus in the overall flow of carbon in the cell. Glycolysis and the conversion of amino acids to carbohydrate and fatty acid intermediates are treated as part of the carbon flow. Thus intermediary metabolism becomes part of the process leading to the production of suitable substrates for mitochondrial oxidations.

Metabolic interrelations are brought to light by emphasizing common intermediates shared by reactions leading to oxidations and reactions leading to the biosynthesis of cell substances. I have tried not to leave the impression that metabolism is a collection of enzymatic reactions without cellular control.

The inclusion of chromatography in the Appendix was dictated by the general applicability and wide scope of the technique in biochemistry. Chromatography is referred to several times in the text, but its full discussion is reserved for the Appendix.

I wish to express my thanks to my colleague Dr. Charles W. Philpott for contributing his thoughts to Chapter 1 and for the electron micrographs illustrating that chapter.

I should also like to express my thanks to Miss Beth Buvens for diligently translating my scribbles into readable typewritten copy and to the editors of Prentice-Hall for advice and suggestions.

J. AWAPARA

# contents :::

# the living cell

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THE AIM OF BIOCHEMISTRY IS TO EXPLAIN BIOLOGICAL PHENOMENA by chemical and physicochemical laws. In this sense it deals with the behavior of living matter at the molecular level.

The substances that make up living matter are not different from any other substance, but they are extremely complex. The proteins and nucleic acids, for example, are macromolecular substances with predictable chemical and physical properties. Their biological properties, however, are not so predictable. Some proteins—the enzymes—are among the most efficient catalysts, but their catalytic activity depends upon structural features that we are unable to recognize at the moment. On the other hand, nucleic acids possess a biological property that has been traced to their unique structure. This biological property is self-replication, and nucleic acids are the only molecules known to display this property.

The study of biological macromolecules is hindered by the distortions introduced when they are liberated from the cell. Because they are organized into the structural elements of the cell, they can be isolated only after complete destruction of cell organization.

Despite this barrier, which prevents research into the molecular

architecture of the cell, great advances have been made by experimentation with isolated cell fragments and by successive approximations to the final state of molecular organization in the living cell.

Much of the progress made in elucidating cell ultrastructure has been due to the introduction of the electron microscope and an array of ingenious techniques for its use. Added to the electron microscope the method of fractional centrifugation has permitted the investigator to isolate intact cell substructures and relate those structures with specific cell functions.

For example, it can be shown that the oxidation of suitable organic molecules in the cell occurs mostly in granular or rod-shaped particles, the *mitochondria.* These can be obtained from cells after disruption and fractional centrifugation. In their isolated state mitochondria have all the necessary components to catalyze complex oxidations that have been predicted to occur in the intact cell. The evidence on hand strongly favors the idea that mitochondrial function in the experimental situation differs little from its function within the cell.

Another example of cell function in isolated cell fragments is the synthesis of proteins in ribosomal complexes. Ribosomes can be isolated by high-speed centrifugation without losing their property of directing protein synthesis when supplied with the necessary ingredients.

The progress made in understanding the chemical activities within the cell has been impressive, but this is just the beginning. The ultimate goal is to find explanations for cell functions in chemical laws.

Up to now the term "living cell" has been used without qualification. There are a multitude of types of cells, most of which differ from one another not only in size and shape but also in their organization and function. In the discussion that follows, we shall not refer to any particular cell; the parts of the cell discussed are those parts common to the majority of living cells.

## 1.1 cell membrane and organelles ::

In Figure 1.1 is shown a composite picture of the cell, its organelles, and its membrane systems in their approximate location and interrelations. The parts distinguished are the cell membrane, the nucleus, the endoplasmic reticulum, the mitochondria, lysosomes, and the Golgi apparatus. Each will be discussed briefly.

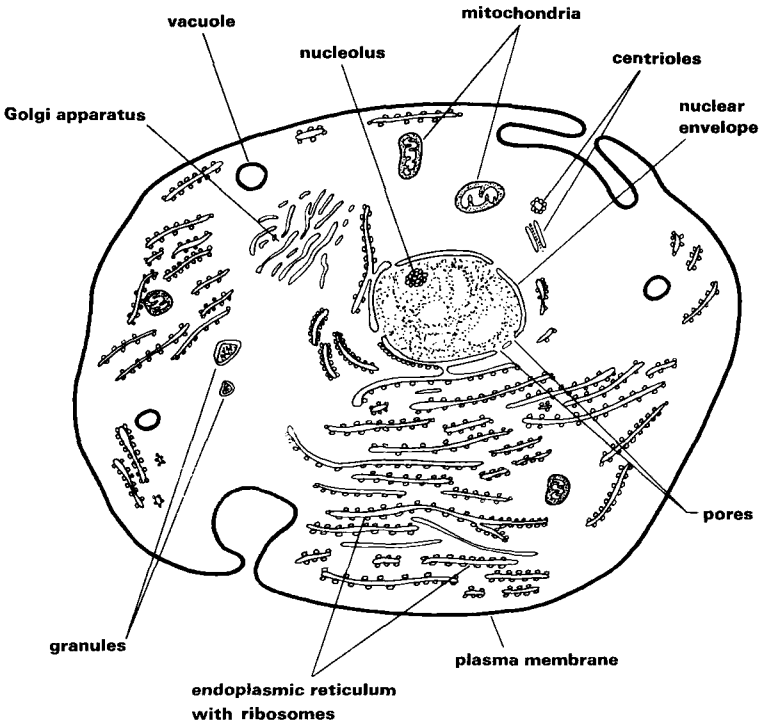


**figure 1.1** :: Composite picture of a living cell.

CELL MEMBRANE : Cells are limited by a membrane that separates the cell's interior from its surroundings. Plant cells, in addition to the membrane, are enveloped by a wall made up mainly of cellulose, but unlike the membrane, the wall is inert.

The *cell membrane* is not inert; its functions are well defined and of supreme importance in the regulation of the cell's interaction with its surroundings. For example, small molecules and ions, which ordinarily would cross an inert membrane, are "selected" by the cell membrane. Only particular molecules and ions can cross. This active process of selection tends to maintain the constant environment observed within the cell.

The basic structure of the cell membrane is complex. E. Gortner and F. Grendel extracted lipid from a known number of erythrocytes and measured the area occupied by the lipid monomolecular layer when it was spread over a surface of water. This experiment revealed that the extracted lipid covered about twice the total surface area of the original cells. From other studies of the surface tension and elastic properties of the cell membrane H. Davson and J. F. Danielli suggested that a layer of protein was attached to each

of the outer surfaces of the bimolecular lipid leaflet. Danielli's model of the cell membrane received independent support by J. D. Robertson, who described the "unit membrane" from electron micrographs. Three distinct layers could be recognized in images that showed membranes sectioned at a right angle to their flat surfaces. The total thickness of the linear profile was about 80 to 100 angstroms (Å). This trilaminar structure consists of two dark lines (protein) separated by an intermediate light line (lipid); each line was about 30 Å thick. Although the trilaminar-structured unit membrane occurs almost universally in cells, one must not mistakenly infer that all the cell membranes have a common origin and similar physiological activities. An electron micrograph of the cell membrane is shown in Figure 1.2.

The cell surface, which is delimited by the cell membrane, or plasmalemma, may be amplified or extended for a particular purpose. Such is the case in certain intestinal cells and cells that form the proximal convoluted tubule of the kidney. The apical surface of these cells bear many fingerlike projections, the *microvilli*, which increase the effective area for absorption. In other instances, cytoplasmic processes from adjacent cells may interdigitate with one another to form a secure attachment.

NUCLEUS : All cells have either a nucleus or chromosomelike bodies endowed with properties similar to those of the nucleus. Blue-green algae and bacteria, for example, do not have an organized nucleus and are generally called *prokaryotic cells*, in distinction to organisms of *eukaryotic cells*, which possess clearly defined nuclei.

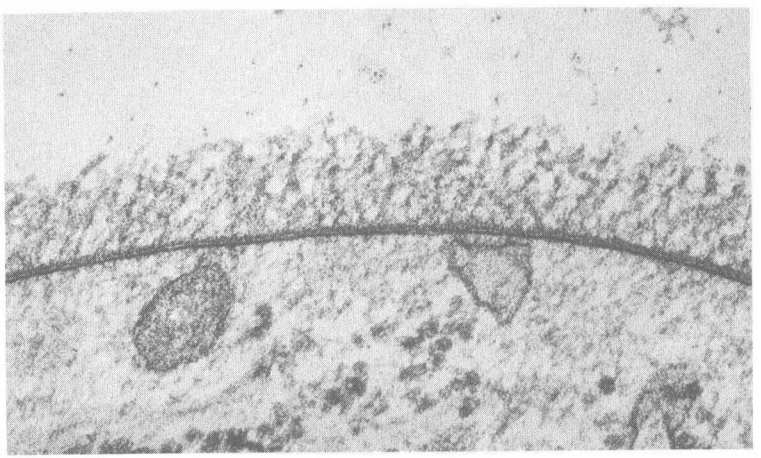

**figure 1.2** :: This micrograph shows a small portion of the cell surface and the cell membrane. The cell membrane, or plasmalemma, appears at high magnification as a trilaminar structure. The dense–light–dense appearance of the plasmalemma is believed to represent the protein–bimolecular lipid–protein layers of the Danielli and Davson cell-membrane model. Most cells have a surface coating that is rich in polysaccharides; in this image the surface coat is represented by a prominent layer of more or less radially oriented filaments. Magnification about 134,000. (*Courtesy of Dr. Charles W. Philpott.*)

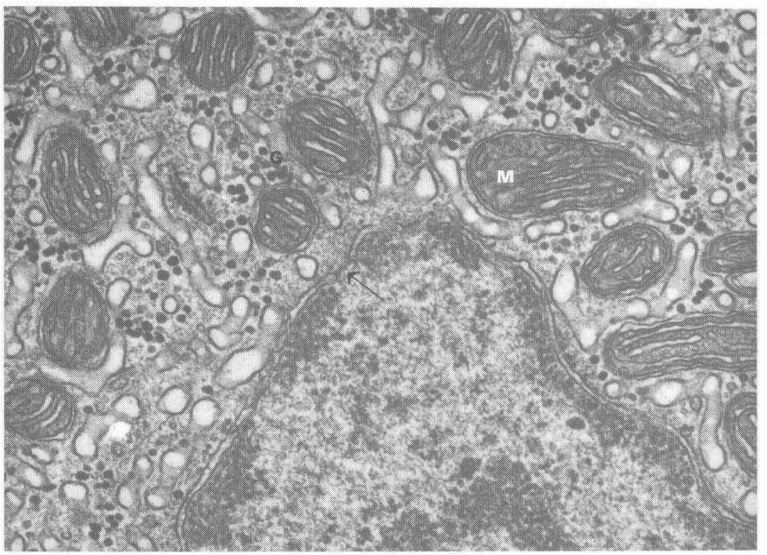


**figure 1.3** :: This micrograph shows portions of the nucleus and cytoplasm of a cell. The arrow points to a nuclear pore, the site which represents an area of continuity between the nuclear and cytoplasmic ground substances. Mitochondria (M) are scattered throughout the cytoplasmic ground substance. The dense granules (G) of the cytoplasm represent glycogen particles. Magnification about 27,000. (*Courtesy of Dr. Charles W. Philpott.*)

The *nucleus* in most cells is a sphere-shaped body surrounded by a membranous envelope interrupted by pores, which have an average diameter of 1,000 Å. The nuclear envelope consists of two lamellae, which enclose the perinuclear space. There is communication between this space and the cisternae, which are sacs formed by the membranes of the endoplasmic reticulum. It should also be emphasized that the nuclear and cytoplasmic ground substances are continuous, owing to the nuclear pores (Figure 1.3).

Depending on the stage of the life cycle of the cell, one may recognize in the interphase cell a small spherical body, the nucleolus, and, in dividing cells, rod-shaped chromosomes that contain most of the deoxyribonucleic acid (DNA) of the cell. Chromosomes, which are unusually large in some insects, are easily observed under the light microscope. They stain with characteristic crossbands. DNA combined with protein is present in the darkly stained crossbands.

Genetic information is stored in the chromosomes as DNA molecules of specified structure. But in addition to the genetic control, there seem to be other controls of cell activity within the nucleus and nucleolus.

ENDOPLASMIC RETICULUM : The nuclear membrane or envelope in many cells extends into the cytoplasm as a complex membrane system, the *endoplasmic reticulum.* In tissue-thin sections the endoplasmic reticulum usually appears either vesicular or as a

flattened sac. The sacs, called *cisternae*, effectively separate the ground substance into two phases, the ground substance proper and the intracisternal substance.

The membranes of the endoplasmic reticulum may be subdivided into two forms. One type has a smooth outer surface and the other is studded with granules having a diameter of about 150 Å. Biochemical analysis reveals that the granules are largely composed of nucleoprotein. The name *ribosome* has been applied to these particles, which occur also unassociated with membranes in the cytoplasmic ground substance.

The ribosomes are parts of aggregates known as *polysomes*, which are the site of protein synthesis. When polysomes dissociate into free ribosomes they lose their capacity to synthesize protein. The electron micrograph of Figure 1.4 reveals the endoplasmic reticulum with its attached ribosomes.

MITOCHONDRION : The images shown in Figure 1.5 are of mitochondria. These are either granular- or rod-shaped bodies distributed throughout the cell. The average cell may have several hundred mitochondria; their size is about 1.5 to 3.0 microns ($\mu$) in length and 0.5 to 1.0 $\mu$ in thickness. Of course, there are variations in the size and the number of the mitochondria that are present in a cell.

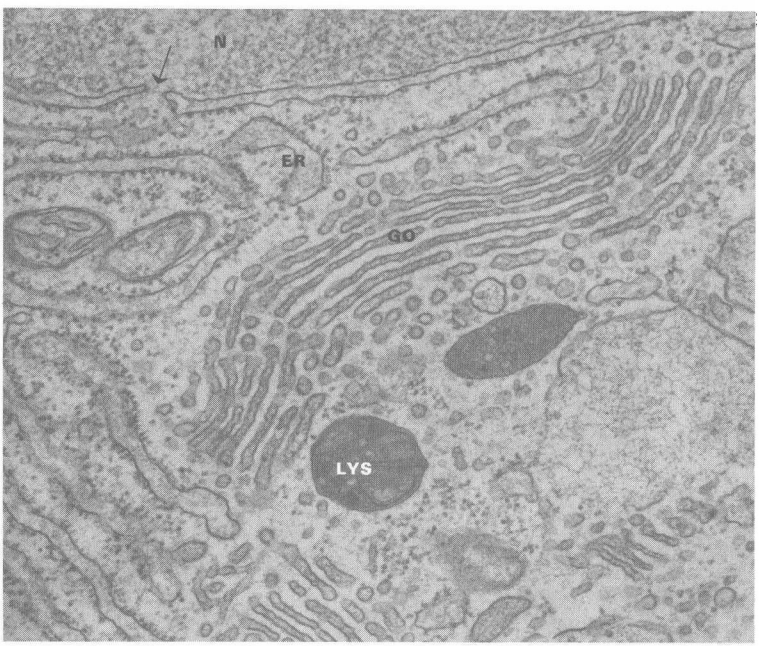


**figure 1.4** :: This micrograph demonstrates several of the common features of cells which are specialized for protein synthesis. In cells that are known to produce protein for export, the ribosomes are associated with the outer surfaces of cisternae of the endoplasmic reticulum (ER). Newly synthesized peptides and protein are then transferred to the Golgi region (GO), where they are packaged into membrane-bound droplets or granules. Also seen in this image are lysosomes (LYS) and a small field of the nucleus (N). The arrow at the top points to a nuclear pore. Magnification about 27,000. (*Courtesy of Dr. Charles W. Philpott.*)

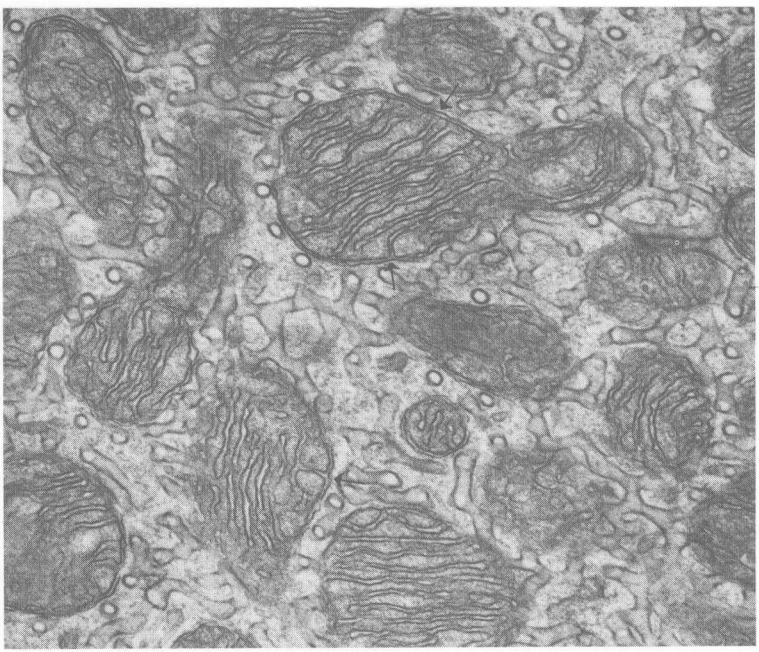

**figure 1.5 ::** Mitochondria are numerous in this limited field of cytoplasm. As can be seen at the arrows, cristae represent shelflike infoldings of the inner mitochondrial membrane. Oxidative enzymes are believed to be associated with the cristae membranes. A situation such as this, where mitochondria are numerous and cristae are closely packed, reflects a high metabolic potential for this cell. Magnification about 40,000. (*Courtesy of Dr. Charles W. Philpott.*)

A mitochondrion has an outer membrane that serves as a sac in which is enclosed a second sac. The inner sac has many foldings extending into the inner portion of the mitochondrion. The infoldings or *cristae* are the sites of numerous enzymes involved in oxidations. The innermost part of the mitochondrion contains a semifluid material consisting of protein and lipid—the *matrix*.

The chemical composition of the mitochondrion has been correlated with structured elements observed with the electron microscope. It is quite rich in lipid into which seem to be embedded proteins, many of which are enzymes. The picture of the mitochondrial architecture is now being drawn in its molecular details.

The lipid, a phospholipid, seems to be responsible for much of the organization of the proteins. For example, it has been shown that random aggregates of mitochondrial enzymes organize spontaneously into vesicles only when mitochondrial phospholipid is present.

The recent discovery that the mitochondrion contains DNA supports the idea that these organelles are endowed with the capacity for self-replication, as opposed to the earlier view that they arise from the ground substance *de novo*.

Other details of mitochondrial properties and mitochondrial function will be discussed in Chapter 8.

GOLGI APPARATUS : The presence of a network of membranes and sacs collectively known as the *Golgi complex* is revealed by special stains (see Figure 1.4). The membranes are only of the smooth type. The Golgi complex or apparatus has been and still is the subject of much speculation and controversy. Its function has not been clearly defined, but the prevailing view is that the Golgi is the place where secretory products made in another part of the cell are stored before they are secreted. For example, in the pancreatic acinar cells, zymogens, a group of proteins, are synthesized by the granular endoplasmic reticulum. The newly synthesized proteins move from the cisternae of the endoplasmic reticulum to the Golgi region, where the mixture is formed into a clearly defined secretory droplet surrounded by membrane derived from the Golgi. Before the zymogens are secreted or extruded from the cell, the membrane enclosing the mature droplet must fuse with the cell membrane at its apical surface.

LYSOSOME : The *lysosome* is a particle that contains a number of enzymes, mainly *acid hydrolases*. This organelle displays considerable morphological heterogeneity, and it is therefore difficult to present a definitive morphological description that would aid recognition with an electron micrograph. Usually verification resides either in a biochemical analysis for *acid hydrolases* in centrifugally isolated particles or in cytochemical staining reactions for the localization of acid phosphatase.

As the name lysosome implies, the particles are rich in lytic enzymes. For this reason, they are believed to play an important role in intracellular digestion. It has been suggested, for example, that following *pinocytosis*,† a lysosome may fuse with the phagosome, which contains material to be digested, to form a digestive vacuole. Digestion then proceeds within the digestive vacuole and breakdown products presumably diffuse into the ground substance. There is also evidence that lysosomes sometimes fuse with "worn-out" organelles within the cell to form an autophagic vacuole. This theory holds that old or damaged organelles are digested and that the products may be reutilized by the cell to form new organelles.

CHLOROPLAST : *Chloroplasts* are organelles of plant cells and cells with the capacity for photosynthesis. They, like the mitochondria, are rich in lipid material. Internally they consist of stacks of

† Pinocytosis is a mechanism for the introduction of large molecules or particles from the outside into the interior of the cell.