Paparella: Volume I: Basic Sciences and Related Disciplines

Section 4: Biochemistry

Chapter 22: Basic Principles of Cellular Metabolism

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Definitions and Basic Assumptions

The word "metabolism" refers to the chemical reactions that occur in living organisms. It is now a fundamental assumption of biochemists that these reactions conform to the same laws of chemistry and physics that also govern the reactions of nonliving matter. This assumption has far-reaching consequences. One consequence is that matter is conserved in living things. Every carbon, nitrogen, or any other atom that an enters an organism can, at all times, be accounted for in one compound or another within the organism until it is finally released to the environment. Living things must balance their chemical equations. Another consequence is that energy is conserved and the laws of thermodynamics are applicable. Converting simple precursors to complicated proteins, polysaccharides, lipids, and other constituents inevitably requires energy, and this energy must come from somewhere. Along with anabolic (synthetic) energy-requiring reactions, organisms must carry out catabolic (degradative) energy-yielding reactions that provide the necessary energy. In addition, catabolism must furnish the energy expended by the organism as mechanical work and also the heat energy and entropy that inevitably must be expended during the operation of essentially irreversible processes. This energy ultimately comes from the sun. Photosynthetic plants are capable of utilizing solar energy to perform anabolic reactions, and it is the resulting plant-produced organic compounds that are, directly or indirectly, the source of energy for animals. These organic compounds are also the precursors or "building blocks" for all the proteins, nucleic acids, polysaccharides, lipids, and other constituents synthesized by the animal.

Machinery of Metabolism

Although living things are made of chemical and conform to the laws of chemistry and physics, they possess fantastic attributes found nowhere in the world of non-living things. Many of these attributes are shared by all living things and can be considered to be the distinguishing features of life. That all organisms share these unique features is undoubtedly true because life originated only once and then developed through an unbroken chain of evolution and diversification that must establish a kinship between all individual organisms, living or dead, and all species, extant or extinct.

Some of the distinguishing features pertaining to the metabolism of all organisms will now be reviewed.

Enzyme Catalysis

One distinguishing feature of living organisms is that almost all of their metabolic reactions are catalyzed by enzymes. The constituents of living things are quite stable under physiologic conditions, that is, in dilute aqueous salt solutions, at pH near neutrality, and at a range of temperatures of which the absolute extremes for a few organisms are near the freezing point or somewhat below the boiling point of water. In fact, stability under these conditions was probably a prerequisite for selection as a component of living matter, and compounds of biologic origin with half-lives as short as a few hours under these conditions in the absence of enzymes are definitely exceptional. All enzymes are proteins (Zubay et al, 1988). Each kind of enzyme has a definite molecular structure with a unique amino acid sequence and a unique three-dimensional conformation of the peptide chain. Each kind of enzyme molecule possesses the specific structure necessary to interact with and catalyze a reaction of a specific compound or group of related compounds. During enzyme catalysis, the reactants (substrates) interact with specific functional groups of the enzyme molecule (the active site). This binding brings the reactants into precise juxtaposition and also alters the chemical reactivity of their functional groups in such a way that a specific reaction takes place that otherwise would occur only rarely under physiologic conditions. The products of the reaction are released from the active site; the free enzyme molecules then have the same structure that they had before the reaction, and they can participate in the conversion of additional molecules of the substrate. Each enzyme shows a remarkable specificity not only for the kind of reaction that it will catalyze but also for the structure and stereochemistry of nonreacting portions of the substrate molecules. This specificity is not necessarily absolute; closely related analogues to the natural substrate sometimes can be found that participate in the enzymatic reaction. However, such analogues often are not present in the living system in which the enzyme operates; the combination of highly specific enzymes and a limited number of natural substrates together makes metabolism in vivo remarkably free from wasteful and purposeless side-reactions.

Coupled Reactions

A feature of living systems that baffled biologists for decades is how these systems derive the energy necessary to conduct their intricate biosynthetic and physiologic feats. Before it was established that total energy is indeed conserved in living things, this problem provided a fruitful area for invoking the intervention of speculative "vital forces". The distinctive manner in which this is now known to take place is by pairs of chemical reactions that share a common intermediate, that is, coupled reactions. In order to participate efficiently in energy transfer, the common intermediate must possess certain properties, and during evolution a limited number of substances were selected for this role. These include the high energy phosphate compounds, typified by ATP (Lipmann, 1941). It happens that the free energy for hydrolysis of either of the two phosphate anhydride linkages in ATP, one yielding ADP and Pi, the other AMP and PPi, is much greater than the free energy change involved in formation of many biologically important phosphate ester linkages and other linkages. If an anabolic reaction such as the formation of glucose-6-phosphate from glucose occurs by a reaction involving hydrolysis of ATP, such as the reaction catalyzed by the enzyme hexokinase,

glucose + ATP --> glucose-6-phosphate + ADP,

the equilibrium constant of this reaction is strongly favorable to synthesis of glucose-6-phosphate. Now if this reaction is coupled with a reaction of a catabolic process that derives sufficient energy from the breakdown of some compound to produce ATP with favorable equilibrium, the catabolic process effectively "drives" the formation of glucose-6-phosphate by participation of the common intermediate ATP. Examples of systems that produce ATP in mammals include the familiar glycolytic pathway and the mitochondrial electron transport system. In the latter system, reduced coenzymes such as NADH and FADH2, formed during catabolism of acetyl CoA by the citric acid cycle or during catabolism of fatty acids by the fatty acid degradative cycle, are reoxidized, and the energy from this is utilized for formation of ATP. The hydrolysis of this ATP, in turn, "drives" a large number of metabolic processes, including polysaccharide synthesis, muscle contraction, and nucleic acid and protein synthesis.

Although ATP occupies a key role in the economy of the cell, it is not the unique intermediary between anabolism and catabolism. In some cases, other high-energy phosphate compounds (eg GTP or phosphoenolpyruvate), nucleotide-sugars (eg UDP-glucose), coenzyme A derivatives (eg palmityl CoA), or nucleotide-lipids (eg CDP-choline) are used. In other cases, reduced coenzymes (eg NADPH) are directly involved instead of being used for ATP synthesis as mentioned previously. In all cases, the basic principle is the same: the intermediate is synthesized by thermodynamically favorable catabolic reactions; it is degraded to provide energy for an anabolic process.

In reality, all so-called anabolic processes run "downhill". A process such as the abundant, spontaneous biosynthesis of the milk sugar lactose, for example, was formerly observed with awe because the equilibrium of the reaction is, as shown, strongly in favor of hydrolysis:

glucose + galactose <-- lactose + H_2O

However, the actual biochemical process is not known to be:

glucose + UDP-galactose .--> lactose + UDP

This reaction occurs with a free energy change favoring lactose synthesis. The high-energy intermediate necessary for this process, UDP-galactose, was synthesized by reactions involving the catabolism of ATP; the ATP, in turn, was derived from a catabolic process.

The Genetic Code

A feature of living things that has long been recognized to be unique is the system for their not-quite-perfect self-duplication: the genetic system. Achieving a general description of the chemical basis for this system has revolutionized biologic knowledge and, in fact, all human thought in a manner analogous to the revolution wrought near the beginning of this century by the physicists' theories of relativity and quantum mechanics. At the center of the system of

heredity is the molecule on which is imprinted the genetic code, DNA (Kornberg, 1980). The position of every amino acid residue in every peptide chain of every protein of an organism is encoded in its molecules of DNA. The sequence of purine and pyrimidine bases of three adjacent nucleotides of a DNA chain provides the code for each amino acid residue. The entire code sequence is arranged in a linear fashion on the DNA chain, with the tripled code "words" arranged in the same sequence as the sequence of amino acids of the protein. At the end of the code for one peptide chain, a terminating codon is present in the DNA molecule; then the code for another peptide chain starts.

The DNA molecule is constructed of two nucleotide chains rigidly bound together in helical structure. One chain is complementary to the other in that an adenine base on one chain is always opposite to a thymine base on the other, and a guanine base on one is opposite to a cytosine base on the other. During multiplication of the cell, the DNA is replicated by a process that involves unwinding of the complementary chains and simultaneous synthesis of two daughter chains, each complementary to its parental chain (Kornberg, 1980). During protein synthesis, the nucleotide code sequence of one of the DNA chains is specifically *transcribed* to a newly synthesized molecule of messenger RNA. The messenger RNA code is *translated* into the correct amino acid sequence during protein synthesis by a biochemical system in which the participants include ribosomes, enzymes and their co-factors, amino acids, high-energy phosphate compounds, and a number of different kinds of transfer RNA, each kind capable of "recognizing" the messenger RNA code for one amino acid (Bodley, 1988). All the components of the nonspecific "factories" for replication, transcription, and translation must be allocated to the daughter cells from the parent so that the DNA code passed on can be interpreted. Given this, the cell can multiply all its protein components, including those of the genetic apparatus itself, and these proteins, in turn, are responsible for synthesis and integration of all other metabolic and structural features of the cell.

Metabolic Controls

Another feature common to all living systems is the group of mechanisms by which metabolism is controlled. The rate of flow of metabolites through each individual enzyme pathway must always be in adjustment to provide the proper balance of materials for the momentary requirements of the organism. Either overproduction or underproduction of intermediates is pathologic or fatal. The demand of the system for a certain metabolite may fluctuate rapidly with changing physiologic circumstances, and these changes must be accommodated. There is, in fact, a hierarchy of controls, each operating by a different mechanism, imposing control at a different level of organizational complexity, and responding to stress with a different rate of change. An outcome of biochemical research has been to rephrase the question of the physiologist concerning the basis for *homeostasis* to the question concerning the mechanism for individual metabolic controls and how these controls interact to produce homeostasis.

One class of metabolic control is by *allosteric effectors* (Monod et al, 1963). Certain key enzymes in metabolic processes possess allosteric sites in addition to their catalytically active

sites. The allosteric sites selectively bind certain metabolites, termed allosteric effectors, when these metabolites attain sufficient intracellular concentration. The three-dimensional structure of the entire enzyme molecule is reversibly altered by attachment or removal of these metabolites from the allosteric sites, and these structural changes alter the capability of the active site to catalyze the enzymatic reaction. Allosteric effectors may be either positive (ie stimulatory) or negative (ie inhibitory) effectors of the enzyme reaction. A single enzyme may include several sites for different positive and negative effectors. In many cases, the key enzyme involved in this process catalyzes the first reaction of a unique metabolic pathway, and one of the end-products of the pathway serves as a negative allosteric effector. Then, when the end-product attains an excess concentration, it effectively inhibits production of its starting material and thus, indirectly, of all its intermediates. A particularly valuable characteristic of allosteric control is its speed of response, which is a fraction of a second. Much of the moment-to-moment balancing of concentrations of metabolic intermediates is allocated to this mechanism.

Another class of control mechanisms operates on the system for synthesis of enzyme protein under the direction of the genetic code. The DNA code of an organism embodies the complete recipe for the amino acid sequence of every protein that the organism can ever make. However, a multiplicity of controls cause this code to be read selectively. In microorganisms, the two related mechanisms of enzyme induction and repression are the principal means for selectively reading the genetic code (Jacob and Monod, 1961). In these organisms, a variety of metabolites bind to specific protein molecules called *repressors.* This interaction causes an allosteric change in the structure of the repressor molecule, altering its properties to give it either greater or lesser affinity for binding to specific regions of the DNA. The interaction of a repressor with DNA blocks transcription of specific species of messenger RNA, and thus blocks synthesis of specific enzyme proteins. If the metabolite-repressor complex is the species with high affinity for DNA, the phenomenon is called repression. In this situation, the presence of excess metabolite prevents synthesis of enzymes involved in its formation. This mechanism may be used to prevent synthesis of an end-product of anabolism, for instance an amino acid, when it is available in the food supply of the organism. If the metabolite-free repressor is the species that binds DNA, the phenomenon is called induction. Enzyme induction may occur when a potential nutrient (eg lactose) is present in the medium. The metabolite-repressor complex "falls off" of the DNA molecule and thus permits the synthesis of enzymes that can catalyze the breakdown of the nutrient by the metabolic machinery of the cell. Microorganisms have extraordinary abilities to respond to environmental changes, and they also can transfer different genetic capabilities from strain to strain via virus-like genetic elements called *plasmids.* These capabilities are of obvious relevance to problems of bacterial pathogenesis and acquisition of resistance to antibiotics by microorganisms.

In multicellular organisms, including humans, a major mechanism for metabolic control is by means of *hormones.* Hormones are specific small molecules that are produced by an endocrine organ and released into the blood in response to a stimulus. The stimulus may be chemical, such as an altered level of a metabolite, or neural. Hormones interact with and modify the metabolic activities of specific target tissues. A given hormone interacts with multiple target tissues and often alters their metabolism in different ways. The overall effect is to mobilize

different tissues to offset the effects of an altered metabolic state such as starvation or stress. The hormone thus acts as a chemical messenger to which different tissues respond in appropriate ways.

The cells of the target tissue contain specific receptor molecules for hormones. All cells embody receptors for numerous different hormones governs the net activities of the cell. Two distinct classes of hormone receptors are now recognized: cell-surface receptors and intra-cellular receptors. The cell-surface receptors are plasma membrane glycoproteins. They interact with hydrophylic hormone molecules, including the catecholamines, and peptide hormones, like insulin. The intracellular receptors interact with hydrophobic hormones, including the steroid hormones and thyroid hormones. The two receptor types also show functional differences. The cell-surface receptors usually effect changes to pre-existing metabolic systems. The intracellular hormone receptors, in contrast, are proteins that reside in the nucleus of the cell. When a steroid or thyroid hormone enters the cell and interacts with one of these receptors, the resulting hormone-receptor complex binds to specific regions of the cellular DNA and thereby regulates synthesis of specific kinds of messenger RNA (Chan and O'Malley, 1978). Thus, these hormones, like the microbial mechanisms of induction and repression, regulate the machinery for protein synthesis.

Since cell-surface receptors may not be in direct contact with the intracellular metabolic machinery, signalling from these receptors to intracellular enzyme systems often occurs via *second messengers.* (The "first messenger" is the hormone that transmits messages from one tissue to another.) The classic second messenger molecule is cyclic adenosine monophosphate (cAMP), and the best documented system for its action is its role in regulating carbohydrate metabolism via hormones, including epinephrine and glucagon.

The cAMP system includes components attached to the plasma membrane of the cell and components in the cytoplasm of the cell. The cell-surface components include receptor molecules of different kinds, each specific for its interaction with one hormone. Another cell-surface component is the enzyme adenyl cyclase, which catalyzes synthesis of cAMP from ATP. Finally, there are the guanine nucleotide-binding proteins that act as intermediaries between the hormone receptors and adenyl cyclase (Gilman, 1984). Hormone-receptor complexes activate these nucleotide-binding proteins by displacing bound GDP and allowing binding of GTP. The GTP nucleotide-binding protein complex either stimulates or inhibits adenyl cyclase activity, depending on the type, either Gs or Gi, of nucleotide-binding protein involved. Thus the minute-to-minute activity of adenyl cyclase depends on the ever-changing blood levels of hormones acting on their receptors and transducing their signals via guanine nucleotide-binding proteins. The product of the adenyl cyclase-catalyzed reaction, cAMP, pervades the cell. It regulates cellular activities by activating *protein kinases,* enzymes that phosphorylate specific amino acid residues in proteins. Many enzymes are activated or inactivated by phosphorylation. Thus, the phosphorylated form of glycogen phosphorylase is the active form, and glycogen breakdown is stimulated by elevated cAMP. In a reciprocal manner, the dephosphorylated form of glycogen synthetase is the active form; thus, glycogen synthesis is inhibited by increased cAMP.

Very recently, the second messenger strategy for regulation has been expanded to include at least five second messengers, and this is probably only the beginning. Figure illustrates this emerging view of cellular regulation. There are two chemically-distinct classes of second messengers: the cyclic nucleotides and those derived from phosphoinositides. The cyclic nucleotides include cAMP and cyclic guanosine monophosphate (cGMP). The phosphoinositides are lipides, and they comprise 2 to 8 per cent of the lipids of the lipid bilayer of mammalian cell membranes. When first examined, they appeared to have a primarily structural role, but it became evident that they also undergo exceptionally rapid metabolic turnover, suggesting other functions. It is now clear that two products of their breakdown, inositol triphosphate and diacylglycerol, are second messengers with distinctive biologic roles. Diacylglycerol is a potent activator of protein kinase C, an enzyme that, like the protein kinase A affected by cAMP, attaches phosphate groups to specific locations in many different protein molecules, thereby enhancing or inhibiting their activity. The most notable role for inositol triphosphate is his ability to release intracellular stores of calcium ions sequestrated in the endoplasmic reticulum into the cytosol. So many intracellular processes are regulated by changes of calcium ion concentration that it, too, is justly regarded as a second messenger. The role of calcium ions in intracellular regulation is also closely tied to the activities of a calcium-binding protein, *calmodulin*(Klee et al, 1980). The calcium-calmodulin complex interacts with a select group of enzymes to modulate their activities.

Phosphoinositide degradation also produces arachidonic acid. This polyunsaturated fatty acid is the precursor of the prostaglandins, prostacyclin, thromboxane, and the leukotrienes. These hormone-like substances elicit rapid and localized cellular responses. They are of extraordinary medical interest because of their involvement in homeostatic mechanisms, including platelet aggregation, inflammation, and allergic responses, which are components of vital defense mechanisms but which often go astray with disastrous results.

The strategy underlying all these systems is one of great flexibility of response. Different hormones, released by numerous stimuli, converge onto the control systems and adjust a multiplicity of responses in a manner that optimizes the metabolic activities of the cell for immediate physiologic demands.

Self-Assembly of Biologic Structures

The genetic code, a linear sequence of nucleotides in a nucleic acid molecule, is a one-dimensional code. Likewise, the amino acid sequences of the peptide chains that it encodes can be expressed as linear, one-dimensional sequences. Yet all biologic structures, from the simplest virus to the human being, are three-dimensional assemblies, often with many precisely and reproducibly oriented subassemblies. These all originate from the expression of the DNA code of a single cell. The manner in which a one-dimensional code gives rise to a three-dimensional organism involves a series of strategies unique to living things and is another of their distinguishing features. The first strategy is the spontaneous folding of a one-dimensional polypeptide chain to a protein of specific three-dimensional structure (Zubay et al, 1988). The amino acid precursors of peptide chains are a group of compounds of diverse chemical properties. Some are strongly hydrophilic with water-loving hydroxyl groups, such as serin; negatively

charged groups, aspartic and glutamic acids; or positively charged groups, such as arginine and histidine. Others are hydrophobic aliphatic amino acids, such as alanine and valine. Some hydrophobic amino acids also have an aromatic character, such as phenylalanine and tryptophan. Also, certain amino acid residues have unusual steric properties. A notable example is proline which always produces a bend in the peptide chain. A nascent peptide chain undergoing synthesis on a ribosome cannot long exist in solution as a random coil. In fact, those proteins actually made by living things are designed to fold into specific compact three-dimensional structures. Each different kind of protein assumes its own unique structure. These are so definite and reproducible that a large number of proteins have now been crystalized, and their three-dimensional structures have been elucidated by the methods of x-ray crystallography. Although the precise rules that lead to specific folding of a peptide chain are not yet understood, some general principles are evident. Thus, most hydrophilic residues are located on the surface in contact with the water of the cell, whereas the hydrophobic groups tend to be in the interior of the molecule. Certain repetitive structures, such as the alpha-helix, at times provide an extensive backbone for the molecule. Thus, the information for forming the three-dimensional protein subunits of living organisms is encoded in their amino acid sequences, and each kind of protein spontaneously folds into its unique structure.

The next step of organization is into multisubunit aggregates. Proteins not only have sites for specific recognition of small molecule substrates or effectors but also sites for specific recognition of other proteins. These are embodied in the three-dimensional structures of the subunits. Thus, for example, once alpha- and beta-chains of hemoglobin have assumed their unique three-dimensional structures, they are ready to interact in pairs with each other to form the alpha2beta2-structure of the hemoglobin molecule. Within a typical cell, there must be at least several thousand different kinds of proteins subunits, but because the recognition sites on each are unique, inappropriate combinations are not formed.

This strategy for self-assembly extends to exceedingly complex structures. For example, the enzyme pyruvate dehydrogenase is constructed from approximately 60 protein subunits of at least three different kinds (Reed, 1974). The entire sequence of reactions for fatty acid synthesis is catalyzed by a multienzyme complex, fatty acid synthetase. The ribosome is even more complex, being constructed from about 60 to 70 different proteins and three RNA molecules. Even this appears to be a self-assembling structure, and the assembly process has been accomplished in vitro (Nomura, 1973). Likewise, viruses, ranging from the size of ribosomes to much larger dimensions, are self-assembling structures.

An even more complex level of organization is evident in biologic membranes. The matrix for the membranes is lipid, not protein, and is assembled in a bilayer structure. Within this structure, the hydrophilic groups of the lipid molecules are oriented to the two outside surfaces, and the hydrophobic groups interact with those of the other layer in the interior of the membrane. Embedded in the lipid bilayer are protein molecules that confer the unique permeability properties and metabolic activities of the membrane (Singer and Nicolson, 1972). Everything in the membrane structure is highly oriented. The lipid layer facing the outside of the cell or organelle differs in composition from that facing the inside. The many different kinds of proteins of the

membrane are probably all specifically oriented. For example, the polysaccharide moieties that are attached to many membrane proteins during assembly are oriented toward the cell exterior, where they play crucial roles in cell-to-cell recognition and as antigenic determinants. Many of these proteins show considerable mobility in the direction of the plane of the membrane; they resemble icebergs floating in the ocean. Thus, the fluid mosaic structure of the membrane permits considerable movement in the plane but a rigidly asymmetric structure with respect to orientation toward inside or outside of the membrane.

Like the multisubunit proteins, the components of membranes are held together entirely by noncovalent interactions. Also, like multisubunit proteins, much of membrane assembly appears to involve spontaneous specific aggregation of the lipid and protein precursors. In the living cell, this aggregation probably occurs on a matrix of pre-existing membrane, but it shows many features of a self-assembling system.

Experimental Approaches to the Study of Metabolism

Physiology

A number of different experimental approaches have been fruitful in elucidating metabolism. One general type of methodology may be termed the physiologic approach. This approach is characterized by the experimental philosophy of dealing with an intact animal or an isolated organ, homogenate, or other metabolizing system as a "black box". This approach involves supplying various well-defined nutrients and perhaps subjecting the system to the action of various drugs, temperature changes, or other controlled inputs and then measuring various outputs either during the experiment or at postmortem. From this information an attempt is made to deduce what is taking place inside the "black box". Some of the earliest meaningful physiologic experiments involved feeding large amounts of a specific nutrient or feeding a material with distinctive chemical properties and isolating the products of metabolism. An early triumph was the work of Knoop who, in 1904, used this approach to deduce that fatty acids are degraded into two-carbon fragments. More recently, techniques using isotopic tracers and the entire modern armamentarium of procedures for tissue culture, cell disruption and fractionation, quantitative separation and analysis of a host of biologically important compounds, and antimetabolites and drugs with high specific action against a single enzyme or enzyme system have given the physiologic approach great power.

Enzymology

The classic approach to metabolism, by which all of the biochemical reactions were elucidated, is by the techniques of enzymology. The enzymes are isolated and separated, and the substrate specificity of each individual enzyme is studied in vitro. From this, the nature of the metabolic pathway in which these enzymes participate in vivo is deduced. It is because of certain favorable characteristic features of living systems that this approach has proved to be uniquely powerful. One characteristic is the unique and sometimes almost singular specificity of each of the biologic catalysts. Another is that, although the cell is subdivided into a number of separate

regions or compartments, these compartments seem to be relatively few in number. Therefore, much of the routing of metabolites through specific pathways is the result of the specificity of the enzymes; and the carefully prepared cell-free homogenate, especially one derived from a specific subcellular fraction, seems in many respects to duplicate the events occurring within the living cell.

Much experience has indicated that physiologic experiments alone are usually ill-suited to the task of elucidating an unknown metabolic pathway. The alternative possible explanations that are consistent with the experimental data are too numerous, and the correct pathway has rarely been included among the list of postulated ones because of the remarkable and still largely unpredictable types of reactions catalyzed by enzymes. On the other hand, the techniques of enzymology, although capable of clarifying the reactions that do take place, cannot often provide satisfactory insight into the importance of these reactions under specific physiologic conditions. However, physiologic studies, when applied to systems with known enzymology, can often do this, and a judicious use of well-designed physiologic experiments, performed on systems with known enzymology, seems to be one of the most promising approaches to learning what is actually going on inside a living cell.

Biochemical Genetics

A third approach to the study of metabolism is that of the techniques of biochemical genetics. Ever since Beadle and Tatum, in 1941, demonstrated that specific mutants of the mold *Neurospora crassa* lacked the ability to catalyze specific enzymatic reactions, this approach has been refined and its use has been broadened (Beadle, 1946).

In recent years, increasing publicity and much effort have centered on the explosive growth of techniques utilizing *recombinant DNA* (Alberts et al, 1989). In essence, these procedures allow investigators to transfer mammalian genes into the DNA of microorganisms where they can be manipulated and dissected in molecular detail. A practical outcome is the rapid development of manufacturing methods for mammalian and human gene products, such as hormones and vaccines, that were previously available only in minute quantities. Of more profound long-term importance is the prospect that the human genome may now be rapidly dissected, mapped, and manipulated, leading to knowledge of mechanisms and cures for conditions such as cancer, genetic defects, and perhaps even aging. The possible societal implications of this technology have engendered not only excitement and anticipation but also apprehension and dread.

Reconstitution

Synthetic systems constructed from isolated enzymes and mimicking intracellular metabolism have long been a goal for biochemists. In the past, such constructs were a necessary step for verifying the biologic relevance of the particular system under study but often gave little information beyond that deduced by measuring the properties of the individual enzymes. Today, reconstitution is proving to be a powerful method for elucidating mechanisms of action of

components associated with biologic membranes. Synthetic lipid bilayer membranes can be constructed that span orifices of approximately 1 mm. Membrane-associated proteins can be isolated, purified, and reconstituted in biologically active form into these membranes. The responses of these systems to electrical stimulation, drugs, hormones, or neurotransmitters can be measured, and the flow of ions or metabolites through membrane channels can be documented. For many purposes, a more easily achieved preparation is constructed from microscopic artificial lipid vesicles. Although the inside of these vesicles is not directly accessible during an experiment, ingenious techniques using radioisotopes or fluorescent labels can report influx or efflux of ions and yield information similar to that obtained from the more difficult preparations of planar bilayers. The rapid development of these techniques gives promise that bioassays of membrane functions will soon be as routine as assays for soluble enzymes.

Metabolic Pathways of Mammals

Intracellular Metabolism

The experimental knowledge of metabolism that has been obtained can be conveniently summarized in the form of metabolic maps. It is not the purpose of this chapter to present any of these in detail; they can be seen in any modern biochemistry textbook. Only the interrelationships of some major pathways in mammals are pointed out.

Glycolysis is a central pathway of carbohydrate catabolism. The net effect of this pathway is to convert hexose phosphates to pyruvate aerobically or to lactate anaerobically. This process is practically irreversible, and it is coupled to the synthesis of ATP. In addition, certain intermediates of glycolysis serve as the starting materials for important processes, including amino acid and lipid synthesis.

Another pathway for catabolism of hexoses is the hexose monophosphate shunt. Enzymes for both glycolysis and the shunt are present in the cytoplasm of many kinds of cells. In addition to providing intermediates such as the ribose-5-phosphate needed for nucleic acid synthesis, a major role for the shunt is the production of NADPH. This compound acts as the reducing agent for a wide variety of important anabolic reactions and thus rivals ATP as a source of energy for anabolism.

The pyruvate produced by catabolism of hexoses may be oxidized to acetyl CoA, a compound with important roles for both carbohydrate and lipid metabolism. One important fate of the two-carbon acetyl group of acetyl CoA is its oxidation to carbon dioxide by the mitochondrial enzymes of the citric acid cycle. The reduced coenzymes produced by the stepwise oxidation of acetyl CoA are reoxidized by the mitochondrial electron transport system. The terminal step of this chain of oxidation-reduction reactions is the reduction of molecular oxygen to water. A major role for oxygen in mammals and other aerobic organisms is in the operation of this system. Coupled to the electron transport system is the apparatus for oxidative phosphorylation, a biochemical process for which the exact mechanism remains to be elucidated. The system for oxidate phosphorylation utilizes the free energy changes that accompany the

oxidation-reduction reactions of the electron transport system for synthesizing ATP from ADP plus inorganic phosphate. The oxidation of acetyl CoA is thus primary source of ATP in mammals.

A major source of acetyl CoA, in addition to carbohydrate metabolism, is fatty acid catabolism. The stepwise process of fatty acid degradation occurs in the mitochondria, and the reduced coenzymes formed by this oxidation are reoxidized by the electron transport system. Thus, both carbohydrates and fatty acids can furnish energy for ATP synthesis by oxidate phosphorylation.

A third source of acetyl CoA is from catabolism of the ketogenic amino acids.

There are three major fates of acetyl CoA in addition to its oxidation by the citric acid cycle. These are for biosynthesis of fatty acids, ketone bodies, and steroids. Certain metabolic disturbances, including diabetes and starvation, result in an excessive rate of production of acetyl CoA in comparison to its rate of utilization. In these circumstances, ketone body synthesis by the liver, which ordinarily is an important but not major source of energy for extrahepatic tissues, is greatly augmented, and the condition of ketosis occurs.

Fatty acid synthesis and steroid synthesis are examples of anabolic processes that require NADPH, whereas polysaccharide synthesis, nucleic acid synthesis, and protein synthesis are examples of processes that require ATP. It seems reasonable to suppose that the supply of NADPH and ATP in the cell may be adjusted by control of the relative rates of flow of carbohydrates through glycolysis and the hexose monophosphate shunt (Landau et al, 1965).

Although the same two compounds that are the key starting material and product for anabolism may also have the reverse roles for catabolism, nevertheless the reactions linking these compounds may have no enzymes or intermediates that are common to the two pathways. Such is the case for the metabolism of saturated fatty acids. The process for degradation of fatty acids to acetyl CoA by a mitochondrial enzyme system involves CoA intermediates and is coupled to the electron transport system. On the other hand, the process for synthesis of fatty acids from acetyl CoA by a cytoplasmic system utilizes acyl carrier protein intermediates and requires NADPH. A similar situation is seen for the metabolism of polysaccharides. In this case the biosynthetic pathways involve high-energy phosphate reactions utilizing nucleotide-sugar intermediates, whereas the degradative pathways involve hydrolytic or phosphorolytic reactions. The processes of glycolysis and gluconeogenesis are not completely distinct but proceed through some reactions common to both pathways and some that are different. In general, for those steps of glycolysis that are practically irreversible, an alternative gluconeogenic reaction is available. This is known to be true for three steps. One is the glycolytic conversion of phosphoenolpyruvate to pyruvate. This irreversible step is circumvented during gluconeogenesis by conversion of pyruvate to oxaloacetate, which, in turn, is converted to phosphoenolpyruvate. Both of these reactions utilize high-energy phosphate compounds and have favorable equilibria for gluconeogenesis. Similarly, the hexokinase and phosphofructokinase reactions of glycolysis are not used for gluconeogenesis. Instead, thermodynamically favorable hydrolytic reactions catalyzed

by glucose-6-phosphatase and fructose-1,6-diphosphatase are used to produce glucose- and fructose-6-phosphate, respectively. It is evident that these reactions are of critical importance for establishing the rate and direction of carbohydrate metabolism. Therefore it is not surprising that the enzymes involved are controlled by mechanisms that serve to adjust the flow of metabolites through glycolysis and gluconeogenesis in response to the momentary requirements of the cell (Scrutton and Utter, 1968).

Closely related to the pathways of carbohydrate metabolism are those for synthesis of amino acids. Of the 11 amino acids that are not required in the diet for adequate human nutrition (nonessential amino acids), the carbon skeletons of nine are shown. The other two, cysteine and tyrosine, are derived from dietary methionine and phenylalanine, respectively.

Catabolic pathways are also needed to dispose of excess dietary amino acids, both essential and nonessential. The catabolism of some nonessential amino acids is caused by reversal of the reaction used for anabolism. Special degradative pathways are provided for some nonessential amino acids and all essential amino acids. These are discussed in biochemistry textbooks. The bulk of the excess amino acid nitrogen that is released by catabolism is converted to urea by the urea cycle and eliminated in the urine. Once the nitrogen has been removed from an amino acid, the carbon skeleton remaining undergoes catabolism. An appreciable fraction of the energy of humans is derived from dietary amino acids. The carbon skeletons of many amino acids, the glycogenic amino acids, can also undergo gluconeogenesis because they are converted to intermediates of the glycolytic pathway of the citric acid cycle.

Metabolic Activities of Membranes

Hormonal regulation of cellular metabolism mediated by hormone receptors of the cell surface membrane has been discussed. This is but one example of a constellation of membrane functions of crucial importance to cellular regulation, metabolism, and physiologic activity. All these functions are mediated by specific proteins embedded in the lipid bilayer matrix of the membrane. The functions of membrane proteins can be considered in the categories of receptors, selective channels, transducers, and enzymes. Many membranes proteins have two or more of these functions, or perhaps membrane protein complexes combine several functions embodied in different subunits. The role of the membrane in hormonal regulation includes the hormone receptor on the outer surface of the membrane and the transducer, which responds to hormone-receptor interaction by a change of conformation. Membranes have a crucial role in determining what substances are allowed to enter or are excluded from the cell. Here selective channels have a critical function. If the flow of metabolite through the channel is from a region of high concentration to one of low concentration, the process can occur spontaneously. This is termed *facilitated diffusion.* If the flow is from low concentration to high concentration, the process requires energy, and this energy must ultimately come from oxidative reactions or the hydrolysis of ATP. This is termed *active transport.*

In the past decade, a clear picture has emerged for the mechanisms of active transport in mammalian cells (Alberts et al, 1989). The key system for such transport is the sodium ion- and

potassium ion-dependent adenosine triphosphatase (Na+, K+-dependent ATPase). This membrane-embedded protein is responsible for the high intracellular concentration of K+ ions and the high extracellular concentration of Na+ ions. Maintaining these ion gradients against the leveling effects of diffusion is a very difficult uphill task. The Na+, K+-dependent ATPase acts like a "molecular engine" to accomplish this. The ions to be transported enter transmembrane ion channels in the molecule, are attached to specific ion-binding sites, and the conformation of the channel is then changed so they are expelled on the other side of the membrane. The energy required for these molecular transitions is obtained from hydrolysis of ATP, which is bound to a catalytic site located on the intracellular surface of the ATPase molecule. For each molecule of ATP that is hydrolyzed, three Na+ ions are expelled from the cytoplasm and two K+ ions are transported into the cell. This metabolic activity is thought to account for about 30 per cent of the daily energy expenditure of our bodies.

Another primary transport system, ie a transport system directly powered by ATP, is the calcium ion transport system of the sarcoplasmic reticulum of muscle cells. This system sequestrates the calcium ions necessary for the contractile process.

The ionic imbalances that result from the operation of the Na+, K+-dependent ATPase are crucial not only for maintaining the normal intracellular and extracellular environment but also in acting as "batteries" to power other physiologic systems. These ion-driven secondary transport systems actively transport other ions or metabolites in and out of cells. Such systems are called *symport* systems if the driving ions and driven ions or molecules flow in the same direction and *antiport* systems if they flow in opposite directions. An example of a symport system is the Na+-glucose symport, which utilizes the flow of Na+ ions down their ionic gradient into the cell to drive glucose from the intestinal lumen into intestinal epithelial cells (Gray, 1981). A similar symport effects resorption of glucose from the renal tubule.

The concentration gradients established by these systems underlie the mechanisms for depolarizing neurons at synapses and propagating action potentials along axons. A large variety of Na+, K+, Ca++, and Cl- channel proteins, located in presynaptic nerve terminal membranes and in postsynaptic membranes, respond to binding of at least 20 different neurotransmitters by opening or closing ion channels. The resulting changes of ionic fluxes hyperpolarize or depolarize the postsynaptic cell. The action potential is initiated by depolarization and propagated by voltage-sensitive Na+ and K+ channel proteins located in axonal membranes. Thus, the underlying strategy for neuronal signalling is now clear, but it will be a formidable task to isolate and document the properties of all these neurotransmitter receptor proteins, especially since the list of neurotransmitter candidates appears to be far from complete.

Extracellular Metabolism

Although the major metabolic systems for utilization of food energy and synthesis of body constituents reside within the living cells, there are some important enzyme-catalyzed transformations that occur in extracellular spaces. Although not often thought of as being a form of metabolism, they are indeed chemical reactions and, therefore, fall within this definition. All

the extracellular enzymes are synthesized inside living cells and exported to the extracellular fluid.

One such system is the machinery for blood clotting. This machinery is composed of dozens of specific proteins. Many of these proteins lack enzymatic activity in the form in which they are exported from the liver but are converted to active enzymes by proteolysis. The process of clotting begins by activation of minute amounts of proteolytic enzymes by foreign solid material or by substances released by platelets. It proceeds through a cascade of proteases activating other proteases until a substantial amount of the soluble protein fibrinogen is converted by proteolysis to insoluble fibrin, the matrix of the clot. The protease thrombin, derived from prothrombin, catalyzes this reaction. The ultimate dissolution of the clot also involves controlled proteolytic reactions. Another cascade of proteolytic enzymes activates the complement system, which is associated with immunologic defense mechanisms. A final example is the renin-angiotensin system, which controls blood pressure and electrolyte balance. Here, the enzyme renin is released by the kidney in response to hypotension and decreased blood electrolyte concentration. It acts on its substrate angiotensinogen, which is exported by the liver to the blood, to ultimately yield a peptide, angiotensin II, which is a potent hypertensive agent and a trigger for production of the steroid hormone aldosterone by the adrenal cortex.

Traces of countless other enzymes exist in the blood, but most of these are probably of no physiologic significance. Their presence is the result of normal breakdown of cells and recycling of cellular constituents. However, some of them are of considerable clinical value, since elevated levels are sensitive diagnostic indicators for many abnormal conditions, including myocardial infarction, liver diseases, and neoplasms.

Extracellular metabolism also occurs in the interstitial fluid, which bathes all cells. Examples of exceptional medical importance are the extracellular enzymes that catabolize neurotransmitters released into the synaptic cleft. Thus acetylcholine, an important transmitter of the central and autonomic nervous systems and of the neuromuscular junction, is inactivated by the extracellular enzyme acetylcholinesterase. Inhibitors of this enzyme are used medically for conditions such as myasthenia gravis in which augmented acetylcholine is therapeutically beneficial.

The present status of biochemistry is that of a mature science with an ever-increasing body of factual knowledge. This knowledge is securely based on the laws of chemistry and physics, and it also has proved to be effective in explaining a great variety of biologic phenomena. However, this knowledge is still of limited value as a predictive tool for deciding what events will occur if a certain physiologic stress is imposed on an organism. It is also of limited value for devising rational therapeutic procedures for most pathologic conditions. Although there are still some blank areas on the metabolic maps, it appears that the greatest limitations of this science are now in understanding the control mechanisms that regulate the various enzymatic pathways and in discerning the operations of the multitudes of systems and components embedded in biologic membranes. Even in these areas, the gaps in knowledge appear mostly to be for specific details; many guideposts to the underlying molecular strategies have

been discerned. Surprises may be in store, but it comes as a profound shock to the basic scientist to realize that one can now conceptualize much of what one observes in living things by simple extrapolation from model systems that have been worked out in thorough detail. Thus, the major tasks remaining in the biochemical realm of knowledge seem less of finding new basic strategies for each of hundreds or thousands of cellular systems. To the basic investigator, the latter task may seem to be devoid of the gripping challenge and excitement of the former. But to the applied investigator, the detailed analysis of many systems will at some point provide the predictive and applicable strategies for alleviation of human disease and management of the living world. And that is what the basic investigators said they were trying to do all along.